

LÍVIA FRANCO DA COSTA

**Taxonomia e Distribuição de Diatomáceas  
Monorrafídeas (Bacillariophyceae) do Estado de São  
Paulo, Brasil**

Tese apresentada ao Instituto de Botânica da Secretaria de Infraestrutura e Meio Ambiente, como parte dos requisitos exigidos para a obtenção do título de DOUTOR em BIODIVERSIDADE VEGETAL E MEIO AMBIENTE, na Área de Concentração de Plantas Avasculares e Fungos em Análises Ambientais.

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ORIENTADORA: DRA. DENISE DE CAMPOS BICUDO  
COORIENTADOR: DR. CARLOS EDUARDO WETZEL

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À Rita e ao Gustavo, minha mãe e meu filho.  
São vocês que me motivam a sempre continuar.*

*“Equipped with his five senses, man explores the universe around him and calls the adventure Science.”*  
Edwin Powell Hubble

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## RESUMO

As diatomáceas monorrafídeas possuem em comum a presença da rafe em apenas uma das valvas que compõe suas frústulas. Elas pertencem a três famílias (Achnanthaceae, Coccneidaceae e Achnanthidiaceae) acomodadas atualmente em duas ordens, Mastogloiales e Coccneidales. Por um longo período todas as diatomáceas monorrafídeas foram descritas dentro de *Achnanthes* “sensu lato”, chegando a incluir mais de 500 táxons. Contudo, com o aprofundamento do conhecimento da diversidade das diatomáceas e a descrição subsequente de novos táxons, muitas espécies foram transferidas à medida que novos gêneros foram sendo propostos. No Estado de São Paulo, apesar de estudos não publicados indicarem maior riqueza desse grupo de diatomáceas, apenas 32 táxons são contribuições reais, ou seja, passíveis de revisão taxonômica. Desta forma, este estudo teve como objetivo ampliar e aprofundar o conhecimento taxonômico das espécies monorrafídeas com base no estudo detalhado das populações encontradas em diferentes ambientes dulcícolas no Estado de São Paulo. O material de estudo inclui 364 lâminas permanentes de diatomáceas confeccionadas a partir de amostras de dois projetos financiados pela FAPESP (Biota e AcquaSed), abrangendo um amplo gradiente de estados tróficos, distintos hábitats (metafítion, plâncton, perifítion e sedimentos superficiais), épocas do ano (verão/inverno) e diferentes profundidades (ambientes rasos a profundos). O aprofundamento taxonômico foi realizado a partir da análise detalhada das populações encontradas em cada ambiente e hábitat, em microscopia de luz e eletrônica de varredura, bem como da revisão taxonômica das citações desses táxons para o Estado de São Paulo e Brasil. A delimitação das populações levou em conta a circunscrição de materiais-tipo em protólogos, bem como reestudos de material-tipo disponíveis na literatura. Para *Achnanthidium*, as preferências ecológicas das espécies foram analisadas a partir da associação da distribuição quantitativa (abundância relativa) dos táxons com variáveis limnológicas (físicas e químicas) disponíveis no banco de dados do projeto AcquaSed. Os resultados das análises foram apresentados em quatro capítulos já formatados para publicação em periódicos. Cinquenta e seis espécies de diatomáceas monorrafídeas pertencentes a dez gêneros foram encontradas nas amostras analisadas: *Achnanthes* (4), *Platessa* (3), *Coccneis* (9), *Achnanthidium* (13), *Gogorevia* (5), *Planothidium* (17), *Psammothidium* (2), *Lemnicola* (1), *Skabitschewskia* (1) e *Karayevia* (1). Dentre elas, nove espécies foram descritas como novidades para a Ciência (oito *Achnanthidium* spp. e *Coccneis amerieuglypta*). Ainda, *C. placentula* var. *acuta*, atual *C. tropicoacuta*, foi elevada a nível de espécie, e sete táxons foram identificados em nível genérico devido, principalmente, à raridade de espécimes encontrados. Das 40 espécies encontradas que são conhecidas na literatura, 20 foram novas citações para o Estado de São Paulo, sendo nove

novas ocorrências também para o país. Dez espécies de *Achnanthidium* tiveram seu ótimo ecológico calculado, tendo a maioria apresentado preferências ecológicas por ambientes oligo a mesotróficos com baixa condutividade. Destaca-se a importância do uso da microscopia eletrônica de varredura para o aprofundamento do conhecimento das espécies, além de sua grande importância na identificação das espécies de *Achnanthidium*. Por fim, ressalta-se a importância de estudos florísticos incluindo as espécies raras para o conhecimento mais amplo a aprofundado das diatomáceas monorrafídeas.

**Palavras-chave:** diversidade, material-tipo, perifítón, plâncton, preferências ecológicas, sedimentos superficiais

## ABSTRACT

Monoraphid diatoms have in common the presence of raphe in one of the valves of its frustules. They belong to three families (Achnanthaceae, Coccconeidaceae and Achnanthidiaceae) currently classified in two orders, Mastogloiales and Coccconeidales. During a long time, all monoraphid diatoms were described as *Achnanthes* "sensu lato", including about 500 taxa. However, with the improving knowledge in diatoms diversity and subsequent description of new taxa, many species were transferred as new genera. In São Paulo State, despite some unpublished studies show a greater richness of monoraphid taxa, only 32 taxa are real contributions, enabling taxonomic review. In this way, this study aimed at expanding and deepening the taxonomic knowledge on monoraphid species based on detailed study of the populations collected from different freshwater environments in the State São Paulo. Studied material includes 364 permanent slides prepared from samples from two projects funded by FAPESP (Biota and AcquaSed), covering a wide range of trophic states, different habitats (metaphyton, plankton, periphyton and surface sediments), climatic seasons (summer/winter), and depths (shallow and deep waters). The taxonomic study was carried out under light and scanning electron microscopy from a detailed analysis of the population gathered from each environment and habitat, as well as from the taxonomic review of the records of taxa in the State São Paulo and Brazil. Delimitation of populations took into account the circumscription of type materials in protogues, and re-studies of type materials available in the literature. In *Achnanthidium*, the ecological preferences of the species were analyzed from the association of the quantitative distribution (relative abundance) of taxa with limnological variables (physical and chemical) available in the database of the Acquased Project. Results were presented in four chapters already formated for publication in journals. Fifty-six species of monoraphid diatoms belonging to 10 genera were found from samples analyzed: *Achnanthes* (4), *Platessa* (3), *Coccconeis* (9), *Achnanthidium* (13), *Gogorevia* (5), *Planothidium* (17), *Psammothidium* (2), *Lemnicola* (1), *Skabitschewskia* (1), and *Karayevia* (1). Among them, nine species were described as new to Science (eight *Achnanthidium* spp., and *Coccconeis amerieuglypta*); *C. placentula* var. *acuta*, currently *C. tropicoacuta*, was raised to species level; and seven taxa were identified at the generic level, mainly due to the rarity of specimens found. From 40 species known in the literature, 20 were new records for the State of São Paulo, and nine also new for the country. Ten *Achnanthidium* species had their ecological optima calculated, and most of them had ecological preferences for oligo to mesotrophic environments with low conductivity. Importance of scaning electron microscopy is highlighted in the knowledge of the species, besides the great support on the identification

of *Achnanthidium* species. Furthermore, the importance of floristic studies including rare species is emphasized for the broader and deepen knowledge of monorraphid diatoms.

**Key-words:** diversity, ecological preferences, type material, periphyton, plankton, surface sediments

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## INTRODUÇÃO

Diatomáceas são algas unicelulares microscópicas que possuem pigmentos fotossintéticos únicos e produtos de armazenamento específicos, como óleos e crisolaminarina (Taylor et al. 2007). Apresentam célula recoberta por uma carapaça silícica (frústula), geralmente formando um tipo de caixa, composta de duas valvas e bandas conectivas também chamadas de cíngulo (Van Heurck 1986). A taxonomia do grupo baseia-se principalmente na análise morfológica dessa parede, a qual exibe padrões espécie-específicos permitindo o reconhecimento dos táxons.

Análises moleculares são ainda pouco utilizadas na taxonomia de diatomáceas, contudo, estudos filogenéticos abrangendo principalmente gêneros vêm se tornando mais comuns (ex. Vanormeligen et al. 2013, Nakov et al. 2014, Dhar et al. 2015, Witkowski et al. 2015, Kulikovskiy et al. 2016, Li et al. 2020, Al-Handal et al. 2019, Tseplik et al. 2021). Ainda, são reconhecidamente utilizadas na classificação supra ordinal do grupo (Medlin & Kaczmarska 2004). O incipiente desenvolvimento da biologia molecular dentro do estudo de diatomáceas provavelmente se deva à dificuldade de cultivo do grupo devido ao seu distinto ciclo celular (Chepurnov et al. 2008). Também, é possível que poucas diferenças nucleotídicas se acumulem entre diferentes espécies, assim pouco poderia ajudar em suas separações (David M. Williams, com. pes. em 03/02/2017).

Apesar de todas suas dificuldades e do lento desenvolvimento do estudo de diatomáceas no campo molecular, técnicas como o barcode, que caracteriza diretamente o genoma da espécie, trariam novos avanços para o estudo do grupo. A aplicação do DNA barcode em diatomáceas tem grande possibilidade de estabilizar sua nomenclatura, além de outras vantagens desse método em relação à identificação microscópica, como minimizar a dificuldade na identificação gerada pela plasticidade fenotípica em algumas espécies. A plasticidade fenotípica em diatomáceas ocorre em resposta a estímulos ambientais, os quais podem levar a uma variação morfológica ou fisiológica dos indivíduos de uma espécie (Andrejic et al. 2019). Com o barcode se identificaria facilmente espécies com polimorfismo, células em estágio de repouso e ainda essas espécies com características alteradas pelo meio; permitiria ainda a identificação em qualquer estágio do ciclo de vida da espécie, o que nem sempre é possível com a visualização em microscopia devido à grande semelhança entre indivíduos menores de diferentes espécies; também, geraria uma comunicação mais fácil entre sequências e sua variação do que descrições e ilustrações; além de que qualquer pessoa com um treinamento adequado em tecnologia de DNA poder identificar diatomáceas com precisão (Mann et al. 2010).

As diatomáceas ocorrem no plâncton ou aderidas a substratos ocupando o hábitat perifítico ou bentônico e formam o grupo mais rico em espécies de algas com pelo menos 30.000 conhecidas e, mais provavelmente, 100.000 espécies existentes (Mann & Vanormelingen 2013). Surgiram há aproximadamente 200 milhões de anos, consistindo nos principais componentes do fitoplâncton, tanto marinho quanto de água doce, sendo um dos fósseis eucarióticos mais conhecidos (Madigan et al. 2016). Constituem um elemento importante nos ecossistemas aquáticos por serem elo fundamental entre produção primária e secundária, além de serem ótimas indicadoras de alterações ambientais recentes e pretéritas e, assim, valiosas no biomonitoramento (Taylor et al. 2007). Reúnem uma série de características fisiológicas e ecológicas essenciais para seu uso como indicadores ecológicos, as quais incluem a facilidade de coleta, preparação e preservação de amostras; ampla distribuição geográfica; elevada riqueza de espécies com diferentes exigências ecológicas; rápida resposta às mudanças ambientais devido ao seu curto ciclo-de-vida (uma a algumas divisões celulares por dia); e envoltório celular de sílica polimerizada que confere boa preservação das valvas no sedimento, permitindo sua identificação mesmo estando depositada por longo período de tempo. Além dessas vantagens, há grande quantidade de informações sobre a autoecologia de diversas espécies, especialmente para regiões de clima temperado (Blanco et al. 2007, Smol & Stoermer 2010, Bennion et al. 2011).

A classificação atual das diatomáceas as enquadra na divisão Bacillariophyta, dentro da qual a classe Bacillariophyceae engloba a maioria de seus representantes (Medlin & Kaczmarska 2004, Adl et al. 2019). A classe reúne dois grupos distintos de diatomáceas penadas, as que possuem rafe (rafídeas) e as que não possuem (arrafídeas). Com grande importância na classificação do grupo, a rafe é uma estrutura formada por uma ou duas fendas longitudinais na valva, que aparentemente tem por função a locomoção das diatomáceas a partir da secreção de mucilagem pela(s) fenda(s) (Round et al. 1990).

Dentre as diatomáceas rafídeas, um grupo se distingue por apresentar essa estrutura em apenas uma das valvas. Essas, conhecidas como diatomáceas monorrafídeas, fizeram parte por muito tempo da ordem Achnanthales P.C. Silva, formada pelas famílias Achnanthaceae Kützing, Coccconeidaceae Kützing e Achnanthidiaceae D.G. Mann (Round et al. 1990).

Apesar do uso da biologia molecular para a identificação e separação de táxons ser ainda pouco empregada dentre as diatomáceas, recentemente, as monorrafídeas foram objeto de um estudo filogenético específico abordando o significado da rafe na evolução e taxonomia deste grupo (Kulikovskiy et al. 2016). Esse e outros trabalhos incluindo monorrafídeas tem mostrado que elas não compõem um único grupo monofilético, estando na verdade dispersas na árvore filogenética (Medlin & Kaczmarska 2004, Kulikovskiy et al. 2016, 2019).

Hoje, a família Achnanthaceae é classificada na ordem Mastogloiales D.G. Mann, juntamente com outros táxons não-monorrafídeos (Cox 2015). Seu agrupamento ao clado da ‘nova’ ordem se deve ao compartilhamento de estruturas semelhantes tanto no protoplasto quanto na frústula de *Achnanthes brevipes* C.A. Agardh (sinônimo de *A. adnata* Bory, tipo de *Achnanthes* Bory sensu stricto) e outros representantes de Mastogloiales, independente da condição monorrafídea (Cox 2006, Cox & Williams 2006). Ainda, as famílias Achnanthidiaceae e Cocconeidaceae passaram, provisoriamente, a fazer parte da ordem Cocconeidales E.J.Cox enquanto novos estudos filogenéticos não forem realizados (Cox 2015).

A grande maioria dos gêneros monorrafídeos é predominantemente encontrado em águas marinhas e salobras ou são exclusivos desses habitats, entretanto, pelo menos 16 gêneros ocorrem em água doce. Por um longo período todas as diatomáceas monorrafídeas foram descritas dentro de *Achnanthes* sensu lato, chegando esse gênero a incluir mais de 500 táxons (Kulikovskiy et al. 2016). Com o aprofundamento do conhecimento da diversidade das diatomáceas e a descrição subsequente de novos gêneros e espécies, que teve início nos anos 90, muitas espécies migraram de *Achnanthes*, sendo combinadas em outros gêneros e famílias à medida que foram sendo descritos (ex. *Achnanthidium* Kützing, *Planothidium* Round & Bukhtiyarova, *Psammothidium* Bukhtiyarova & Round). Assim, *Achnanthes* está atualmente restrito a táxons que ocorrem principalmente em águas marinhas ou salobras e que possuem aréolas conspícuas ocluídas por cribra (Toyoda et al. 2005). Ainda dentro da família Achnanthaceae, *Platessa* Lange-Bertalot possui valvas pequenas e elípticas, estrias predominantemente bisseriadas e aréolas ocluídas por hymen (Romero 2016). Já *Platebaikalia* Kulikovskiy, Glushchenko, Genkal & Kociolek, gênero monoespecífico que foi descrito recentemente a partir de sistema dulcícola, no Lago Baikal, Rússia, pode ser facilmente diferenciado pelas estrias multisseriadas (Kulikovskiy et al. 2020).

Na família Cocconeidaceae a maioria dos táxons também é preferencialmente ou exclusivamente marinha, com apenas dois gêneros (*Cocconeis* Ehrenberg e *Anorthoneis* Grunow) ocorrendo também em água doce (Kociolek et al. 2018a). *Cocconeis* foi o primeiro gênero descrito da família e alguns outros surgiram a partir da transferência de suas espécies (*Bennettella* Holmes, *Vikingea* Witkowski, Lange-Bertalot & Metzeltin e *Amphicocconeis* M.De Stefano & D.Marino). Atualmente, muitas espécies de *Cocconeis* vêm sendo descritas (De Stefano et al. 2000, De Stefano & Marino 2001, Riaux-Gobin et al. 2007, 2010, 2015, Romero & López-Fuerte 2013, Riaux-Gobin & Witkowski 2017, Costa et al. 2019) e materiais-tipo sendo reexaminados (Romero & Riaux-Gobin 2014, Riaux-Gobin et al. 2016, 2017).

Espécimes de *Coccconeis* apresentam valvas elípticas ou quase circulares, valva rafídea usualmente menos convexa do que a arrafídea e estrias usualmente unisseriadas com aréolas cobertas por hymen com perfurações lineares (Round et al. 1990). Apesar de sua descrição “simples”, a grande variabilidade morfológica vista em estudos recentes o tem colocado entre um dos táxons mais estruturalmente complexos (Car et al. 2012), provavelmente devido à estrutura de sua valvocópula, apresentando fimbrias com morfologia espécie-específica (Holmes et al. 1982).

*Anorthoneis* é um gênero menor e apenas uma de suas espécies foi descrita de água doce em rios da Flórida, Estado Unidos. *Anorthoneis dulcis* Hein já foi encontrado também no México e no Brasil (Garcia & Talgatti 2008, Tremarin & Ludwig 2008, López-Fuerte et al. 2011). Representantes do gênero possuem valvas elípticas, ambas com a área axial excêntrica (não centralizada) não atingindo os pólos, além de estrias unisseriadas se tornando bisseriadas na margem valvar (Round et al. 1990).

Em Achnanthidiaceae a maior representatividade é de táxons dulcícolas: *Achnanthidium*, *Eucoccconeis* Cleve ex Meister, *Planothidium*, *Psammothidium*, *Rossithidium* Bukhtiyarova & Round, *Lemnicola* Round & Basson, *Kolbesia* Round & Bukhtiyarova ex Round, *Karayevia* Round & Bukhtiyarova ex Round, *Gliwiczia* Kulikovskiy, Lange-Bertalot & Witkowski, *Crenotia* Wojtal, A.Witkowski & C.Riaux-Gobin e *Skabitschewskia* Kuliskovskiy & Lange-Bertalot, *Gogorevia* Kulikovskiy, Glushchenko, Maltsev & Kociolek (Kociolek et al. 2018b).

A respeito da filogenia da família Achnanthidiaceae, os gêneros *Achnanthidium*, *Psammothidium*, *Rossithidium* e também *Lemnicola* formam um clado monofilético e seu agrupamento também é consistente morfologicamente (Kulikovskiy et al. 2016). Contudo, *Karayevia* sensu Bukhtiyarova (2006) faz parte, juntamente com *Madinithidium* C.Desrosiers, A.Witkowski & C.Riaux-Gobin (monorrafídea marinha), de uma linhagem independente no clado da família Stauroneidaceae e não de Achnanthidiaceae (Kulikovskiy et al. 2019). *Kolbesia* foi incluído em *Karayevia* por Bukhtiyarova (2006) devido à correspondência do tipo do gênero (*Achnanthes kolbei* Hustedt) com a descrição de *Karayevia* atualizada pela autora. Apesar disso, o nome continua em uso e novas espécies de *Kolbesia* foram descritas recentemente (*K. sichuanensis* P. Yu, Q-M. You & Q-X Wang e *K. sinica* Krzywda, Witkowski & Ch. Li). Yu et al. (2019b) descreveram a primeira espécie e aparentemente ignoraram o gênero *Karayevia* em seu trabalho, o qual não foi mencionado. Já Witkowski et al. (2016) mencionam a diferença entre a estriação de ambos os gêneros: *Karayevia* apresenta pequenas aréolas circulares, enquanto *Kolbesia* possui macroaréolas cobertas externamente por sílica deixando visíveis duas pequenas aréolas alongadas transapicalmente.

*Karayevia*, *Rossithidium* e *Planothidium* foram descritos conjuntamente por Round & Bukhtiyarova (1996) a partir de espécies originalmente propostas como *Achnanthes* [*A. clevei* Grunow, *A. (linearis* var.?) *pusilla* Grunow e *A. lanceolata* (Bréb.) Grunow] e várias combinações novas foram feitas nesse trabalho. Segundo descrições originais dos autores, *Rossithidium* apresenta valvas lineares, com estrias paralelas geralmente contínuas no centro da valva arrafídea, presença ou não de estauro na valva rafídea e terminações da rafe curvadas para o mesmo lado. *Planothidium* possui estrias bisseriadas, interrompidas ou não na região central da valva arrafídea por depressão característica ('cavum' ou 'sinus') e também, terminações da rafe curvadas para o mesmo lado (Round & Bukhtiyarova 1996).

*Gliwiczia* e *Skabitschewskia* foram descritas do Lago Baikal (Rússia) e apresentam estrutura semelhante a *Planothidium* na área central. Em *Gliwiczia*, o 'cavum' é comum em ambas as valvas (rafídea e arrafídea), as estrias são unisseriadas e as terminações da rafe são curvadas em direções opostas (Kulikovskiy et al. 2013). Em *Skabitschewskia* as estrias são unisseriadas na valva rafídea e bisseriadas na valva arrafídea, a mesma que apresenta o 'cavum' (Potapova 2019).

Ainda dentro de Achnanthidiaceae, *Lemnicola* é um gênero monoespecífico caracterizado por possuir estrias bisseriadas, estauro largo e assimétrico na valva rafídea e bem reduzido ou ausente na valva arrafídea (Round & Basson 1997). Na última década, outras espécies foram transferidas e descritas para o gênero, como *Lemnicola exigua* (Grunow) Kulikovskiy, Witkowski & Plicski, *L. rostellata* (Cleve) Chudaev e *L. uniseriata* Y. Shi & B.-H. Kim (Plicski & Witkowski 2011, Chudaev et al. 2015, Shi et al. 2018). Contudo, estudo mais recente baseado em morfologia e dados moleculares colocou essas espécies em um novo gênero denominado *Gogorevia*.

*Crenotia* também é um gênero pequeno, suas seis espécies descritas (Kociolek et al. 2020a) são caracterizadas por possuírem estrias bisseriadas que terminam internamente em uma depressão superficial coberta por uma camada fina de sílica, além de não possuírem ornamentação na margem valvar e elementos do cíngulo (Rioual et al. 2019).

*Eucocconeis* engloba um número maior de espécies, com pelo menos 31 conhecidas (Enache & Potapova 2012). Ele foi primeiramente considerado subgênero por Cleve (1895) e então estabelecido como gênero em Meister (1912). Round et al. (1990) comentam sobre a rejeição do gênero ocasionada pelo contínuo de variação da curvatura da rafe, de reta em *Achnanthidium* a fortemente sigmóide em *Eucocconeis*, mas reafirma sua validação por outras estruturas da frústula. Além da rafe sigmoide, espécies do gênero apresentam frústulas curvadas na região mediana no plano transapical e torcidas no eixo apical, as estrias são unisseriadas com aréolas ocluídas por hymen (Round et al. 1990).

*Psammothidium* foi estabelecido a partir de *Achnanthes marginulata* Grunow (Bukhthiyarova & Round 1996) e apesar de ser bastante semelhante a *Achnanthidium*, já foi comprovado serem táxons independentes em estudo molecular (Kulikovskiy et al. 2016). Um conjunto de características morfológicas distingue o gênero dos demais: pequenas valvas elípticas, valva rafídea convexa, área central distinta e retangular na valva rafídea, terminações da rafe curvadas para lados opostos ou ausentes (retas), estrias usualmente unisseriadas e aréolas com diferentes formatos (Bukhthiyarova & Round 1996, Spaulding & Edlund 2008). A distinção entre as espécies do gênero é muitas vezes feita com o uso de microscopia eletrônica de varredura para verificar a forma do esterno/rafe e das aréolas (Bukhthiyarova & Round 1996). Com o maior número de táxons da família, incluindo mais de 200 espécies, variedades e formas taxonômicas (Fourtanier & Kocolek 2011), *Achnanthidium* é encontrado em ambientes marinhos e de água doce (Procopiak et al. 2006), constituindo um dos principais componentes da comunidade bentônica (ex. Ponader & Potapova 2007, Novais et al. 2011). O gênero também possui representantes planctônicos de vida colonial como *A. catenatum* (Bíly & Marvan) Lange-Bertalot (Bíly & Marvan 1959).

O gênero passou por muitas mudanças desde sua proposição, dificultando a taxonomia do grupo. Descrito por Kützing (1844), foi considerado por Reimer um subgênero (Patrick & Reimer 1966). Então, foi reestabelecido mais tarde como gênero e incluído na família Achnanthidiaceae juntamente com *Eucocconeis* em Round et al. (1990). Posteriormente, sua redefinição foi proposta de forma a confinar o conceito de *Achnanthidium* apenas aos táxons do complexo *Achnanthes minutissima* (Round & Bukhtyarova 1996). Assim, alguns táxons foram realocados a outros gêneros da família já mencionados (*Rossithidium*, *Planothidium* e *Karayevia*).

Além da inconstância em sua classificação, a problemática na taxonomia de *Achnanthidium* deve-se também ao seu pequeno tamanho, dificultando a visualização de estruturas em microscopia óptica (i.e. estrias, aréolas) e, principalmente, pela falta de critérios morfológicos que definam os limites das espécies do gênero (Hlúbková et al. 2011). Atualmente, a circunscrição das espécies baseia-se nos caracteres morfológicos (i.e. formato valvar, área central, estrias) das duas valvas, rafídea e arrafídea, cuja visualização muitas vezes requer o uso de microscopia eletrônica de varredura (MEV). Assim, as características diagnósticas do gênero *Achnanthidium* são bastante amplas: células pequenas usualmente menores do que 30 µm em comprimento e 5 µm em largura; valvas linear-lanceoladas a linear-elípticas; estrias unisseriadas, radiais ou quase transversas, levemente espaçadas no centro (às vezes ausentes ou reduzidas) e mais próximas em direção aos ápices; vista lateral em forma de V profundo (Round & Bukhtyarova 1996).

*Achnanthidium* é amplamente distribuído e tem ganhado maior atenção atualmente, permitindo diversas descrições de espécies novas para a ciência, principalmente em regiões temperadas da Europa (Potapova 2006, Rimet et al. 2010, Novais et al. 2011, 2015, Wojtal et al. 2011, Van de Vijver et al. 2011a, Wetzel et al. 2019), mas também, em menor escala, nas regiões tropicais, subtropicais (Morales et al. 2011; Marquardt et al. 2017; Krahn et al. 2018, Yu et al. 2019a, 2019b; You et al. 2019) e polar (Taylor et al. 2014). No total, mais de 50 táxons foram propostos como novos nos últimos 10 anos, demonstrando o conhecimento em expansão, mas ainda incipiente deste gênero. Além disso, um estudo molecular foi realizado com *Achnanthidium*, incluindo diversas populações de *A. minutissimum* (Kützing) Czarnecki e suportando a separação de uma nova espécie (Pinseel et al. 2017).

Na América do Sul (exceto Brasil), estudos taxonômicos com foco em monorrafídeas de água doce são escassos. Maidana (2000) estudou amostras de distintos corpos d'água continentais de Tierra del Fuego, Argentina, como parte de um projeto de flora criptogâmica, em busca do conhecimento da diversidade de Achnanthaceae. Considerando a classificação mais atual das famílias, a autora observou 24 representantes de Achnanthidiaceae (cinco de *Achnanthidium*, uma *Karayevia*, 11 *Planothidium* e sete *Psammothidium*), além de três de Achnanthaceae (*Achnanthes*) e três de Cocconeidaceae (*Cocconeis*), incluindo também novas combinações. Já em rios na Bolívia, Morales et al. (2011), identificou 12 espécies de *Achnanthidium*, descreveu nova espécie (*A. cadimae* E.Morales, E.Fernández & Ector) e deu novo nome a *Achnanthes kryophiles* Hohn (*Achnanthidium peruvianum* E.Morales & Ector).

Além dos mencionados, representantes do grupo são bastante citados em floras de diatomáceas na Argentina, Bolívia, Chile, Colômbia, Ecuador, Uruguai e Venezuela (Oliveira & Steinitz-Kannan 1992, Sala 1996, Metzeltin & Lange-Bertalot 1998, Rumrich et al. 2000, Maidana 2000, Metzeltin & García-Rodríguez 2003, Metzeltin et al. 2005, Morales & Vis 2007, Alvial et al. 2008, Sala et al. 2008, García & Maidana 2015) e trabalhos ecológicos (Lobo et al. 2004, Ramírez & Plata-Díaz 2008, Tapia 2008, Bere & Tundisi 2011, Castillejo et al. 2018). Dentre as espécies citadas, *A. minutissimum* apresenta a maior distribuição, ocorrendo em grande parte dos países da região.

No Brasil, na última década também foram propostas novas espécies monorrafídeas como *Achnanthidium*, *Planothidium* e *Cocconeis* (Wetzel & Ector 2014a, Marquardt et al. 2017, Costa et al. 2019, Wetzel et al. 2019, Morais et al. 2020). Segundo a ‘Flora do Brasil 2020 em construção’ (Flora do Brasil 2020), já foram encontrados no Brasil: um táxon de *Lemnicola*, um de *Rossithidium*, quatro de *Anorthoneis*, oito de *Planothidium*, 13 de *Achnanthidium*, 34 de *Cocconeis* e 35 de *Achnanthes*. *Karayevia*, *Kolbesia*, *Psammothidium*, *Platebaikalia*, *Platessa*, *Eucocconeis*, *Gliwiczia*, *Skabitschewskia* e *Crenotia* não apresentaram citações. O

site considerou espécies marinhas e de água doce, além de sinônimos, ainda, o mesmo também não é atualizado há alguns anos pois está em construção.

Através de revisão da literatura do país, considerando estudos publicados com informações taxonômicas (i.e. descrições, medidas e/ou ilustrações), em águas dulcícolas foram observadas várias espécies de *Achnanthes*, muitas atualmente fazendo parte de outros gêneros (ex. *A. lanceolata*, *A. salvadoriana*, *A. hungarica*, *A. minutissima*). Ainda pertencente a este gênero, *Achnanthes inflata* (Kützing) Grunow foi a espécie mais encontrada em águas continentais brasileiras (Rodrigues & Moreira-Filho 1990, Bicudo et al. 1993, Moro & Furstenberger 1993, Oliveira et al. 2001, Ferrari & Ludwig 2007, Moutinho et al. 2007, Bicudo et al. 2009, Bertolli et al. 2010). *Platessa* foi menos diversa, sendo encontrado o parátipo de *P. guianensis* R.Le Cohu, L.Tudesque & C.E.Wetzel no Rio Negro (Amazonas) (Tudesque et al. 2016); *P. hustedtii* (Krasske) Lange-Bertalot no Rio Grande do Sul (Bes et al. 2012); *P. oblongella* (Østrup) C.E.Wetzel, como *Karayevia oblongella* (Østrup) Aboal, também no RS (Rosa & Garcia 2014); e *P. conspicua* (A.Mayer) Lange-Bertalot em Santa Catarina, como *Achnanthes conspicua* A.Mayer (sem ilustrações, Rodrigues 1984). Além disso, citações de *Achnanthes rupestroides* Hohn para o Sul do país também parecem corresponder a *P. hustedtii* (ex. Oliveira et al. 2002, Ferrari & Ludwig 2007, Moresco et al. 2011).

*Anorthoneis dulcis* foi citado para o Brasil e América do Sul pela primeira vez em amostras de sedimento arenoso no Pará e da Lagoa dos Patos no Rio Grande do Sul (Garcia & Talgatti 2008, Tremarin & Ludwig 2008). Já *Coccneis* é mais amplamente citado apesar de predominantemente representado por espécies do complexo-*Coccneis placentula* Ehrenberg em quase todo o país: *C. placentula*, *C. euglypta* Ehrenberg, *C. lineata* Ehrenberg e *C. tropicoacuta* L.F. Costa & C.E. Wetzel (Moreira 1975, Rodrigues & Moreira-Filho 1990, Oliveira et al. 2001, Ferrari & Ludwig 2007, Schneck et al. 2008, Fontana & Bicudo 2012, Bartozek et al. 2013, Faustino et al. 2016, Nardelli et al. 2016, Costa et al. 2019).

Em relação à Achnanthidiaceae, *Lemnicola hungarica* e *Gogorevia exigua* (como *Achnanthes exigua* ou *Achnanthidium exiguum*) são bastante comuns em águas continentais nacionais (Bicudo et al. 2009, Carneiro & Bicudo 2007, Ferrari & Ludwig 2007, Faria et al. 2010, Bes et al. 2012, Marra et al. 2016, Costa et al. 2017, Bartozek et al. 2018). Também bastante frequente, o gênero *Planothidium* é representado principalmente por *P. dubium* (Grunow) Round & Bukhtiyarova, *P. lanceolatum* (Brébisson ex Kützing) Lange-Bertalot e *P. rostratum* (Østrup) Lange-Bertalot, alguns ainda citados como representantes de *Achnanthes* em trabalhos mais antigos (Rodrigues 1984, Torgan 1985, Rodrigues & Moreira-Filho 1990, Bicudo et al. 1993, Magrin & Senna 1997, Bertolli et al. 2010, Fontana & Bicudo 2012,

Bartozek et al. 2013, 2018 Marra et al. 2016). Recentemente, *P. brasiliense* C.E.Wetzel & S.Blanco foi descrito de amostra do Estado de São Paulo e *P. xinguense* K.S.Morais, C.E.Wezel & C.E.M.Bicudo foi descrito do Rio Xingu, Amazônia (Wetzel et al. 2019, Morais et al. 2020). Mais raros, *Psammothidium subatomoides* (Hustedt) Lange-Bertalot já foi observado em amostras epilíticas (Schneck et al. 2008) e epifíticas no sul do Brasil (sem ilustrações, Santos et al. 2011), enquanto *P. ventralis* foi observado no Paraná (como *Navicula ventralis*, Contin 1990).

Dentres as espécies de *Achnanthidium* já encontradas no Brasil, assim como no resto da América do Sul, *A. minutissimum* é também a mais amplamente distribuída, sendo citada para os estados de São Paulo (Magrin & Senna 1997, Bicudo et al. 2009, Bere 2010, Faustino et al. 2016), Rio Grande do Sul (Oliveira et al. 2001, Lobo et al. 2004, 2010, Raupp et al. 2006), Santa Catarina (Rodrigues & Moreira-Filho 1990), Paraná (Fürstenberger & Valente-Moreira 2000, Ferrari & Ludwig 2007, Bertolli et al. 2010, Faria et al. 2010, Bartozek et al. 2013) e Goiás (Nogueira et al. 2008). *Achnanthidium* distribuiu-se em lagos, represas e rios, principalmente no habitat perifítico, em ambientes de águas neutras a levemente alcalinas (pH 7.1–7.5), condutividade moderada ( $133\text{--}114 \mu\text{s cm}^{-1}$ ) e oligotróficas a eutróficas (Nogueira et al. 2008, Silva et al. 2010, Faustino et al. 2016).

Na região Sul, das 13 espécies/variedades de *Achnanthidium* que foram citadas pelo catálogo de diatomáceas de Tremarin et al. (2009), apenas dois representantes, *A. minutissimum* e *Achnanthidium* sp., foram publicados com inclusão de informações taxonômicas (Fürstenberger & Valente-Moreira 2000, Moro & Fürstenberger 1993, Ferrari & Ludwig 2007). Mais recentemente, outros trabalhos reportaram as mesmas espécies, além de *Gogorevia constricta* (Torka) Kulikovskiy & Kocielek como *A. exiguum* var. *constrictum* (Grunow) Andresen, Stoermer & Kreis (Bertolli et al. 2010, Faria et al. 2010, Santos et al. 2011, Bartozek et al. 2013). Na região como um todo, além dos táxons já mencionados, outros dois foram reportados nos Estados do Rio Grande do Sul e Santa Catarina: *A. exiguum* var. *heterovalvum* (Krasske) Czarnecki (Torgan 1985, Rodrigues & Moreira-Filho 1990) e *A. microcephalum* Kützing, reportada apenas no Rio Grande do Sul (Torgan 1985, Raupp et al. 2006, Schneck et al. 2008).

Contrastando com a quantidade de trabalhos conhecidos para o Sul do país, as outras regiões são pobramente representadas. Ainda sobre *Achnanthidium*, na região Centro-Oeste, o catálogo de diatomáceas da região por Da Silva et al. (2011) indica cinco estudos publicados contendo oito táxons que ocorreram no Estado de Goiás e no Distrito Federal (Contin & Oliveira 1993, Souza & Oliveira 2007, Nogueira et al. 2008).

Na região Sudeste do país, especificamente no Estado de São Paulo, a contribuição mais ampla de monorrafídeas foi a dissertação de Carneiro (2003), a qual reporta 46 táxons (10 de *Achnanthes*, 11 de *Achnanthidium*, uma de *Lemnicola*, 10 de *Planothidium*, três de *Psammothidium*, duas de *Rossithidium*, nove de *Cocconeis*) observados em distintos ecossistemas e hábitats a partir do levantamento florístico da ordem Achnanthales no Estado. Além dessa, dissertações e teses mais recentes sobre ecologia de comunidades de diatomáceas possibilitaram o reconhecimento de vários outros táxons (Wengrat 2011, Almeida 2012, Nascimento 2012, Rocha 2012, Faustino 2013, Oliveira 2015, Bartozek 2017). Dessa forma, a pequena representação do grupo em trabalhos publicados para o Estado de São Paulo leva à subestimativa de sua biodiversidade, sendo assim, apenas 32 táxons são contribuições reais, passíveis de revisão taxonômica, para o Estado (Bicudo et al. 1993, Magrin & Senna 1997, Bicudo et al. 2009, Costa-Böddeker et al. 2012, Fontana & Bicudo 2012, Faustino et al. 2016, Bartozek et al. 2018, Marquardt et al. 2017, 2018, Costa et al. 2019). Nove desses foram observados no epipélon da cachoeira da Reserva Biológica do Alto da Serra de Paranapiacaba (Bicudo et al. 2009). Ainda dentro desse número de táxons, o presente trabalho já trouxe importante contribuição com a publicação de estudo sobre a diversidade de *Cocconeis* no Estado, resultando assim na descrição de uma nova espécie, novo status e nome de *Achnanthes placentula* var. *acuta* Ehrenberg, elevado à categoria de espécie (*C. tropicoacuta*) e a citação total de nove representantes do gênero (Costa et al. 2019).

Em síntese, em âmbito nacional é possível verificar que há maior ocorrência de diatomáceas monorrafídeas nas regiões Sul e Sudeste, cenário esse que se deve principalmente à concentração de especialistas trabalhando com diatomáceas nessas regiões do país. Especificamente para o Estado de São Paulo, a maior parte dos táxons conhecidos não foi publicada e há necessidade de revisão taxonômica com base na avaliação de características ultraestruturais (i.e. estrias, aréolas, terminações da rafe). Dentre os estudos publicados, apenas os mais recentes apresentaram ilustrações em microscopia eletrônica de varredura (MEV) de alguns táxons, incluindo o estudo resultante desse projeto (Marquardt et al. 2017, 2018, Costa et al. 2019). Finalmente em âmbito global, a resolução de problemas taxonômicos dentro de complexos de espécies é de extrema importância necessitando de estudos específicos com diferentes ferramentas (ex. MEV, análise morfológica, morfogeométrica, molecular).

Dessa forma, visando ampliar e aprofundar o conhecimento das diatomáceas monorrafídeas de água doce para regiões tropicais, há necessidade de se definir as características diagnósticas e a melhor circunscrição dos táxons inventariados, aumentar a resolução da observação da decoração das valvas e avaliar complexos de espécies de forma a resolver

problemas taxonômicos. Cabe mencionar, ainda, que informações autoecológicas sobre espécies monorrafídeas são ainda muito escassas para o país, tendo início com Marquardt et al. (2017) que determinaram o ótimo ecológico de *Achnanthidium tropicocatenatum* G.C.Marquardt, C.E.Wetzel & Ector. Tais informações alicerçadas em taxonomia sólida podem subsidiar trabalhos de biomonitoramento e reconstrução ambiental.

## **OBJETIVOS**

### **Objetivo Geral**

Revisar, ampliar e aprofundar o conhecimento taxonômico e ecológico das diatomáceas monorrafídeas pertencentes às famílias Achnanthaceae, Achnanthidiaceae e Cocconeidaceae de regiões tropicais a partir do estudo detalhado em microscopia óptica e eletrônica de varredura das populações encontradas em ambientes dulcícolas do Estado de São Paulo.

### **Objetivos Específicos**

- Identificar e caracterizar a variabilidade morfológica das espécies e variedades encontradas em níveis de microscopia de luz e eletrônica de varredura com base em análise populacional e de material-tipo sempre que possível;
- Buscar a resolução de problemas taxonômicos a partir do reestudo do protólogo dos táxons inventariados;
- Revisar as identificações taxonômicas das espécies já registradas para o Estado de São Paulo e passíveis de reestudo taxonômico;
- Avaliar a distribuição no Brasil dos táxons identificados em âmbito do Estado de São Paulo;
- Avaliar a preferência ecológica das espécies e variedades de *Achnanthidium* considerando variáveis limnológicas de represas situadas em cinco bacias hidrográficas das regiões Sul e Sudeste do Estado de São Paulo.

## **MATERIAL E MÉTODOS**

### **Área de estudo**

O Estado de São Paulo apresenta área total de 248.222,362 km<sup>2</sup> em que estão distribuídos 645 municípios e uma população estimada em mais de 44 milhões de habitantes (IBGE 2015). A cobertura florestal compreende 2.662.203 ha de remanescente da Mata Atlântica, incluindo a maior extensão de restinga (206.698 ha) e a terceira maior de mangue (25.016 ha) no país (INPE 2014). Insere-se em duas grandes Regiões Hidrográficas brasileiras, Paraná e Atlântico

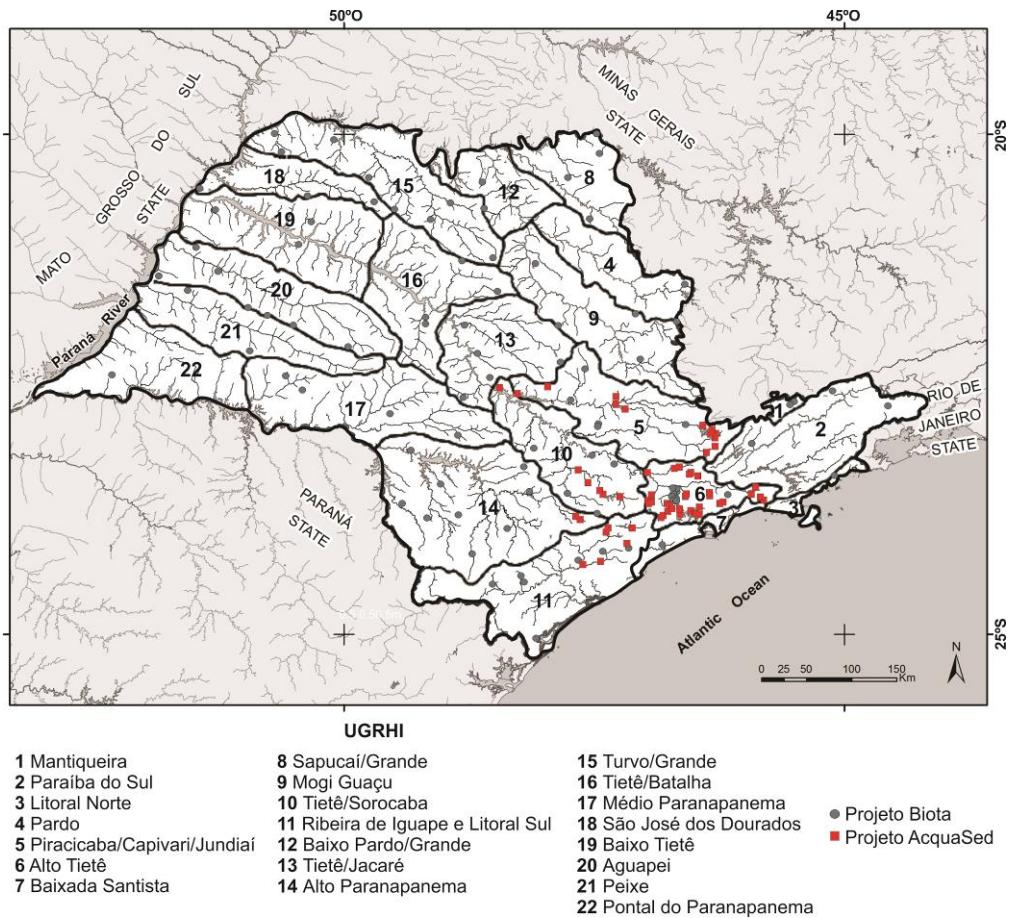
Sudeste e apresenta 22 bacias hidrográficas, que abrangem ecossistemas aquáticos protegidos, ainda com poucos impactos antrópicos, e altamente urbanizados.

O Estado apresenta a maior demanda por recursos hídricos do País, atingindo 141,2 m<sup>3</sup>/s (ANA 2015). A SABESP (Companhia de Saneamento Básico do Estado de São Paulo) é responsável pelos serviços de abastecimento de água em 366 municípios do Estado, sendo 31 deles pertencentes à Região Metropolitana de São Paulo (RMSP) com suas demandas atuais atendidas por oito sistemas produtores do Sistema Integrado de Abastecimento de Água da companhia (ANA 2015). Tais sistemas produtores estão principalmente distribuídos nas bacias do Alto Tietê e do Piracicaba, Capivari e Jundiaí (PCJ).

A presente proposta insere-se em dois projetos mais amplos financiados pela FAPESP. O projeto “Flora Ficológica do Estado de São Paulo” (Biota), que tem por objetivo o conhecimento da biodiversidade das algas em âmbito do Estado, e o “Projeto AcquaSed”, que visa avaliar a qualidade ecológica de 32 represas situadas em áreas de mananciais da Bacia do Alto Tietê e bacias vizinhas (Figura 1), bem como aumentar o conhecimento da autoecologia de diatomáceas, subsidiando seu uso na bioindicação de represas tropicais.

### **Material para análise**

No conjunto, foram analisadas 364 amostras, distribuídas em 150 estações de amostragem (Figura 1). No âmbito do Projeto AcquaSed, foram selecionadas 224 amostras (Tabela 1) a partir de análise preliminar, de forma a contemplar as 32 represas e que incluem distintas épocas do ano (verão/inverno), profundidades (represas profundas e rasas), habitats (fitoplâncton, perifítion e sedimento superficial) e gradiente de estado trófico (ultraoligotrófico a hipereutrófico). No âmbito do Projeto Biota, 171 amostras vêm sendo utilizadas para o levantamento de distintos grupos de diatomáceas (ex. Tavares 2001, Morandi 2002, 2008, Carneiro 2003, 2007, Rocha 2008, Marquardt 2012, Ferreira 2016). Dessas, 140 amostras (Tabela 2) foram selecionadas para análise por conterem diatomáceas monorrafídeas, principalmente representantes de *Achnanthidium*. A seleção baseou-se em Carneiro (2003) e em análise preliminar.



**Figura 1.** Localização das estações de amostragem distribuídas nas 22 bacias hidrográficas do Estado de São Paulo. Círculos cinza: estações de amostragem do Projeto Biota. Quadrados vermelhos: estações de amostragem do Projeto AcquaSed.

**Tabela 1.** Amostras analisadas (SP) do projeto AcquaSed com suas respectivas estações amostrais georeferenciadas distribuídas em 32 reservatórios do Estado de São Paulo.

| Reservatório | Estação amostral | Latitude (S) | Longitude (O) | Número de Herbário (SP)                |
|--------------|------------------|--------------|---------------|--|
| Paineiras    | PI1              | 23°51'13.8"  | 47°36'54.8"   | 469212, 469405, 469425, 469439, 469450 |
| Paineiras    | PI3              | 23°50'34.8"  | 47°38'26.2"   | 469214, 469407, 469427, 469441, 469452 |
| Barra Bonita | BB2              | 22°36' 42"   | 48°19'14.9"   | 469244, 469515, 469520, 469545, 469554 |
| Barra Bonita | BB5              | 22°31'56.04" | 48°27'37.3"   | 469247, 469518, 469523, 469553, 469557 |
| Hedberg      | HB1              | 23°25'55.86" | 47°35'33.06"  | 469240, 469508, 469511, 469534, 469539 |
| Ipaneminha   | IP3              | 23°32'34.62" | 47°31'8.88"   | 469239, 469504, 469507, 469530         |
| Itupararanga | IT1              | 23°37'11.58" | 47°13'59.34"  | 469232, 469492, 469497, 469526         |
| Itupararanga | IT5              | 23°36'53.1"  | 47°23'34.7"   | 469236, 469496, 469501                 |

*Continua*

**Tabela 1. Cont.**

| <b>Reservatório</b>      | <b>Estação amostral</b> | <b>Latitude (S)</b> | <b>Longitude (O)</b> | <b>Número de Herbário (SP)</b>         |
|--------------------------|-------------------------|---------------------|----------------------|--|
| Santa Helena             | SH2                     | 23°34'58.56"        | 47°25'50.52"         | 469230, 469454, 469490, 469524, 469525 |
| Billings (Rio Grande)    | RG2                     | 23°45'59.46"        | 46°30'35.7"          | 401560, 401584, 427898                 |
| Billings (Rio Grande)    | RG4                     | 23°43'43.74"        | 46°26'31.62"         | 401562, 401574, 401586, 427900, 427907 |
| Billings (Rio Pequeno)   | RP6                     | 23°47'41.34"        | 46°28'21.66"         | 401564, 401576, 401588                 |
| Billings (Rio Pequeno)   | RP7                     | 23°47'1.62"         | 46°26'11.28"         | 401565, 401577, 401589, 427902, 427909 |
| Billings (Corpo Central) | CC9                     | 23°45'40.92"        | 46°38'54.06"         | 401567, 401579, 401591, 427903, 427910 |
| Billings (Taquacetuba)   | TQ10                    | 23°48'44.1"         | 46°37'51.24"         | 401568, 401580, 401592, 427904, 427911 |
| Cabuçu                   | CB1                     | 23°23'25.6"         | 46°31'42.8"          | 428921, 428936, 428938, 428939, 428942 |
| Cabuçu                   | CB3                     | 23°24'0.6"          | 46°31'56.6"          | 428923, 428941                         |
| Cachoeira da Graça       | CG2                     | 23°39'17.68"        | 46°57'57.75"         | 427584, 427591, 427598, 469558         |
| Guarapiranga             | GU1                     | 23°46'29.76"        | 46°47'13.2"          | 469455, 469469, 428507                 |
| Guarapiranga             | GU2                     | 23°45'17.7"         | 46°46'11.22"         | 469456, 469470, 428508                 |
| Guarapiranga             | GU6                     | 23°45'0.72"         | 46°43'36.9"          | 469460, 469474, 428512                 |
| Guarapiranga             | GU7                     | 23°43'38.82"        | 46°43'25.38"         | 469461, 469475, 428513                 |
| Guarapiranga             | GU8                     | 23°42'58.14"        | 46°43'36.72"         | 469462, 469476, 428514                 |
| Guarapiranga             | GU12                    | 23°41'53.1"         | 46°44'40.38"         | 469466, 469480, 428518                 |
| Jundiaí                  | JU1                     | 23°38'50.2"         | 46°09'48.1"          | 427988, 427989, 427996, 468830, 468849 |
| Jundiaí                  | JU2                     | 23°39'07.2"         | 46°11'34.3"          | 427997, 468831, 468850                 |
| Lago das Garças          | LG1                     | 23°38'44.4"         | 46°37'29.9"          | 469483, 469484, 469485, 469486         |
| Lago das Ninféias        | NI1                     | 23°38'19.9"         | 46°37'20.3"          | 469315, 469316, 469317, 469318, 469576 |
| Paiva Castro             | PC1                     | 23°19'39"           | 46°36'42"            | 469259, 469281, 469303, 469369, 469379 |
| Paiva Castro             | PC4                     | 23°19'49"           | 46°40'37"            | 469262, 469284, 469306, 469551, 469552 |
| Paraitinga               | PA1                     | 23°31'22.0"         | 45°54'17.8"          | 427984, 427985, 427992, 427994, 468847 |
| Pedro Beicht             | PB3                     | 23°42'58.07"        | 46°57'46.16"         | 427580, 427587, 427594                 |
| Pedro Beicht             | PB4                     | 23°43'44.06"        | 46°58'5.10"          | 427581, 427588, 427595, 469559         |
| Pedro Beicht             | PB5                     | 23°42'55.07"        | 46°58'19.28"         | 427582, 427589, 427596, 469560         |
| Ponte Nova               | PN2                     | 23°36'04.3"         | 45°55'44.04"         | 427923, 427926, 427983, 468845         |
| Rasgão                   | RA2                     | 23°23'28.59"        | 47°01'19.03"         | 427990, 427991, 468860                 |
| Ribeirão do Campo        | RC1                     | 23°39'31.8"         | 45°49'23.22"         | 427916, 427919, 468841                 |
| Ribeirão do Campo        | RC3                     | 23°38'15.2"         | 45°50'0.78"          | 427921, 427982, 468843                 |

*Continua*

**Tabela 1.** Cont.

| <b>Reservatório</b> | <b>Estação amostral</b> | <b>Latitude (S)</b> | <b>Longitude (O)</b> | <b>Número de Herbário (SP)</b>         |
|---------------------|-------------------------|---------------------|----------------------|--|
| Taiaçupeba          | TA1                     | 23°36'25.9"         | 46°15'59.5"          | 427986, 427987, 468833, 468835, 468857 |
| Taiaçupeba          | TA2                     | 23°34'40.9"         | 46°16'50.3"          | 468858                                 |
| Tanque Grande       | TG3                     | 23°22'29"           | 46°27'31"            | 428920, 428926, 428929, 428932, 428935 |
| Atibainha           | AT1                     | 23°08'50"           | 46°18'49"            | 469253, 469275, 469297                 |
| Atibainha           | AT3                     | 23°11'12"           | 46°22'51"            | 469255, 469277, 469299                 |
| Cachoeira           | CA2                     | 23°00'37"           | 46°17'11"            | 469249, 469271, 469293                 |
| Cachoeira           | CA3                     | 23°01'56"           | 46°17'20"            | 469250, 469272, 469294                 |
| Jacareí             | JC6                     | 22°58'33.37"        | 46°20'39.26"         | 428846, 428855, 428864                 |
| Jacareí             | JC7                     | 22°57'16.03"        | 46°18'52.24"         | 428847, 428856, 428865                 |
| Jaguari             | JA1                     | 22°54'57.18"        | 46°24'19.76"         | 428839, 428848, 428857                 |
| Salto Grande        | SG1                     | 22°43'43"           | 47°13'56"            | 469263, 469285, 469307, 469372, 469381 |
| Salto Grande        | SG4                     | 22°41'59"           | 47°16'33"            | 469266, 469288, 469310, 469375, 469384 |
| Represa do Tatu     | TU1                     | 22°38'42"           | 47°17'10"            | 469267, 469289, 469311, 469376, 469385 |
| Cachoeira da Fumaça | FU2                     | 24°00'17.1"         | 47°15'44.8"          | 469200, 469393, 469413                 |
| Cachoeira do França | FR3                     | 23°55'53.4"         | 47°10'34.4"          | 469197, 469390, 469410, 469430, 469443 |
| Jurupará            | JP1                     | 23°56'00"           | 47°22'18.0"          | 469208, 469401, 469421, 469435, 469446 |
| Jurupará            | JP4                     | 23°57'36.7"         | 47°23'43.6"          | 469211, 469404, 469424, 469438, 469449 |
| Salto do Iporanga   | SI3                     | 24°05'54.5"         | 47°43'25.8"          | 469207, 469400, 469420                 |
| Serraria            | SE3                     | 24°08'28.8"         | 47°32'32.3"          | 469204, 469397, 469417                 |

**Tabela 2.** Amostras analisadas (SP) do projeto Biota incluindo informações de coleta: data, coletores, coordenadas (se disponível), município e outras informações do local de coleta.

| <b>Número Herbário</b> | <b>Data da coleta</b> | <b>Coletores</b>                             | <b>Município</b> | <b>Coordenadas</b> | <b>Outras informações</b>  |
|------------------------|-----------------------|--|------------------|--------------------|--|
| SP164898               | 22/03/1982            | Azevedo, M.T.P., Sant'Ana C.L. & Vital, D.M. | Águas da Prata   | -                  | Praça Basílio Sisquim, cachoeira Estrada da Lagoinha, estância “Marrequinho” Rodovia Marechal Rondon |
| SP239234               | 15/01/1992            | Branco, L.H.                                 | Andradina        | -                  |  |
| SP239239a              | 15/01/1992            | Branco, L.H.                                 | Araçatuba        | -                  |  |
| SP239090,<br>SP255756  | 20/07/1991            | Bittencourt-Oliveira, M.C.                   | Assis            | -                  | SP-333, km 435, lagoa  |

*Continua*

**Tabela 2. Cont.**

| <b>Número<br/>Herbário</b> | <b>Data da<br/>coleta</b> | <b>Coletores</b>   | <b>Município</b>     | <b>Coordenadas</b>            | <b>Outras<br/>informações</b>            |
|----------------------------|---------------------------|--|----------------------|-------------------------------|--|
| SP255759a,<br>SP255773a    | 10/09/1991                | Castro, A.A.J.,<br>Bicudo, C.E.M. &<br>Marques-Lopes,<br>M.R.  | Avaré                | -                             | SP-255, km<br>278, Represa<br>Jurumirim  |
| SP255742                   | 02/01/1991                | Branco, L.H.   | Barra Bonita         | -                             | Rio Tietê                                |
| SP255772a                  | 28/02/1990                | Branco, L.H.   | Barretos             | -                             | Região dos<br>lagos                      |
| SP239096a,<br>SP255761     | 16/11/1991                | Castro, A.A.J.   | Batatais             | -                             | SP-330, km<br>355,5, Represa             |
| SP188208                   | 17/11/1988                | Castro, A.A.J. &<br>Bicudo, C.E.M.   | Bragança<br>Paulista | -                             | SP-381, km<br>12, charco                 |
| SP355356,<br>SP355357      | 08/08/2000                | Bicudo, C.E.M.,<br>Carneiro, L.A. &<br>Faustino, S.M.M.<br>Castro, A.A.J.,<br>Bicudo, C.E.M. &<br>Bicudo, D.C. | Caconde              | 21°34'39,9"S,<br>46°37'31,0"O | Represa de<br>Caconde                    |
| SP239041                   | 27/08/1990                | Bicudo, C.E.M. &<br>Bicudo, D.C.   | Campos do<br>Jordão  | -                             | Horto<br>Florestal,<br>córegos           |
| SP255736                   | 27/07/1990                | Castro, A.A.J. &<br>Bicudo, C.E.M.   | Campos do<br>Jordão  | -                             | Horto<br>Florestal,<br>córegos           |
| SP255737                   | 27/07/1990                | Castro, A.A.J.,<br>Bicudo, C.E.M. &<br>Bicudo, D.C.  | Campos do<br>Jordão  | -                             | Horto<br>Florestal,<br>áçude             |
| SP130812                   | -                         | -  | Cananéia             | -                             | -  |
| SP365693a                  | 18/07/2000                | Bicudo, C.E.M.,<br>Cordeiro, F. &<br>Morandi, L.L.   | Capão Bonito         | 23°53'37,3"S,<br>48°15'21,3"O | SP-127, km<br>199,9, Rio<br>Paranapanema |
| SP239044,<br>SP255723      | 20/03/1990                | Castro, A.A.J. &<br>Bicudo, C.E.M.   | Capivari             | -                             | SP-308, km<br>132, charco                |
| SP188327                   | 17/10/1989                | Castro, A.A.J. &<br>Bicudo, C.E.M.   | Casa Branca          | -                             | SP-340, km<br>228,5, charco              |
| SP371174a                  | 18/05/2001                | Bicudo, C.E.M.,<br>Costa, D.L. &<br>Faustino, S.M.M.   | Colina               | 20°45'41,1"S,<br>48°30'3",6"O | SP-326, km<br>399, riacho                |
| SP355389a,<br>SP355404     | 24/04/2001                | Bicudo, C.E.M.,<br>Costa, D.L. &<br>Faustino, S.M.M.   | Cosmorama            | 20°30'18,4"S,<br>49°46'14,4"O | SP-320, km<br>496, riacho                |
| SP255758a                  | 21/07/1991                | Bittencourt-<br>Oliveira, M.C.   | Dracena              | -                             | SP-563, km<br>111, riacho                |
| SP239137,<br>SP255765a     | 29/12/1991                | Bicudo, C.E.M. &<br>Bicudo, D.C.   | Eldorado             | -                             | Ribeirão das<br>Ostras                   |
| SP239171                   | 22/12/1991                | Bicudo, C.E.M. &<br>Bicudo, D.C.   | Eldorado             | -                             | Ribeirão das<br>Ostras                   |
| SP239241                   | 05/12/1991                | Branco, L.H.   | General<br>Salgado   | -                             | SP-310                                   |
| SP371176a                  | 20/09/2000                | Morandi, L.L. &<br>Schety, S.  | Guaréí               | 23°19'54,8"S,<br>48°11'11,8"O | SP-157, km<br>43, riacho                 |

*Continua*

**Tabela 2. Cont.**

| <b>Número<br/>Herbário</b>         | <b>Data da<br/>coleta</b> | <b>Coletores</b>  | <b>Município</b>    | <b>Coordenadas</b>            | <b>Outras<br/>informações</b>  |
|------------------------------------|---------------------------|---|---------------------|-------------------------------|--|
| SP239091                           | 20/07/1991                | Bittencourt-Oliveira, M.C.<br>Bicudo, C.E.M.,<br>Carneiro, L.A. &<br>Faustino, S.M.M.               | Inubia<br>Paulista  | -                             | SP-294, km<br>578, riacho<br>Rodovia<br>vicinal,<br>Iporanga-Apiaí   |
| SP355393a                          | 13/09/2000                | Faustino, S.M.M.<br>& Schety, S.P.<br>Castro, A.A.J.,<br>Bicudo, C.E.M. &<br>Marques-Lopes,<br>M.R. | Iporanga            | 24°35'40,5"S,<br>48°37'12,4"O | SP-249, km<br>114, lago<br>SP-255, km<br>308,3, Represa<br>Jurumirim |
| SP355392a                          | 26/07/2000                |   | Itaberá             | 23°51'11,3"S,<br>49°09'10,8"O |  |
| SP239094,<br>SP255760              | 10/09/1991                |   | Itaí                | -                             |  |
| SP255771,<br>SP239143a             | 22/02/1992                | Bicudo, C.E.M. &<br>Bicudo, D.C.  | Itaju               | -                             | SP-304, km<br>347,5, açude   |
| SP188434                           | 12/03/1990                | Castro, A.A.J. &<br>Bicudo, C.E.M.  | Itanhaém            | -                             | SP-55, km<br>332,7, charco   |
| SP239042,<br>SP255740,<br>SP255741 | 11/11/1990                | Castro, A.A.J. &<br>Bicudo, C.E.M.  | Itapetininga        | -                             | SP-270/127,<br>km 171, lago  |
| SP371180a                          | 18/05/2000                | Bicudo, C.E.M.,<br>Morandi, L.L. &<br>Firmino, F.S.   | Itapeva             | 23°58'28,0"S,<br>48°55'02,5"O | SP-258, km<br>289, rio<br>Taquari                                    |
| SP355358,<br>SP355359              | 26/07/2000                | Faustino, S.M.M.<br>& Schety, S.P.  | Itaporanga          | 23°42'24,3"S,<br>49°28'15,6"O | SP-255, km<br>358, rio   |
| SP355388a,<br>SP370964a            | 16/05/2001                | Bicudo, C.E.M. &<br>Bicudo, D.C.  | Itapura             | 22°16'41,0"S,<br>51°48'16,5"O | SP-595, km<br>21,5, Rio Tietê  |
| SP163994                           | 18/09/1979                | Aulino, O.  | Itirapina           | -                             |  |
| SP188435,<br>SP255725              | 20/03/1990                | Castro, A.A.J. &<br>Bicudo, C.E.M.  | Itu                 | -                             | SP-312, km<br>112,5, Fazenda<br>Potiguara,<br>represa<br>SP-304, km  |
| SP239142,<br>SP255768              | 22/02/1992                | Bicudo, C.E.M. &<br>Bicudo, D.C.  | Jaú/Bariri          | -                             | 317,5, Fazenda<br>Santa Fé,<br>açude                                 |
| SP365692a                          | 11/07/2000                | Bicudo, C.E.M. &<br>Faustino, S.M.M.  | Juquiá              |                               | SP-79, km<br>211, tanque   |
| SP239236a                          | 22/02/1992                | Bicudo, C.E.M. &<br>Bicudo, D.C.  | Lençóis<br>Paulista | -                             | SP-300, km<br>299,5, lago  |
| SP355366a                          | 25/04/2001                | Bicudo, C.E.M.,<br>Costa, D.L. &<br>Faustino, S.M.M.  | Macedônia           | 20°08'19,5"S,<br>50°11'56,4"O | Rodovia<br>Alberto Faria,<br>açude<br>SP-333,                        |
| SP239086,<br>SP255753a             | 20/07/1991                | Bittencourt-Oliveira, M.C.  | Marília             | -                             | Riacho Água<br>da cobra  |
| SP188433,<br>SP255722a             | 28/02/1990                | Branco, L.H.  | Matão               | -                             | SP-310, km<br>309, brejo   |
| SP255763a                          | 29/11/1991                | Bicudo, C.E.M. &<br>Bicudo, D.C.  | Miracatu            | -                             | BR-116, km<br>383, Rio Itariri                                       |

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**Tabela 2. Cont.**

| <b>Número<br/>Herbário</b>                      | <b>Data da<br/>coleta</b> | <b>Coletores</b>                                  | <b>Município</b>   | <b>Coordenadas</b>            | <b>Outras<br/>informações</b>                              |
|---|---------------------------|---|--------------------|-------------------------------|--|
| SP255728  | 10/04/1990                | Bicudo, C.E.M. & Bicudo, D.C.                     | Mirassol           | -                             | SP-310, km 410,7, charco                                   |
| SP188211  | 20/03/1989                | Castro, A.A.J. & Bicudo, C.E.M.                   | Mogi das Cruzes    | -                             | SP-310, km 220, lago Fazenda Campaninha                    |
| SP255732,<br>SP255733,<br>SP255734,<br>SP255735 | 12/05/1990                | Bicudo, D.C.                                      | Mogi Guaçu         | -                             | Rodovia Monte Alto - Vista Alegre, lago Estrada            |
| SP239233  | 20/02/1992                | Branco, L.H.                                      | Monte Alto         | -                             | Vicinal Monte Aprazível – Eng. Balduíno, km 0,5, charco    |
| SP355374,<br>SP355375                           | 24/04/2001                | Bicudo, C.E.M., Costa, D.L. & Pereira, F.C.       | Monte Aprazível    | 20°45'18,0"S,<br>49°42'13,9"O | SP-593, km 179,5, Charco                                   |
| SP370963a                                       | 16/05/2001                | Bicudo, C.E.M. & Bicudo, D.C.                     | Nova Independência | 21°03'32,0"S,<br>51°26'30,1"O | SP-322, km 441, charco                                     |
| SP371177  | 23/08/2000                | Bicudo, C.E.M., Faustino, S.M.M. & Schety, S.     | Olímpia            | 20°44'18,3"S,<br>48°51'7,0"O  | SP-294, km 623, açude Usina do Porto Primavera, Rio Paraná |
| SP370962a                                       | 15/05/2001                | Bicudo, C.E.M. & Bicudo, D.C.                     | Pacaembu           | 21°32'50,2"S,<br>51°18'25,1"O | A 1km da SP-425, charco                                    |
| SP371185a                                       | 25/10/2001                | Bicudo, C.E.M., Carneiro, L.A. & Faustino, S.M.M. | Panorama           | 21°21'35,1"S,<br>51°52'08,7"O | Fazenda Sobrado, riacho                                    |
| SP239085  | 21/07/1991                | Bittencourt-Oliveira, M.C.                        | Paraguaçu Paulista | -                             | SP-284, km 457, riacho                                     |
| SP371186a                                       | 15/05/2001                | Bicudo, C.E.M. & Bicudo, D.C.                     | Parapuã            | -                             | Estrada Lorena-Piquete, km 65, riacho                      |
| SP255738  | 02/09/1990                | Castro, A.A.J.                                    | Pedregulho         | -                             | Manoel de Nóbrega, km 370, açude                           |
| SP371181  | 11/07/2000                | Bicudo, C.E.M. & Faustino, S.M.M.                 | Pedro de Toledo    | 24°16'48,8"S,<br>47°12'6,2"O  | SP-79, km 133,3, córrego represso                          |
| SP255766a,<br>SP255767a                         | 30/12/1991                | Bicudo, C.E.M. & Bicudo, D.C.                     | Piedade            | -                             | SP-330, km 393,25, lagoa                                   |
| SP239038,<br>SP255739                           | 02/09/1990                | Castro, A.A.J.                                    | Pioneiros          | -                             | Estrada Lorena-Piquete, km 65, riacho                      |
| SP355362  | 19/09/2001                | Bicudo, C.E.M., Costa, D.L. & Pereira, F.C.       | Piquete            | 22°37'24,2"S,<br>45°09'40,1"O | Continua   |

**Tabela 2. Cont.**

| <b>Número<br/>Herbário</b>         | <b>Data da<br/>coleta</b> | <b>Coletores</b>  | <b>Município</b>              | <b>Coordenadas</b>            | <b>Outras<br/>informações</b>                       |
|------------------------------------|---------------------------|---|-------------------------------|-------------------------------|---|
| SP239095                           | 29/10/1991                | Castro, A.A.J.  | Piracicaba                    | -                             | Rio Piracicaba,<br>cachoeira                        |
| SP239140a                          | 21/01/1992                | Bicudo, D.C. &<br>Figueiredo, D.M.                      | Piraju                        | -                             | Represa<br>Jurumirim                                |
| SP188207                           | 17/11/1988                | Castro, A.A.J. &<br>Bicudo, C.E.M.                      | Porangaba                     | -                             | SP-162/SP-<br>141, alagado                          |
| SP371182a                          | 20/09/2000                | Morandi, L.L. &<br>Schety, S.                           | Porto Feliz                   | 23°12'46,9"S,<br>47°30'3,6"O  | SP-300, km<br>129, rio                              |
| SP371178a                          | 15/08/2000                | Bicudo, C.E.M.,<br>Faustino, S.M.M.<br>& Morandi, L.L.  | Pradópolis                    | 21°21'14,0"S,<br>48°03'53,7"O | SP-291,<br>rancho                                   |
| SP239087,<br>SP255754              | 21/07/1991                | Bittencourt-<br>Oliveira, M.C.                          | Rancharia                     | -                             | SP-457  |
| SP239144,<br>SP255769,<br>SP255770 | 22/02/1992                | Bicudo, C.E.M. &<br>Bicudo, D.C.                        | Reginópolis                   | -                             | SP-331, km<br>115,2, brejo                          |
| SP239244                           | 28/04/1992                | Castro, A.A.J. &<br>Bicudo, C.E.M.                      | Ribeirão<br>Branco            | -                             | SP-249, km<br>56,7                                  |
| SP371175a                          | 30/05/2000                | Bicudo, C.E.M. &<br>Bicudo, D.C.                        | Rifaína                       | 20°04'24,8"S,<br>47°25'2,5"O  | Ponte de<br>Rifaína-<br>Araxá, rio                  |
| SP188219,<br>SP255726              | 17/07/1989                | Bicudo, C.E.M. &<br>Bicudo, D.C.                        | Rio Claro                     | -                             | Horto florestal<br>Navarro de<br>Andrade, lago      |
| SP371179                           | 22/07/2000                | Bicudo, C.E.M.,<br>Faustino, S.M.M.<br>& Schety, S.     | Santa Adélia                  | 21°11'48,8"S,<br>48°47'59,4"O | Rodovia João<br>Colombro                            |
| SP355385a                          | 24/04/2001                | Bicudo, C.E.M.,<br>Costa, D.L. &<br>Faustino, S.M.M.    | Santa<br>Albertina            | 20°3'20,1"S,<br>50°46'01"O    | Estrada vicinal<br>Vereador Ítalo<br>Biani, riacho  |
| SP355370a                          | 27/03/2001                | Bicudo, C.E.M.,<br>Carneiro, L.A. &<br>Faustino, S.M.M. | Santa Cruz do<br>Rio Pardo    | 22°45'24,8"S,<br>49°29'07,7"O | SP-255, km<br>309, açude                            |
| SP355386a                          | 25/04/2001                | Bicudo, D.C.,<br>Costa, D.L. &<br>Faustino, S.M.M.      | Santo Antônio<br>do Arancangá | 20°50'30,2"S,<br>50°27'14,4"O | SP-320, km<br>463, lago                             |
| SP188212,<br>SP255727              | 20/03/1989                | Castro, A.A.J. &<br>Bicudo, C.E.M.                      | São Carlos                    | -                             | SP-310, km<br>220, lago                             |
| SP188322                           | 21/11/1989                | Castro, A.A.J. &<br>Bicudo, C.E.M.                      | São José do<br>Barreiro       | -                             | SP-64, km 0,8,<br>brejo                             |
| SP188210                           | 21/02/1989                | Castro, A.A.J. &<br>Bicudo, C.E.M.                      | São José dos<br>Campos        | -                             | SP-99, km 8,<br>Vila São<br>Judas, charco           |
| SP255749a                          | 18/07/1991                | Bicudo, D.C.,<br>Ludwig, T.A.V. &<br>Figueiredo, D.M.   | São Paulo                     | -                             | PEFI, Jardim<br>Botânico,<br>Córrego<br>Pirarungáua |

*Continua*

**Tabela 2. Cont.**

| <b>Número<br/>Herbário</b>          | <b>Data da<br/>coleta</b> | <b>Coletores</b>   | <b>Município</b>   | <b>Coordenadas</b>           | <b>Outras<br/>informações</b>                          |
|-------------------------------------|---------------------------|--|--------------------|------------------------------|--|
| SP255743,<br>SP255744               | 18/07/1991                | Bicudo, D.C.,<br>Ludwig, T.A.V. &<br>Figueiredo, D.M.<br>Bicudo, D.C., | São Paulo          | -                            | PEFI, Lago<br>das Ninféias                             |
| SP255745,<br>SP255746               | 18/07/1991                | Ludwig, T.A.V. &<br>Figueiredo, D.M.<br>Bicudo, D.C.,                  | São Paulo          | -                            | PEFI,<br>hidrofitotório                                |
| SP255747,<br>SP255748               | 18/07/1991                | Ludwig, T.A.V. &<br>Figueiredo, D.M.<br>Bicudo, D.C.,                  | São Paulo          | -                            | PEFI, Lago<br>dos Bugios                               |
| SP255749,<br>SP255750               | 18/07/1991                | Ludwig, T.A.V. &<br>Figueiredo, D.M.<br>Bicudo, D.C.,                  | São Paulo          | -                            | PEFI, Córrego<br>Pirarungáua                           |
| SP255751,<br>SP255752               | 18/07/1991                | Ludwig, T.A.V. &<br>Figueiredo, D.M.<br>Bicudo, D.C.,                  | São Paulo          | -                            | PEFI, Lago<br>das Garças                               |
| SP294899,<br>SP294900               | 07/11/1996                | Bicudo, D.C. &<br>Morandi, L.L.  | São Paulo          | -                            | PEFI, Lago<br>das Ninféias                             |
| SP294902                            | 07/11/1996                | Bicudo, D.C. &<br>Morandi, L.L.  | São Paulo          | -                            | PEFI, Lago<br>dos Bugios                               |
| SP294903                            | 07/11/1996                | Bicudo, D.C. &<br>Morandi, L.L.  | São Paulo          | -                            | PEFI,<br>hidrofitotório                                |
| SP294904,<br>SP294905               | 14/01/1997                | Bicudo, D.C. &<br>Morandi, L.L.  | São Paulo          | -                            | PEFI, Lago<br>das Garças                               |
| SP294906,<br>SP294907,<br>SP294908  | 15/01/1997                | Bicudo, D.C. &<br>Morandi, L.L.  | São Paulo          | -                            | PEFI, lago do<br>Centro de<br>Ciências e<br>Tecnologia |
| SP294909                            | 01/03/1997                | Bicudo, D.C. &<br>Morandi, L.L.  | São Paulo          | -                            | PEFI, Jardim<br>Botânico,<br>Lago do<br>Monjolo        |
| SP427341,<br>SP427342a,<br>SP427343 | 07/06/2011                | Silva, P.D.A.,<br>Marquardt, G.C.,<br>Wengrat, S. &<br>Pellegrini, B.  | São Paulo          | -                            | PEFI, Jardim<br>Botânico,<br>Hidrofitotório            |
| SP427344,<br>SP427346               | 07/06/2011                | Silva, P.D.A.,<br>Marquardt, G.C.,<br>Wengrat, S. &<br>Pellegrini, B.  | São Paulo          | -                            | PEFI, Jardim<br>Botânico                               |
| SP188436,<br>SP255724               | 20/03/1990                | Castro, A.A.J. &<br>Bicudo, C.E.M.                                     | São Pedro          | -                            | SP-304, km<br>127,<br>Restaurante do<br>Lago, lago     |
| SP371173a                           | 20/09/2000                | Morandi, L.L. &<br>Schety, S.  | Sarapuí            | 23°34'1,4"S,<br>47°52'55,4"O | SP-270, 146,5<br>km, riacho                            |
| SP239136                            | 08/12/1991                | Bittencourt-<br>Oliveira, M.C.   | Teodoro<br>Sampaio | -                            | Ribeirão<br>Inhana                                     |

*Continua*

**Tabela 2. Cont.**

| <b>Número Herbário</b> | <b>Data da coleta</b> | <b>Coletores</b>  | <b>Município</b> | <b>Coordenadas</b> | <b>Outras informações</b>                           |
|------------------------|-----------------------|---|------------------|--------------------|---|
| SP239088,<br>SP255755  | 20/07/1991            | Bittencourt-Oliveira, M.C.<br>Castro, A.A.J.,<br>Bicudo, C.E.M. &<br>De-Lamonica-Freire, E.M. | Tupã             | -                  | SP-294, brejo<br>SP-55, km 72,<br>Praia da Lagoinha |
| SP188344               | 27/11/1988            |   | Ubatuba          | -                  |   |
| SP239043,<br>SP255729  | 10/04/1990            | Bicudo, D.C. &<br>Bicudo, C.E.M.  | Uchoa            | -                  | SP-310, km 410,7, charco                            |
| SP239237               | 05/12/1991            | Branco, L.H.  | Urânia           | -                  | SP-300 Rodovia vicinal                              |
| SP371184               | 16/08/2000            | Bicudo, C.E.M.,<br>Faustino, S.M.M. & Morandi, L.L.   | Viradouro        | -                  | Viradouro - Thomé Francisco dos Reis, riacho        |

### **Coleta e fixação do material**

As coletas das amostras selecionadas a partir do Projeto Biota foram realizadas no período de 1979 a 2011 e as do Projeto AcquaSed ocorreram de 2011 a 2014. O material fitoplanctônico foi coletado com garrafa de Van Dorn e/ou rede de plâncton (20 µm de abertura de malha). O material perifítico foi coletado a partir de substratos naturais, como pedras ou macrófitas aquáticos, por meio de raspagem ou espremidos manuais de plantas. O sedimento superficial foi obtido com testemunhador de gravidade (UWITEC), aproveitando-se os dois primeiros centímetros superficiais que usualmente integram de um a dois anos de informação (Smol 2008). Por integrar uma escala maior de tempo, o sedimento superficial foi amostrado apenas no período de inverno.

O material foi fixado em laboratório com solução aquosa de formalina a 4% (Bicudo & Menezes 2006). Ainda, subamostras de sedimento úmido foram mantidas em geladeira e subamostras liofilizadas, em freezer.

Todas as amostras brutas fixadas estão depositadas no Herbário Científico do Estado “Maria Eneyda P. Kauffmann Fidalgo” (SP) do Instituto de Botânica, Secretaria do Meio Ambiente do Estado de São Paulo. As lâminas permanentes de diatomáceas encontram-se no laminário do Núcleo de Pesquisa em Ecologia desta Instituição.

### **Análise do material**

As amostras passam por um processo de oxidação para remoção da matéria orgânica a fim de permitir a visualização das estruturas das frústulas em microscopia de luz e microscopia eletrônica de varredura. Para tanto, dois métodos foram utilizados: a) adição de H<sub>2</sub>O<sub>2</sub> 35% e

HCl 37% em amostra aquecida no bloco digestor, conforme o protocolo do European Committee for Standardization (ECS 2003) para as amostras do projeto AcquaSed; e b) adição de K<sub>2</sub>MnO<sub>4</sub> e HCl segundo Simonsen (1974) modificado por Moreira-Filho & Valente-Moreira (1981), para a maior parte das amostras do projeto Biota.

O material lavado com água deionizada foi montado em lâminas permanentes utilizando-se Naphrax (*IR* = 1,73) ou Hyrax (*IR* = 1,67) como meios de inclusão. A maioria do material analisado já estava montado em lâminas permanentes, contudo algumas amostras precisaram ser re-oxidadas e novas lâminas foram montadas durante o curso do trabalho.

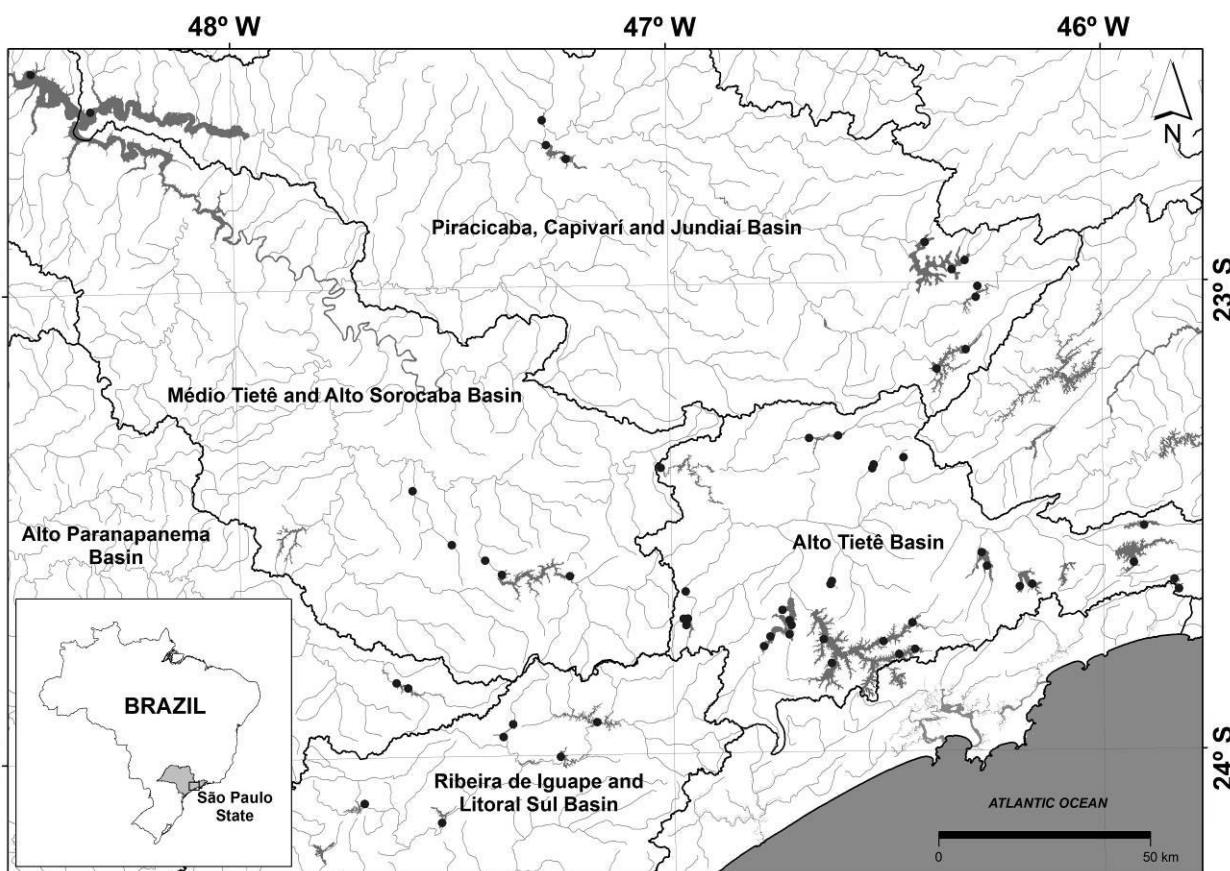
As lâminas permanentes foram observadas por meio de microscópio óptico binocular Zeiss, Axio Imager A2, equipado com contraste-de-fase, ocular micrometrada digital, luz polarizada circular em contraste (DIC) e sistema de captura de imagem com câmara acoplada (MRc5). Para análise ao microscópio eletrônico de varredura (MEV), parte do material oxidado foi filtrado com água deionizada em membranas de policarbonato Isopore™ com 3 µm de abertura de malha. Os filtros foram montados sobre “stubs” de alumínio e posteriormente cobertos com paládio a 100 mA durante 30 segundos em um metalizador BAL-TEC MED 020 (Wetzel & Ector 2014a). A análise foi feita utilizando-se MEV Hitachi SU-70, operado a 5kV e com 10 mm de distância de trabalho.

O exame taxonômico foi baseado em análise populacional (*n* = 20, exceto táxons mais raros) de cada ambiente e hábitat de forma a registrar a variabilidade das características morfológicas e métricas por unidade amostral. A delimitação das populações considerou o protólogo sempre que possível. Os táxons foram identificados com auxílio de periódicos especializados, obras clássicas, modernas e trabalhos específicos de monografias (ex. Patrick & Reimer 1966, Krammer & Lange-Bertalot 1991, Rumrich et al. 2000, Cantonati & Lange-Bertalot 2006, Monnier et al. 2007, Ponader & Potapova 2007, Potapova & Hamilton 2007, Hlúbková et al. 2011, Jüttner et al. 2011, Morales et al. 2011, Van de Vijver et al. 2011b, Wojtal et al. 2011, Novais et al. 2015). Também foram utilizados sites especializados em diatomáceas como ‘Diatoms of North America’ e ‘Diatom New Taxon File’ (<https://diatoms.org/>, Potapova et al. 2020). Ainda, a padronização dos nomes botânicos foi feita conforme o ‘Catálogo de Nomes de Diatomáceas’ e o ‘DiatomBase’ (Fourtanier & Kociolek 2011, Kociolek et al. 2020b).

Finalmente, foi feita revisão da distribuição dos táxons encontrados na área de estudo em âmbito do Estado de São Paulo com base em informações disponíveis em literatura, como descrição e ilustrações.

## Distribuição e autoecologia das espécies

Para avaliar a representatividade e a distribuição dos táxons monorrafídeos, as amostras preparadas nas lâminas permanentes foram quantificadas. As amostras referentes ao Projeto Biota não foram contadas devido à ausência de informações ecológicas das mesmas. A quantificação foi realizada em aumento de 1000X, utilizando microscópio óptico. A unidade básica de contagem considerada foi a valva, frústulas completas foram consideradas como duas e fragmentos foram incluídos quando foi possível identificar a espécie e visualizar, pelo menos, 50% da valva (Battarbee et al. 2001). A contagem foi feita em transecções longitudinais na lâmina com limite de contagem de pelo menos 500 valvas no total por amostra. Os resultados foram expressos em abundância relativa (% de dada espécie em relação ao total de valvas na amostra).



**Figura 2.** Mapa indicando a localização das 57 estações de amostragem do Projeto Acquased distribuídas em 5 bacias hidrográficas do Estado de São Paulo.

O estudo autoecológico foi realizado apenas para os táxons de *Achnanthidium* por apresentarem maior diversidade e distribuição. Foi baseado nas amostras do Projeto AcquaSed que abrange 32 represas com amplo gradiente trófico e 57 unidades amostrais

distribuídas em 5 bacias hidrográficas (Figura 2). Tais amostras estão acompanhadas de variáveis limnológicas disponibilizadas no banco de dados do projeto AcquaSed. As informações incluem o índice de estado trófico (Lamparelli 2004) e variáveis físicas e químicas da água de cada unidade amostral (temperatura, pH, condutividade elétrica, oxigênio dissolvido, transparência da água, alcalinidade, sílica solúvel reativa, nitrito, nitrato, nitrogênio amoniacial, ortofosfato, fósforo total dissolvido, nitrogênio e fósforo total). Os métodos analíticos realizados em laboratório estão detalhados em Wengrat & Bicudo (2011) e padronizados conforme APHA (2005).

O estudo autoecológico permite determinar um valor de concentração ideal para a distribuição de dada espécie, compreendendo o cálculo e a avaliação dos ótimos ecológicos das espécies em função de variáveis limnológicas. O ótimo ecológico foi calculado a partir do valor médio das concentrações da variável ambiental de interesse ponderado pela abundância da espécie em todas as unidades amostrais, conforme ter Braak & van Dam (1989) e aplicado em Bicudo et al. (2016) para *Aulacoseira*:

$$u_k = \frac{\sum_{i=1}^n y_{ik} x_i}{\sum_{i=1}^n y_{ik}}$$

$u_k$  = ótimo ecológico da espécie k

$y_{ik}$  = abundância da espécie k na amostra i

$x_{ik}$  = concentração da variável de interesse na amostra i

As variáveis de interesse foram: condutividade, pH, nitrogênio total e fósforo total. O cálculo foi realizado no software R v.3.5.2 com a função *wa()* do pacote analogue (Simpson 2007). Foi calculado para cada habitat separadamente e apenas para as espécies com frequência de ocorrência igual ou maior do que 10% nas unidades amostrais do habitat em questão.

## ORGANIZAÇÃO DA TESE

A tese está organizada em partes gerais (resumo, abstract, introdução, material e métodos e considerações finais) e os resultados estão apresentados em quatro capítulos, um publicado, um submetido e outros dois que serão submetidos à publicação. Com exceção do capítulo 4, todos estão apresentados na formatação da revista. Finalmente, são apresentados apêndices para consulta com informações mais detalhadas do estudo. Os capítulos são:

Capítulo 1: “Freshwater *Cocconeis* species (Bacillariophyceae) from Southeastern Brazil, and description of *C. amerieuglypta* sp. nov.” publicado na Botany Letters (Costa et al. 2019).

Capítulo 2: “New *Achnanthidium* species (Bacillariophyta, Achnanthidiaceae) from southeastern Brazil” submetido a European Journal of Taxonomy.

Capítulo 3: “Criptógamos do Parque Estadual das Fontes do Ipiranga, São Paulo, SP. Algae, 46: Bacillariophyceae (Achnanthaceae, Coccconeidaceae, Achnanthidiaceae)” a ser submetido na Hoehnea.

Capítulo 4: “Monoraphid diatoms diversity in freshwater environments from São Paulo State, Brazil”.

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## CAPÍTULO 1

### Freshwater *Coccconeis* species (Bacillariophyceae) from Southeastern Brazil, and description of *C. amerieuglypta* sp. nov.

Lívia F. Costa<sup>a, b,\*</sup>, Carlos E. Wetzel<sup>b</sup>, Luc Ector<sup>b</sup> and Denise C. Bicudo<sup>a</sup>

<sup>a</sup>Ecology Department, Instituto de Botânica, São Paulo, Brazil; <sup>b</sup>Environmental Research and Innovation (ERIN) Department, Luxembourg Institute of Science and Technology (LIST), Belvaux, Luxembourg

\*corresponding author: [livia.fcosta@hotmail.com](mailto:livia.fcosta@hotmail.com), Ecology Department, Instituto de Botânica, Av. Miguel Estéfano 3687, 04301–012, São Paulo, SP, Brazil

#### Abstract

*Coccconeis* Ehrenberg is a monoraphid diatom genus included in the Family Coccconeidaceae. It occurs in marine and freshwater environments, living attached to a substrate by the raphid valve. Through the documentation of the taxa found in LM and SEM analyses, this study aims to increase the knowledge about freshwater *Coccconeis* species occurring in Brazilian waters. In addition, one new species is formally described here. Seven samples were chosen from a total of 346 samples investigated for monoraphid diatoms in São Paulo state, Brazil, due their representativeness for *Coccconeis* biodiversity. These samples include periphytic, metaphytic and surface sediments materials. One sample from Ivory Coast was also analysed to compare a similar taxon with the taxa found. The biodiversity and the occurrence of the genus *Coccconeis* were low in our study material (nine taxa) despite of the great amount of samples analysed for monoraphid taxa. Nevertheless, one taxon was considered a new species, being described here. Finally, this study was based on morphological characters of the frustule observed in LM and SEM analysis, showing the importance of both to better understand the variability of each species and to separate similar taxa.

Keywords: biodiversity; diatoms; metaphytic; monoraphid; periphytic; surface sediments

#### Introduction

The Family Coccconeidaceae Kützing, Order Coccconeiales E.J.Cox (after Cox 2015), includes until the present moment ten monoraphid genera: *Amphicoccconeis* M.De Stefano & D.Marino, *Anorthoneis* Grunow, *Bennettella* R.W.Holmes, *Campyloneis* Grunow, *Coccconeopsis* Witkowski, Lange-Bertalot & Metzeltin, *Coccconeis* Ehrenberg, *Epipellis* R.W.Holmes, *Psammococcconeis* M.Garcia, *Vikingea* Witkowski, Lange-Bertalot & Metzeltin, and *Xenococcconeis* Riaux-Gobin (Guiry & Guiry 2019).

The first genus described was *Cocconeis* (Ehrenberg 1838), and most of the genera were later separated from it. All the genera are predominantly found in marine environments and some of them are exclusive from this habitat (*Bennettella*, *Epipellis*, *Xenococconeis*) (Kociolek et al. 2018). *Cocconeis* species are also predominantly found in marine waters, receiving great attention nowadays with the description of several new taxa (e.g. De Stefano, Marino, and Mazella 2000; De Stefano and Marino 2001; Riaux-Gobin, Witkowski, and Romero 2007; Riaux-Gobin et al. 2010; Romero and López-Fuerte 2013; Riaux-Gobin, Compère, and Jordan 2015; Riaux-Gobin and Witkowski 2017) and the re-examination of some type materials (e.g. Romero and Riaux-Gobin 2014; Riaux-Gobin et al. 2016, 2017). Moreover, the genus is also common in freshwater, living attached to a substrate by the raphid valve.

Specimens of *Cocconeis* present elliptical or almost circular valves; raphe valve usually less convex than the sternum valve; mantle and girdle view shallows; striae usually uniseriate, and poroids closed by hymenes with linear perforations (Round, Crawford, and Mann 1990). Despite its ‘simple’ description, the great morphological variability shown in recent studies place the genus among the most structurally complex (Car et al. 2012), probably due to the complex structure of the valvocopulae, presenting fimbriae with species-specific morphology (Holmes, Crawford, and Round 1982).

In Brazil, the genus is mentioned in several diatom surveys (e.g. Bertolli, Tremarin, and Ludwig 2010; Faria, Tremarin, and Ludwig 2010; Fontana and Bicudo 2012; Bartozek et al. 2013) and ecologic studies (e.g. Lobo et al. 2004; Hermann et al. 2006; Burliga et al. 2014), being normally represented by one or two taxa, and never receiving special attention.

Therefore, as part of a survey examining the monoraphid diatoms in São Paulo state, the aim of this study is to increase the knowledge about freshwater *Cocconeis* species occurring in Southeastern Brazilian waters, through the documentation of the taxa found in LM and SEM analyses. One new species is formally described (*C. amerieuglypta* sp. nov.). *Cocconeis schroederi* Foged from Ivory Coast is illustrated here to compare it to a similar taxon found in Brazil: *C. fluviatilis* J.H.Wallace. Additionally, *Cocconeis placentula* var. *acuta* F.Meister, a common species reported to Brazil, is erected to the species level.

## **Material and Methods**

Seven samples were chosen from a total of 346 samples investigated for monoraphid diatoms in São Paulo state, Brazil, due their representativeness for *Cocconeis* biodiversity. These samples include periphytic (epilithic, epiphytic, and unknown substrate), metaphytic and surface sediments materials (Table 1). One sample (BR-4376, Botanic Garden Meise,

Belgium) from Mborou, M'Borou River, Ivory Coast (Agneby hydrographical basin, 5°58'N 4°31'W, N'Guessan et al. 2014) was also analysed to illustrate *C. schroederi*.

The preparation of permanent slides was carried out from the digestion of the samples with concentrated 35% H<sub>2</sub>O<sub>2</sub> and 37% HCl, followed by cleaning and dilution with deionized water (ECS 2003). The material was dried onto coverslips and mounted on slides using Naphrax®. Light microscopy (LM) analysis was performed using a Leica microscope equipped with phase-contrast. The cleaned material was also mounted on aluminium stubs and coated with platinum. For that, part of the oxidized samples were filtered through a polycarbonate membrane filter (3 µm), and washed with deionized water. Scanning electron microscopy (SEM) analysis was performed with a Hitachi SU-70, and micrographs were taken at 5 kV and 10 mm working distance. Plates containing light and scanning electron microscopy images were created using CorelDraw X8.

All the samples analysed and the holotype slide from Brazilian material are deposited in the Herbário Científico do Estado Maria Eneyda P. Kauffmann Fidalgo, Instituto de Botânica, São Paulo, Brazil (SP). The isotype slide is deposited in the Botanic Garden Meise, Belgium (BR).

For all the Brazilian species, a detailed description was presented considering the population found. In addition, ecological information concerning the sampling site of occurrence was given whenever possible (Table 1). The terminology used for the description of frustule features followed Round, Crawford, and Mann (1990) and current literature. The valves were identified as SV (sternum valve) and RSV (raphe-sternum valves) following Riaux-Gobin et al. (2013).

## Results and Discussion

The biodiversity and the occurrence of the genus *Cocconeis* were low in our study material, despite of the great amount of samples analysed for monoraphid diatom taxa. Nevertheless, nine taxa were found; one of them is described as a new species and a variety is erected to the status of species.

### *Cocconeis amerieuglypta* L.F.Costa, C.E.Wetzel & Ector, sp. nov. (Figures 1–12, 25–30)

#### Description

Elliptic to sub-round valves, with rounded apices (Figures 1–12); 13.2–27.6 µm long, 8.4–16.4 µm wide. RSV: axial area very narrow, linear and slightly thickened; central area small rounded (Figures 25–27). Internally thickened (Figure 27, arrow), inner hyaline ring

interrupting the striae all along the valves, and forming a submarginal area (Figure 1–6). Raphe filiform and straight. Proximal raphe endings close, internally slightly bent to opposite sides (Figures 25–27), and externally straight (Figure 28). Distal raphe endings terminating at inner hyaline area, usually interrupting the rows of areolae at the submarginal area (Figures 25–28), and externally drop-like expanded (Figure 28, arrow). Small helictoglossae (Figure 26, arrow). Transapical striae uniserial, finely punctate and radiate towards the apices, 24–26 striae in 10 µm. Submarginal striae formed by 1–5 areolae (Figures 25–27). Areolae round. SV: sternum narrow, widening at the centre, and internally thickened; central area small, apically elliptic (Figure 29) to not differentiated. Transapical striae uniserial, radiate and strongly curved at the apices, 24–26 striae in 10 µm. Areolae externally dash-like (Figure 29), and internally rounded (Figure 30).

#### *Type*

**Holotype.** Slide SP355388, Herbário Científico do Estado Maria Eneyda P. Kauffmann Fidalgo, Instituto de Botânica, São Paulo, Brazil.

**Isotype.** Slide BR-4567, Botanic Garden Meise, Belgium.

#### *Type locality*

Brazil, São Paulo state, Itapura, Tietê River, SP-595, km 21.5. Collected from metaphyton by C.E.M. Bicudo and D.C. Bicudo on 16 May 2001.

#### *Etymology*

The specific epithet refers to the occurrence of the taxon in South American (Brazilian) waters and to its similarity with *Coccconeis euglypta* Ehrenberg.

#### *Remarks*

The new species shares several morphological features with species belonging to the *Coccconeis placentula* group, mainly resembling *C. euglypta* (Table 2). In LM, the striae and areolae density easily distinguish the taxa. *Coccconeis amerieuglypta* presents greater striae density and more areolae per stria. In SEM, the differences occur in both valves: the internal view of RSV is quite similar, however in *C. euglypta* there are only 1–2 rows of areolae at the submarginal area, while in *C. amerieuglypta* there are 1–5. For SV, our species presents an elliptical central area, while in *C. euglypta* it is not differentiated from the axial area in this view. Furthermore, the areolae shape is slightly distinct in both views of SV (see Figures 29, 30, 33–36).

*Coccconeis amerieuglypta* was identified in the past as *C. placentula* var. *placentula* Ehrenberg in Brazilian literature (e.g. Moro and Fürstenberger 1993; Faria, Tremarin, and Ludwig 2010), probably due to their similar RSV and the problematic taxonomic history regarding the latter taxon. However, the SV is distinct, presenting different striae configuration in *C. placentula* with areolae arranged in longitudinal undulated rows, besides the larger axial area. In addition, the striae density in the SV is greater in *C. amerieuglypta* sp. nov. (*C. placentula* var. *placentula*: 15–16 in 10 µm, Jahn, Kusber, and Romero 2009). Also being part of this complex, *Coccconeis placentula* var. *acuta* differs from *C. amerieuglypta* sp. nov. in LM mainly by its cuneate apices, and metric features: the first taxa presents larger valves and lower striae density.

### *Coccconeis euglypta* Ehrenberg (Figures 13–24, 31–36)

#### Description

Elliptic valves, with slightly cuneate apices (Figures 13–24); 13.4–29.5 µm long, 8.3–16.6 µm wide. RSV: axial area very narrow and linear; central area small elliptical (Figure 31) or rounded (Figure 32). Internally thickened (Figure 32), inner hyaline ring interrupting the striae all along the valves, and forming a submarginal area (Figure 13–18). Raphe filiform and straight. Proximal raphe endings close, internally bent to opposite sides (Figure 32), and externally straight and slightly expanded (Figure 31). Distal raphe endings terminating at inner hyaline area, externally drop-like expanded (Figure 31). Small helictoglossae (Figure 32). Transapical striae uniseriate, finely punctate and radiate towards the apices, 19–24 striae in 10 µm. Submarginal striae formed by 1–2 areolae (Figures 31, 32). Areolae round, becoming smaller near the axial area (Figure 31). SV: sternum narrow, with some depressions externally (Figure 34, arrow), and internally larger (Figures 35, 36); central area not differentiated. Valve margins strongly silicified centrally divided by a medium depressed area (Figure 35, arrow). Transapical striae uniseriate, radiate and strongly curved at the apices (Figure 33, arrow), 22–24 striae in 10 µm. Striae apically arranged in 4–6 undulated (zigzag) rows (Figures 19–24, 35, 36). Areolae externally irregular dash-like (Figures 33, 34), and internally rounded (Figures 35, 36).

#### Remarks

*Coccconeis euglypta* is already known from South America, also cited as *C. placentula* var. *euglypta* (Ehrenberg) Grunow (e.g. Morales and Vis 2007; Bartozek et al. 2013; Nardelli et al. 2016). The taxonomy of this taxon was confused in literature for a long time due to the lack

of knowledge about Ehrenberg's type materials (Monnier et al. 2007). Romero and Jahn (2013) restudied it trying to solve this problematic and allowing a more precise identification of the taxon by also providing an epitype. In this way, our specimens present correspondent morphometric features as observed in the epitype both in LM and SEM, despite the distal raphe endings expanded in the external view of RSV (Table 2).

*Coccconeis euglypta* is similar to *C. lineata* Ehrenberg in morphology, but they can be distinguished by the striae organization pattern and density, showed between their epitypes. *Coccconeis euglypta* shows a typical zigzag apical areolar arrangement and lower striae density than *C. lineata* (see *C. lineata*).

### ***Coccconeis feuerbornii* Hustedt (Figures 80–84)**

#### *Description*

Elliptic valves, with rounded apices (Figures 80–84); 19–22 µm long, 10.9–12.3 µm wide. RSV: axial area relatively narrow and linear; central area bow-tied shape (Figure 80). Raphe filiform. Proximal raphe endings distant and expanded (Figure 80). Transapical striae uniseriate, finely punctate and radiate towards the apices, 22 striae in 10 µm. SV: sternum narrow, expanding at the centre; central area small rounded (Figure 82) or not differentiate (Figures 81, 83). Transapical striae uniseriate, finely punctate, and radiate towards the apices, 21–22 striae in 10 µm.

#### *Remarks*

*Coccconeis feuerbornii* is cited here for the first time in Brazilian environments, but it is also rarely mention in general literature. The species has been found in the Amazon, French Guiana (Tudesque, Le Cohu, and Wetzel 2016), Kerguelen Islands (Le Cohu and Maillard 1983), and more recently in Sardinia, Italy (Lai et al. 2019). This taxon was firstly published from a waterfall in Tjibeureum, Java in Schmidt (1936) without any description, and posteriorly validate by Hustedt (1937). Simonsen (1987) provided illustrations in LM from the type material and our population agrees with all the morphometric parameters presented also by the protologue (17–25 µm long, 10–12 µm wide, 20 striae in 10 µm, Hustedt 1937). When compared to other *Coccconeis* species from freshwater, the morphology of *C. feuerbornii* is peculiar due to its striae finely punctate in both valves and the central area bow-tied shape in the RSV, similar to *Psammothidium* species. In SEM analysis it was not possible to find the species due its rarity in the sample.

***Cocconeis fluviatilis* J.H.Wallace** (Figures 48–58, 65–70)

*Description*

Elliptic valves, with rounded apices (Figures 51, 52); 18.5–25.0 µm long, 11.1–14.6 µm wide. RSV: axial area very narrow and linear, central area very small rounded (Figures 51, 52). Inner hyaline ring interrupting the striae all along the valves, and forming a submarginal area (Figures 65, 66). Raphe filiform and straight. Proximal raphe endings close, externally straight and slightly expanded. Distal raphe endings terminating with the striae, externally round expanded. Small helictoglossa (Figure 66, arrow). Transapical striae uniseriate, finely punctate and radiate towards the apices, 14–15 striae in 10 µm. Submarginal striae formed by 2–3 areolae. Areolae round or elliptic, becoming smaller close to the axial area, and bigger and elongate at the submarginal area. SV: sternum very narrow, with small depressions (Figure 68, arrow); central area not differentiated. Transapical striae radiated and curved towards the apices, 13 striae in 10 µm. Areolae conspicuously large with complex organization; externally formed by 2–5 poroids occluded by hymens; and internally formed by elliptic areolae occluded by perforated hymens.

*Remarks*

*Cocconeis fluviatilis* was described from South Carolina, North America (Wallace 1960), and two draws were provided (Figure 48) besides the description of the species. This type material was reexamined in Potapova (2010) and the LM (Figures 49, 50) and SEM illustrations expanded the knowledge about the taxon. Unfortunately, only one RSV external valve view was illustrated in SEM. Our populations showed the same morphological known features, despite the slightly smaller and narrower valves (23–24 µm long, 13–19 µm wide, Wallace 1960).

Several studies including *C. fluviatilis* in the South of Brazil (e.g. Rodrigues and Moreira-Filho 1990; Hermann et al. 2006; Ferrari and Ludwig 2007; Torgan, Salomoni, and Bicca 2009; Bartožek et al. 2013) are not in agreement with our populations here illustrated. The illustrations and measurements in the mentioned publications show a lower striae and areolae density, in this way being more similar to *C. schroederi* (Figures 37–47, 59–64). Both species also differ in SEM by the complex areolae structure: flower-shaped in the latter taxon, with smaller and more grouped poroids (Figures 62, 64), while in *C. fluviatilis* they are greater and more spaced distributed forming an elongated areola (Figures 68, 70).

***Cocconeis lineata* Ehrenberg** (Figures 71–79)

### Description

Elliptic valves, with rounded apices (Figures 71–79); 12.7–19.6 µm long, 7.9–10.9 µm wide. RSV: axial area very narrow and linear; central area small rounded (Figures 71, 72). Narrow inner hyaline ring interrupting the striae all along the valve, and forming a submarginal area (Figures 71, 72). Raphe filiform and straight. Proximal raphe endings close. Transapical striae uniseriate, finely punctate and radiate towards the apices, 21–22 striae in 10 µm. SV: sternum linear and very marked, slightly widening towards central area; central area not differentiated (Figures 73–79). Transapical striae uniseriate, radiate and strongly curved at the apices, 25–32 striae in 10 µm.

### Remarks

The species has been recently typified by Romero and Jahn (2013). An epitype was designated since new material was collected to established unicellular *Cocconeis* strains. In this sense, it was an important step to clarify the taxonomic blurriness of some varieties within the *C. placentula* group.

Despite the slightly longer valves presented by the latter authors (18.6–22.7 µm), the length:width ratio of our specimens (1.6–1.8) is in agreement with the mentioned study (1.5–1.8), including other morphometric features observed in LM.

### *Cocconeis neodiminuta* Krammer (Figures 89–94)

### Description

Small elliptic valves, with rounded apices (Figures 89–94); 13–17 µm long, 7–10 µm wide. RSV: axial area very narrow and linear; central area small rounded (Figure 89). Narrow inner hyaline ring interrupting the striae all along the valve, forming a submarginal area (Figure 89). Raphe filiform and straight. Proximal raphe endings very close. Transapical striae uniseriate, finely punctate and radiate towards the apices, 30 striae in 10 µm. SV: wide sternum lanceolate (Figures 90–92) or linear (Figures 93, 94); central area not differentiated. Transapical striae uniseriate, radiate towards the apices, 13–15 striae in 10 µm. Areolae conspicuously large.

### Remarks

The taxon was rare in the analysed samples, and was not found in SEM. It is barely mentioned in grey literature (i.e. one mention), but it is rather frequently cited from European

and North American waters (e.g. Camburn and Charles 2000; Wojtal 2009; Siqueiros Beltrones and Argumedo Hernández 2014; Szabó et al. 2017; Peeters & Ector 2018).

All the morphometric parameters are in agreement with the protologue despite our *Coccconeis neodiminuta* population presents slightly wider valves (6–9 µm wide in Krammer 1990). *Coccconeis crozetensis* O.E.Romero & Van de Vijver is a similar taxon, which can be differentiated in the RSV by the conspicuous areolae in LM; the slightly narrower axial area, and higher striae density in the SV (22–26 in 10 µm, Romero and Van de Vijver 2011).

### ***Coccconeis neothumensis* Krammer (Figures 85–88)**

#### *Description*

Small elliptic valves, with rounded apices, slightly cuneate (Figures 85–88); 11.2–12.2 µm long, 7–7.4 µm wide. RSV: axial area very narrow, and linear; central area not visible. Narrow inner hyaline ring interrupting the striae all along the valve, and forming a submarginal area (Figure 85). Raphe filiform and straight. Proximal raphe endings very close. Transapical striae visible in LM only on margins, finely punctate and radiated towards the apices, 23 striae in 10 µm. SV: sternum relatively narrow and straight. Transapical striae uniseriate radiate towards the apices, 26 striae in 10 µm.

#### *Remarks*

*Coccconeis neothumensis* was described with *C. neodiminuta* in Krammer (1990). In the protologue, the author mentioned the confused taxonomy involving *C. diminuta* Pantocsek and *C. thumensis* Ant.Mayer, species with type material and type slides lost, defending the invalidity of the names and describing two small new species. Both species are difficult to separate by the RSV, however *C. neothumensis* presents more delicate striae; the SV is easily distinguished by the areolae conspicuously larger in *C. neodiminuta*, and finely punctate in the other.

*Coccconeis neothumensis* can also be distinguished from *C. pseudothumensis* by the RSV striae visible only at the margin in the latter taxa, the striae finely punctate and narrow axial area in the SV.

### ***Coccconeis neuquina* Frenguelli (Figures 95–98)**

#### *Description*

Small elliptic to sub-round valves, with rounded apices (Figures 95–98); 8.8–14.6 µm long, 6.3–8.6 µm wide. RSV: axial area very narrow and linear; central area not differentiated (Figure 95). Raphe filiform and straight. Proximal raphe endings very close. Transapical striae visible in LM only on margins, finely punctate and radiated towards the apices, 28 striae in 10 µm. SV: sternum broadly linear-lanceolate (Figures 96–98). Transapical striae uniserial, radiate and strongly curved at the apices, 16–18 striae in 10 µm. Areolae conspicuously large (Figures 96–98).

#### *Remarks*

Belonging to the small *Coccconeis* species group, *Coccconeis neuquina* was described from Argentina by Frenguelli (1942), and can be easily confounded with the European taxon *C. pseudothumensis* E.Reichardt. The mainly difference in LM between them is the axial area of the SV, being broader and elliptic-lanceolate in the latter taxon, and linear-lanceolate in *C. neuquina* (García et al. 2018).

The taxon is also similar to *C. neodiminuta* due to their SV with conspicuously large areolae, but they differ in the shape and width of the axial area, being more lanceolate and wider in the first. In addition, the RSV can be differentiated by the more circular valve outline, and the striae visible only at the margin in *C. neuquina*. *Coccconeis margaritata* Riaux-Gobin & Al-Handal also shows similarities with *C. neuquina* and *C. neodiminuta*, however, it is easily distinguished by the narrow axial area in the SV (Riaux-Gobin et al. 2010).

Unfortunately, only few valves of *C. neuquina* were observed in LM and we could not find the species in SEM analysis.

#### ***Coccconeis tropicoacuta* L.F.Costa & C.E.Wetzel, nom. nov., stat. nov. (Figures 99–114)**

Basionym: *Coccconeis placentula* var. *acuta* F Meister 1935, Berichte der Schweizerischen Botanischen Gesellschaft vol. 44, p. 99; fig. 60, nec *Coccconeis acuta* Ehrenberg 1854, *nomen nudum*, p. 68, 69.

#### *Description*

Elliptic valves, with cuneate apices (Figures 99–108); 22–38.4 µm long, 11–18.5 µm wide. RSV: axial area narrow, linear and externally thickened (Figure 110); central area very small rounded (Figures 99–103). Internally strongly thickened (Figure 110, arrow), narrow inner hyaline ring interrupting the striae all along the valves, and forming a submarginal area (Figures 109, 110). Raphe filiform and straight. Proximal raphe endings close, internally bent

to opposite sides (Figure 110), and externally straight and round expanded (Figure 109). Distal raphe endings externally terminating at the submarginal area, interrupting the last row of areolae with a great drop-like expansion (Figure 109, arrow). Small helictoglossae (Figure 110). Transapical striae uniserial, finely punctate and radiate towards the apices, 15–17 striae in 10 µm. Submarginal striae formed by one areola. Areolae round. Valvocopula margins ornamented with undulating regular and short fimbriae (Figure 111, arrow). SV: sternum linear, slightly widening towards central area; central area not differentiated (Figures 104–108). Transapical striae uniserial, radiate and curved at the apices, apically slightly undulated, 15–17 striae in 10 µm. Areolae foramen externally rounded to elongated, and slit-like, sometimes located in apical depressions crater-like on the valve face (Figure 113, arrow); the depressions vary in size, comprising one to several areolae. Areolae internally rounded or elliptic (Figure 114).

#### *Remarks*

*Coccconeis placentula* var. *acuta* was described from China in Meister (1935). It is widely cited in South of Brazil (e.g. Burliga et al. 2005; Raupp, Torgan, and Baptista 2006; Ferrari and Ludwig 2007; Schneck, Torgan, and Schwarzböld 2008; Santos, Tremarin, and Ludwig 2011; Bartožek et al. 2013; Nardelli et al. 2016), however none of the illustrations presented in these studies correspond to the type illustration. Some of these specimens present a stronger longitudinal undulated striae pattern and lower areolae density (see Nardelli et al. 2016). Populations found in São Paulo samples are more similar to the protologue's illustration, despite other taxonomic information are missing (e.g. description, measurements), and preventing a better comparison. Nevertheless, our specimens are in agreement with other population occurring in China, which are represented only by greater specimens (36–40 µm long, 18–20 µm wide, 15–16 striae in 10 µm, Liu et al. 2016).

The restudy of the Chinese type material of *Coccconeis placentula* var. *acuta* is necessary to better understand Brazilian populations and their similar taxa. However, according to our recent attempts to find Meister material, it is "very likely there is no material left for SEM – since Meister kept not every raw sample or some vials are broken" (L. Taxböck, pers. comm.). In this way, a new name is proposed here and SEM illustrations are provided for the Brazilian population. The specific epithet "acuta" was not retained as it was previously used by Ehrenberg (1854, *Coccconeis acuta* Ehrenberg, *nomen nudum*).

In the USA, from mountain streams, one species was recently described possessing similar features when compared with *C. tropicoacuta*: *Coccconeis cascadiensis* Stancheva presents great valves and corresponding metric features, including the striae density in the

RV. Besides these, they are also similar in SEM due the presence of depressions on the valve face of the SV, where the areolae are located. Despite these similarities, *C. cascadensis* has lower striae density in the SV (7–12 in 10 µm, Stancheva 2019) and it presents the depressions arranged transapically, while in *C. tropicoacuta* the arrangement is apical. Both RV valvocopulae possess marginal fimbriae. However, in our taxon it is regular and undulated, and in the North American taxon it is formed by irregular indentations.

### **Final considerations**

The SEM analysis is indispensable since some significant features for taxon discrimination, such as the fine structure of striae are not revealed in LM (Romero and Van de Vijver 2011). Despite this great importance, the rarity of some taxa could not let us to analyse the SEM ultrastructure of them, including the valvocopulae features. However, most Brazilian taxa are easily distinguished in LM by the characteristic sternum, the areolae shape and the valve size. The central area is also considered as a key diagnostic feature. It shows some degree of variability in *Cocconeis* species, while in the biraphid diatoms it seems to be a conservative feature (Holmes, Crawford, and Round 1982).

The specific study of some diatom genera contributes to enhance the biodiversity knowledge with the description of new species (e.g. Morales et al. 2011, Riaux-Gobin, Compère, and Jordan 2015, Costa et al. 2017). This is of great importance for countries or regions (e.g. Tropical, Antarctic) where the study of diatoms is scarce and still developing. Finally, this study gather the greater diversity of *Cocconeis* species found in Brazilian freshwaters.

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The authors reported no potential conflict of interest.

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## **Notes on contributors**

### ***Lívia F. Costa***

PhD student in plant biodiversity and the environment at the Botanic Institute of São Paulo. She has written five scientific articles, including co-authorships and one volume of *Bibliotheca Diatomologica*, all of them related to diatom taxonomy and ecology from Brazilian environments. Contribution: preparation of images, bibliographic research, writing and editing of the manuscript.

### ***Carlos E. Wetzel***

Botanist and researcher at the Luxembourg Institute of Science and Technology (LIST) has written over 90 scientific articles related to diatoms. He has been working on diatoms in rivers, lakes and soils, worldwide. Carlos E. Wetzel has been running numerous training courses on diatom ecology and taxonomy designed for biologists, technicians and ecologists, contributing to the continuous improvement in the Water Framework Directive implementation in Europe. Contribution: scanning electron microscopy, bibliographic research, revision and editing of the manuscript.

### ***Luc Ector***

Botanist and senior researcher at the Luxembourg Institute of Science and Technology. He has written over 200 articles and has been working on diatoms in rivers, lakes and soils for the last 30 years. He was the President of the “Association des Diatomistes de Langue Française (ADLaF)”, which organizes annual meetings on diatom taxonomy, ecology and related subjects. Over the last 20 years, he has been organizing and teaching numerous training courses on diatom ecology and taxonomy designed for biologists, technicians and ecologists, contributing to the continuous improvement in the Water Framework Directive implementation in Europe. Contribution: discussion of results, bibliographic research, revision and editing of the manuscript.

### ***Denise C. Bicudo***

Botanist and researcher at the Botanic Institute of São Paulo. She has written over 80 articles, 30 books and chapters, and has been working on limnology, paleolimnology and diatom ecology for the last decades. She has been leading masters and PhD students for 20 years, including the first author of this study. Contribution: discussion of results, bibliographic research, revision and editing of the manuscript.

## ORCID

- Lívia F. Costa <https://orcid.org/0000-0001-7148-9964>  
 Carlos E. Wetzel <http://orcid.org/0000-0001-5330-0494>  
 Luc Ector <http://orcid.org/0000-0002-4573-9445>  
 Denise C. Bicudo <https://orcid.org/0000-0002-4248-3261>

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Table 1. Herbarium number, locality, collected habitat and taxa found from samples analysed in LM and SEM. Nd: no data.

| Samples  | Locality  | Habitat          | Ecological information  | Taxa found  |
|----------|---|------------------|---|---|
| SP255742 | Tietê River, Barra Bonita, SP, Brazil           | Epiphyton        | Nd  | <i>C. lineata</i>                                       |
| SP255753 | Água da Cobra stream, Marilia, SP, Brazil       | Periphyton       | Nd  | <i>C. euglypta</i>                                      |
| SP255758 | Peixe River, Dracena, SP, Brazil                | Periphyton       | Nd  | <i>C. neodiminuta</i>                                   |
| SP255765 | Ribeirão das Ostras, Eldorado, SP, Brazil       | Epilithon        | Nd  | <i>C. neuquina</i>                                      |
| SP355388 | Tietê River, Itapura, SP, Brazil                | Metaphyton       | Low conductivity ( $40 \mu\text{S.cm}^{-1}$ ), and slightly acid pH (6)   | <i>C. amerieuglypta</i> sp. nov., <i>C. fluviatilis</i> |
| SP469207 | Salto do Iporanga reservoir, Juquiá, SP, Brazil | Surface sediment | Low conductivity ( $53 \mu\text{S.cm}^{-1}$ ), slightly alkaline pH (7.7), and oligotrophic condition (total phosphorus $14.6 \mu\text{g L}^{-1}$ ) | <i>C. tropicoacuta</i> nom. nov., stat. nov.            |
| SP469524 | Santa Helena reservoir,                         | Epilithon        | Low conductivity ( $105 \mu\text{S.cm}^{-1}$ )  | <i>C. feuerbornii</i> and <i>C.</i>                     |

|  |           |  |
|--|-----------|--|
| Votorantim, SP, Brazil                           |           | <sup>1</sup> ), neutral pH (7), and<br>oligotrophic condition (total<br>phosphorus < 14 µg L <sup>-1</sup> ).<br><i>neothumensis</i> |
| BR-4376<br>M'Borou River, Mborou, Ivory<br>Coast | Epilithon | Nd<br><i>C. schroederi</i>   |

Table 2. Comparisons between *Coccconeis amerieuglypta* sp. nov., *C. euglypta*, and other similar *Coccconeis* taxa. Nd: no data.

|                            | <i>C. amerieuglypta</i><br>sp. nov.               | <i>C. euglypta</i>                           | <i>C. euglypta</i>                  | <i>C. lineata</i>                   | <i>C. placentula</i> var.<br><i>placentula</i>  |
|----------------------------|---|--|-------------------------------------|-------------------------------------|---|
| Reference                  | This study  | This study                                   | Romero and Jahn<br>(2013) – epitype | Romero and Jahn<br>(2013) – epitype | Jahn, Kusber, and<br>Romero (2009) –<br>epitype |
| Valve outline              | Elliptic to sub-<br>round, with<br>rounded apices | Elliptic, with<br>slightly cuneate<br>apices | Elliptical                          | Elliptical                          | Elliptical to<br>somewhat linear-<br>elliptical |
| Length (μm)                | 13.2–27.6   | 13.4–29.5                                    | 15.9–29.5                           | 18.6–22.7                           | 10.9–36.2                                       |
| Width (μm)                 | 8.4–16.4  | 8.3–16.6                                     | 9.8–17.7                            | 6.4–13.1                            | 7.2–25.7  |
| <b>Raphe-sternum valve</b> |   |  |                                     |                                     |   |
| Valve curvature            | Concave   | Concave                                      | Concave                             | Concave                             | Nd  |
| Central area               | Small rounded                                     | Small elliptical or<br>rounded               | Small elliptical                    | Elliptical or orbicular             | Small, more or<br>less oval                     |

|                    | Filiform and<br>straight  | Filiform and<br>straight  | Rectilinear  | Filiform  | Filiform       |
|--------------------|---|---|--|---|----------------|
| Raphe              |   |   |  |   |                |
| Raphe endings:     |   |   |  |   |                |
| Proximal ends      | Close, and<br>externally straight   | Close, externally<br>straight and slightly<br>expanded                                | Nd   | Coaxial   | Close          |
| Distal ends        | Drop-like<br>expanded   | Drop-like<br>expanded   | Nd   | Coaxial   | Straight       |
| Helictoglossae     | Small and straight  | Small and straight  | Small  | Nd  | Nd             |
| Striae:            |   |   |  |   |                |
| Arrangement        | Almost parallel in<br>the centre, radiate<br>and slightly curved<br>toward the apices | Almost parallel in<br>the centre, radiate<br>and slightly curved<br>toward the apices | Parallel in the middle,<br>radiate toward the<br>apices, slightly curved | Parallel and straight in<br>the valve center, radiate<br>towards the valve ends | Curved radiate |
| Density (in 10 µm) | 24–26   | 19–24   | 17–22  | 20–28   | 18–26          |

## Areolae:

|  |         |   |   |  |         |
|--|---------|---|---|--|---------|
| External opening                           | Rounded | Rounded, smaller<br>near the axial area | Nd  | Circular or ovoid                      | Nd      |
| Internal opening                           | Rounded | Rounded                                 | Nd  | Circular or ovoid                      | Nd      |
| Location of the occlusion                  | Nd      | Internal                                | Nd  | Nd                                     | Nd      |
| Type of the occlusion                      | Nd      | Hymen                                   | Nd  | Nd                                     | Nd      |
| Submarginal hyaline area                   | Present | Present                                 | Present   | Present                                | Present |
| Rows of areolae at the<br>submarginal area | 1–5     | 1–2                                     | 1–2   | 1–3                                    | 3–4     |
| Valvocopulae                               | Nd      | Nd                                      | Opened with short,<br>slightly laterally<br>expanded fimbriae | Open at one end,<br>devoid of fimbriae | Nd      |

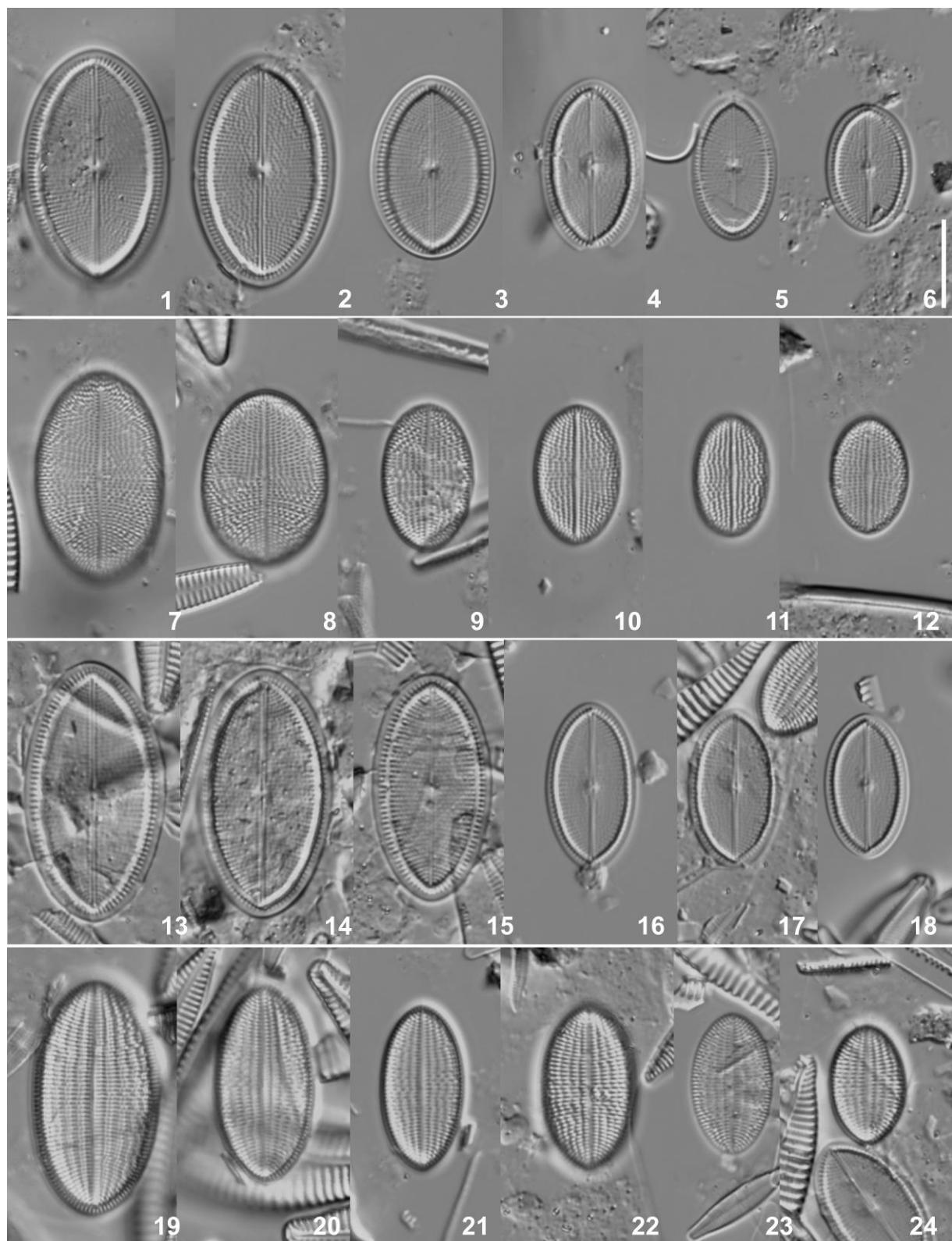
**Sternum valve**

|                 |        |        |         |                 |    |
|-----------------|--------|--------|---------|-----------------|----|
| Valve curvature | Convex | Convex | Concave | Slightly convex | Nd |
|-----------------|--------|--------|---------|-----------------|----|

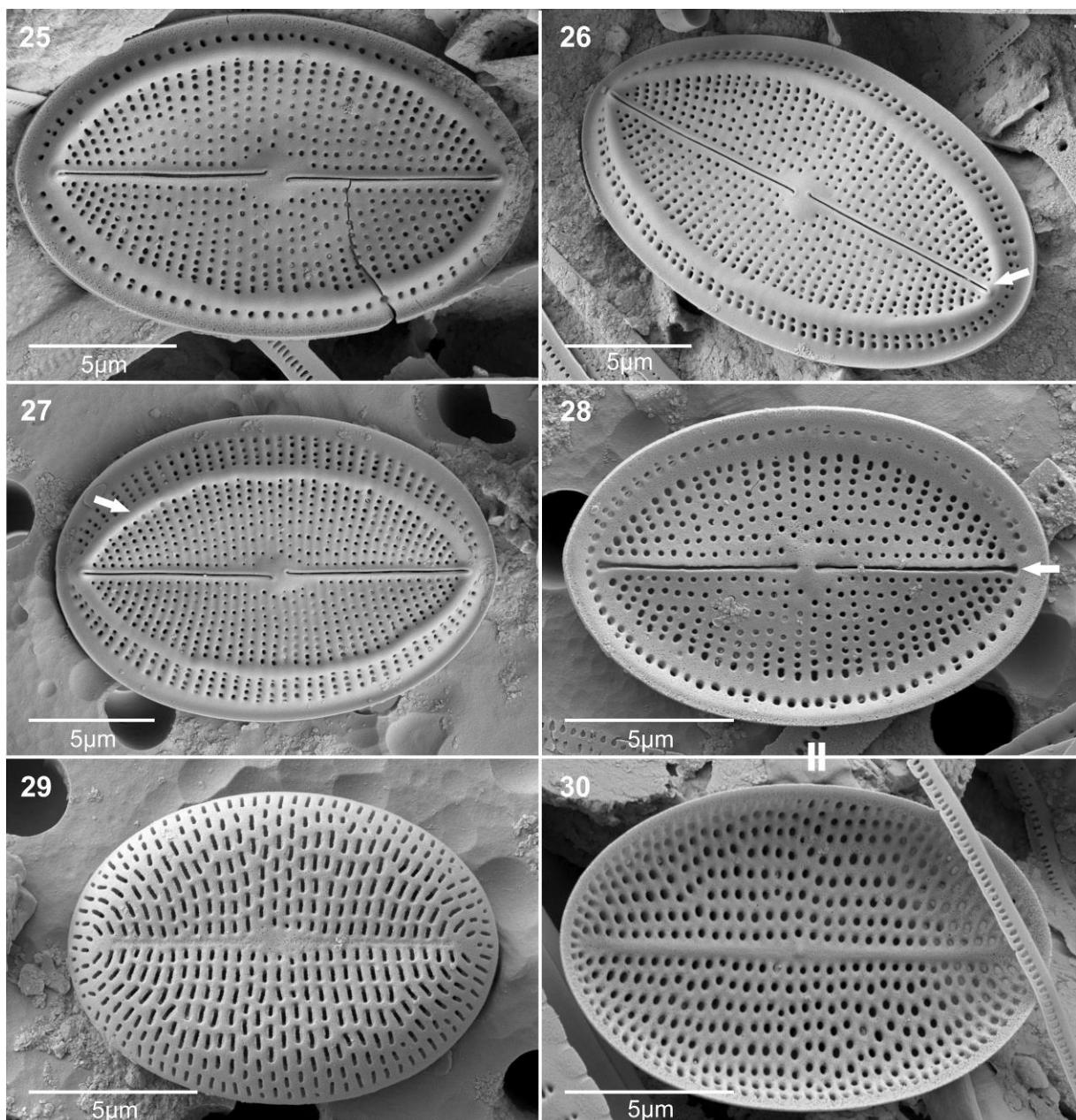
|                  |  |   |  |   |  |
|------------------|--|---|--|---|--|
| Sternum shape    | Narrow, widening at the centre, and internally thickened | Narrow, with some depressions externally, and internally larger | Narrow, linear                                       | Narrow, linear/linear-lanceolate                                | Very narrow and linear, surrounded by a wide axial area                                  |
|                  | Striae:  |   |  |   |  |
|                  | Radiate and strongly curved toward the apices            | Radiate and strongly curved toward the apices                   | Mostly radiate, more curved towards the valve apices | the valve center, although radiate and curved toward the apices | Parallel and straight in the valve center, although radiate and curved toward the apices |
| Arrangement      | 24–26  | 22–24   | 18.5–24  | (7)10–15 (apically)   | Curved and radiate   |
|                  | Density (in 10 µm)                                       |   |  |   |  |
|                  | Areolae:   |   |  |   |  |
| External opening | Dash-like  | Irregular dash-like   | Alveoli  | Dash-shape  | Dash -like, arranged in longitudinally   |

|                           |          |          |   |                                      |   |
|---------------------------|----------|----------|---|--------------------------------------|---|
|                           |          |          |   |                                      | undulating rows                                       |
| Internal opening          | Rounded  | Rounded  | Rounded   | Ovoid                                | Rounded   |
| Location of the occlusion | External | External | Nd  | Nd                                   | Nd  |
| Type of the occlusion     | Nd       | Hymen    | Nd  | Nd                                   | Nd  |
| Valvocopulae              | Nd       | Nd       | Open at one end, bears<br>very short digitiform<br>fimbriae | Open at one end,<br>lacking fimbriae | margins with<br>additional<br>secondary<br>structures |

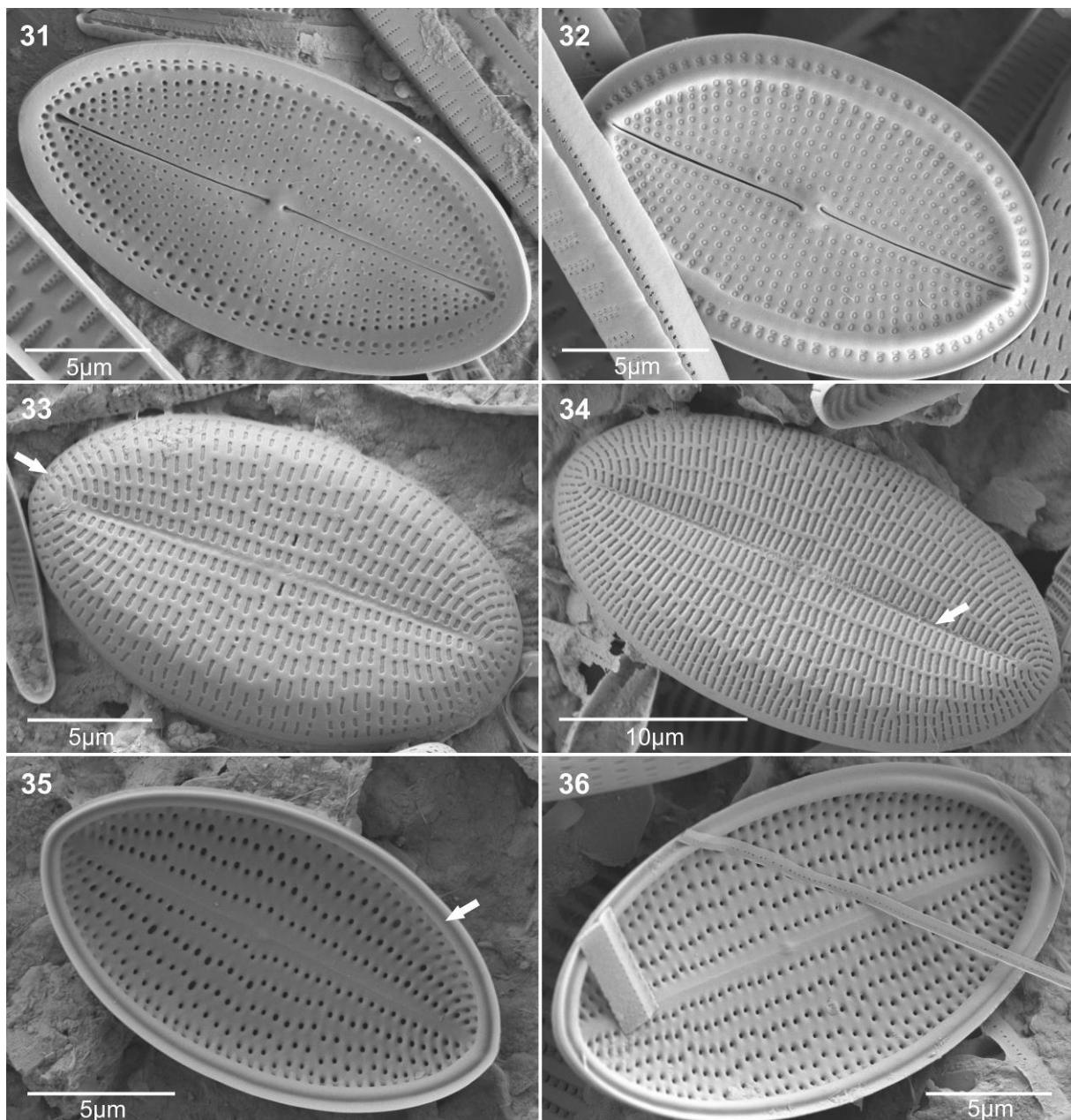
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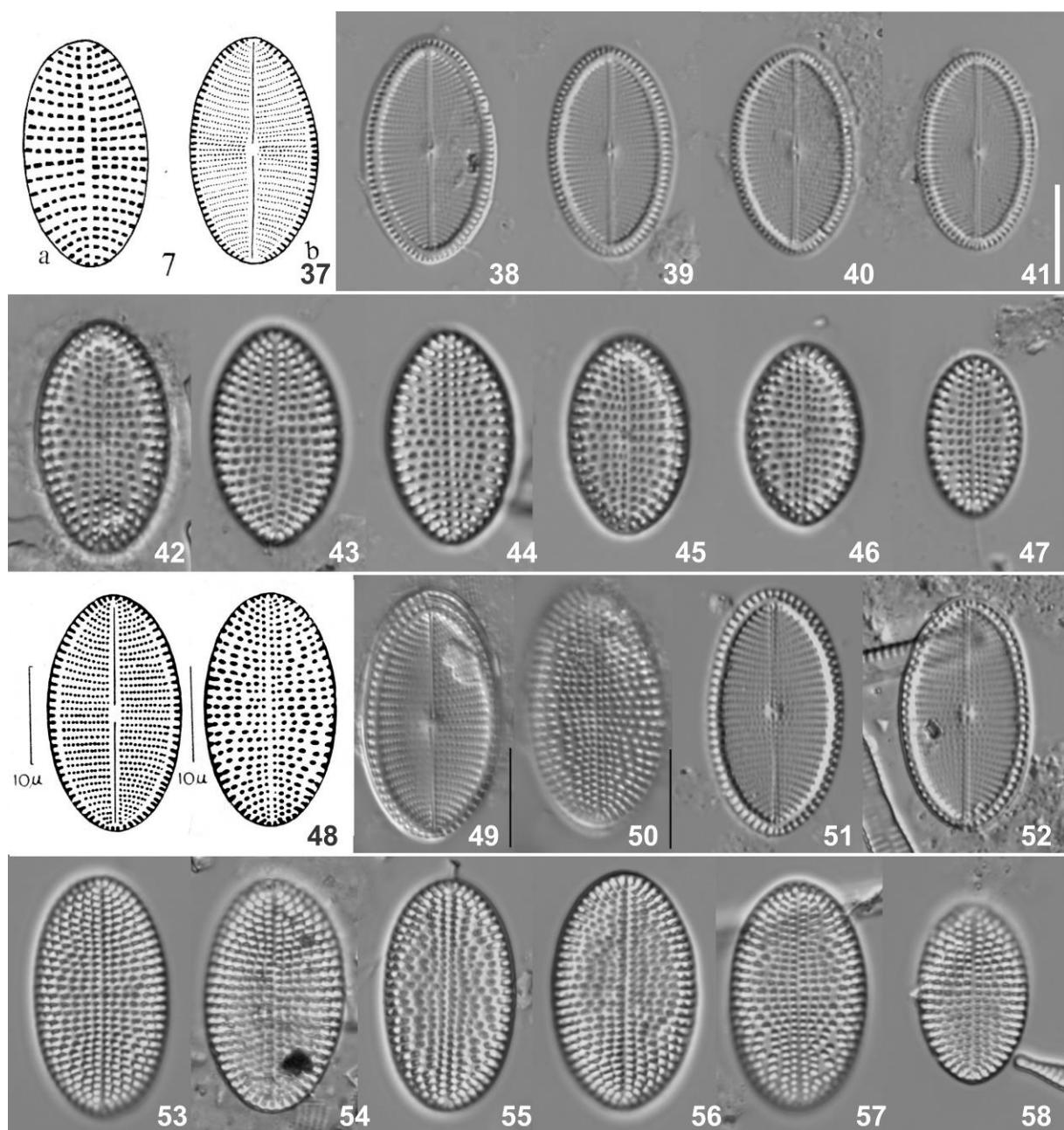
Figures 1–24. Valve diminution series showing the size variability and shape outline in LM. **1–12.** *Cocconeis amerieuglypta* sp. nov. **1–6.** RSV. **7–12.** SV showing the great striae and areolae density. **13–24.** *Cocconeis euglypta*. **13–18.** RSV. **19–24.** SV showing the zigzag apical areolar arrangement. Scale bar = 10  $\mu\text{m}$ .



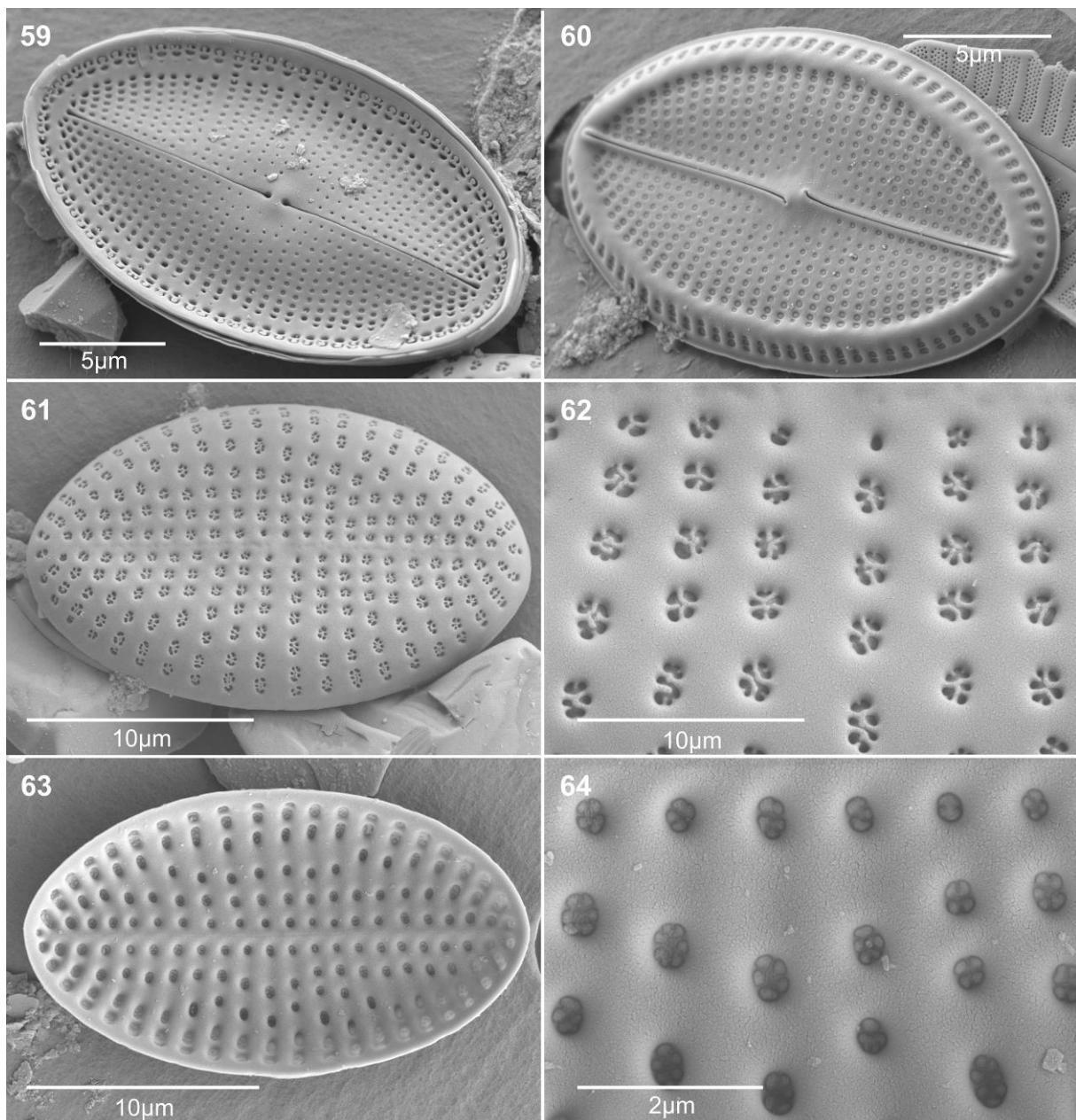
Figures 25–30. *Cocconeis amerieuglypta* sp. nov., SEM. **25–27.** Internal views showing the helictoglossae (**25**, arrow), and the proximal raphe ends deflected to opposite sides. Note the prominent hyaline ring interrupting the areolae (**27**, arrow) and the submarginal areolae varying from 1 to 5. **28.** External view showing the terminal raphe endings drop-like shaped (arrow). **29.** External valve view showing the dash-like areolae and the small elliptical central area. Note the apical striae strongly curved. **30.** Internal view showing the rounded areolae and the prominent sternum.



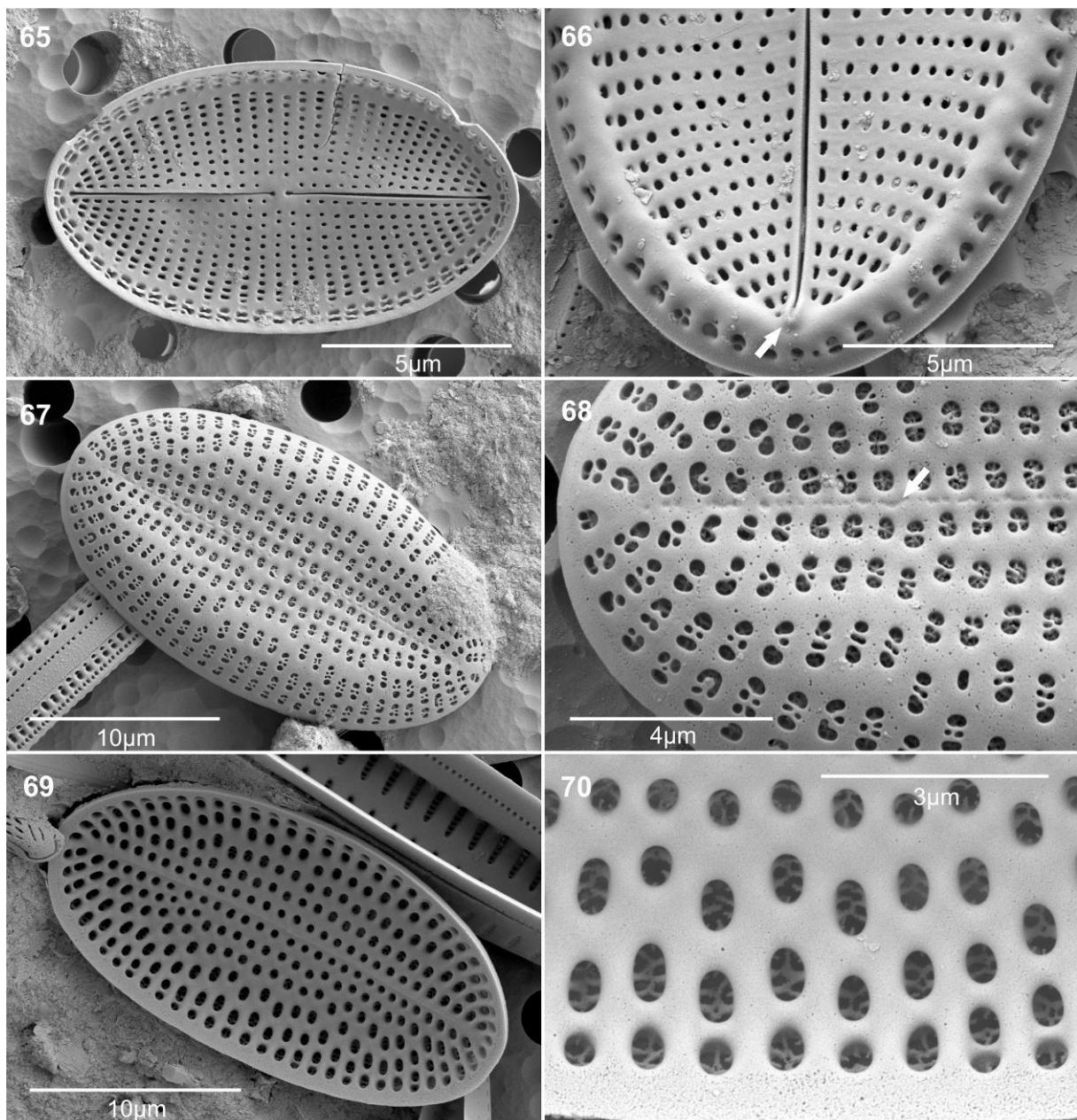
Figures 31–36. *Cocconeis euglypta*, SEM. **31.** External view showing the hyaline ring interrupting the areolae, and the terminal and proximal raphe ends drop-like shaped. **32.** Internal view showing the helictoglossae and the proximal raphe ends deflected to opposite sides. Note the strongly silicified hyaline ring. **33, 34.** External views showing the irregular dash-like areolae, the strongly curved striae at the apices (**33**, arrow), and the depressions on the sternum (**34**, arrow). **35, 36.** Internal views showing the zigzag apical arrangement of the areolae. Note the valve margins strongly silicified, and centrally divided by a medium depressed area (**35**, arrow).



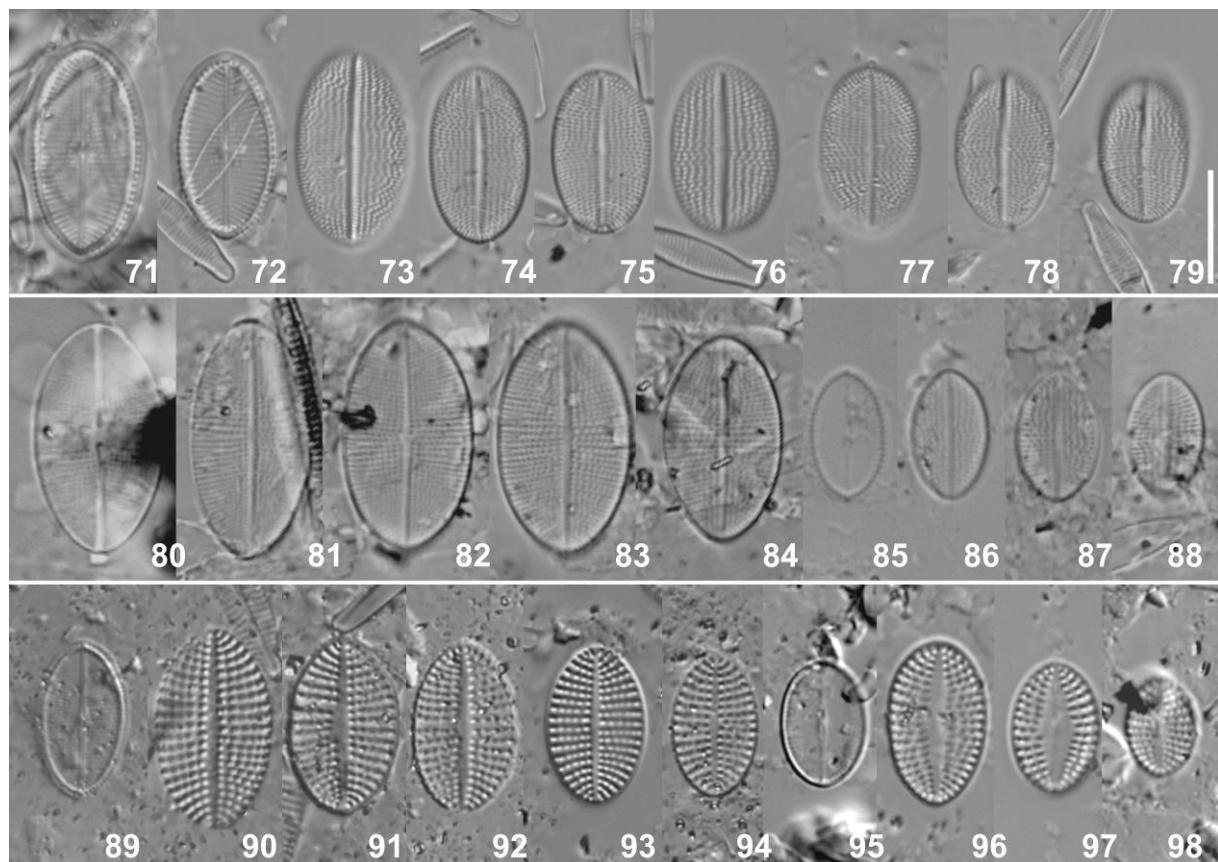
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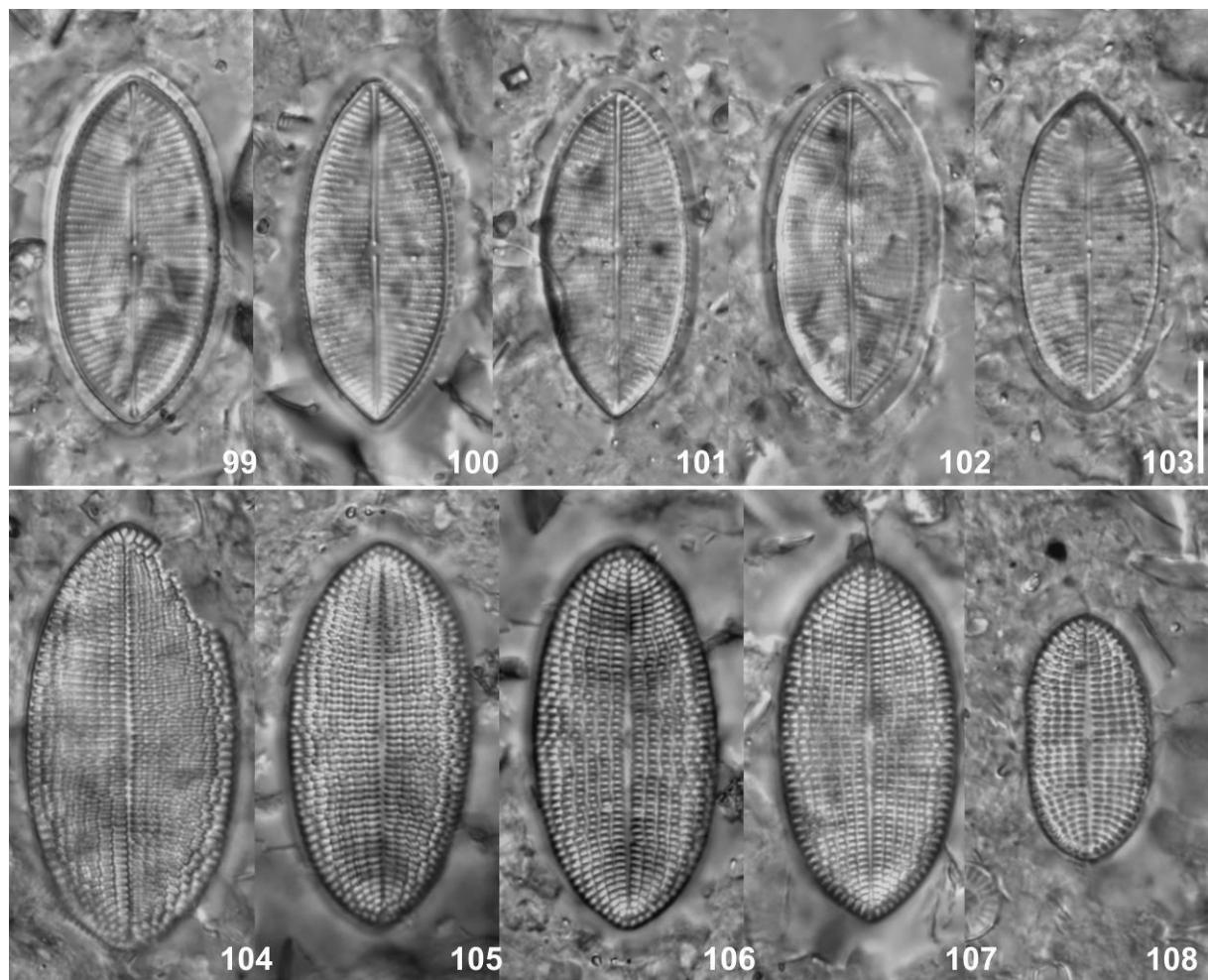
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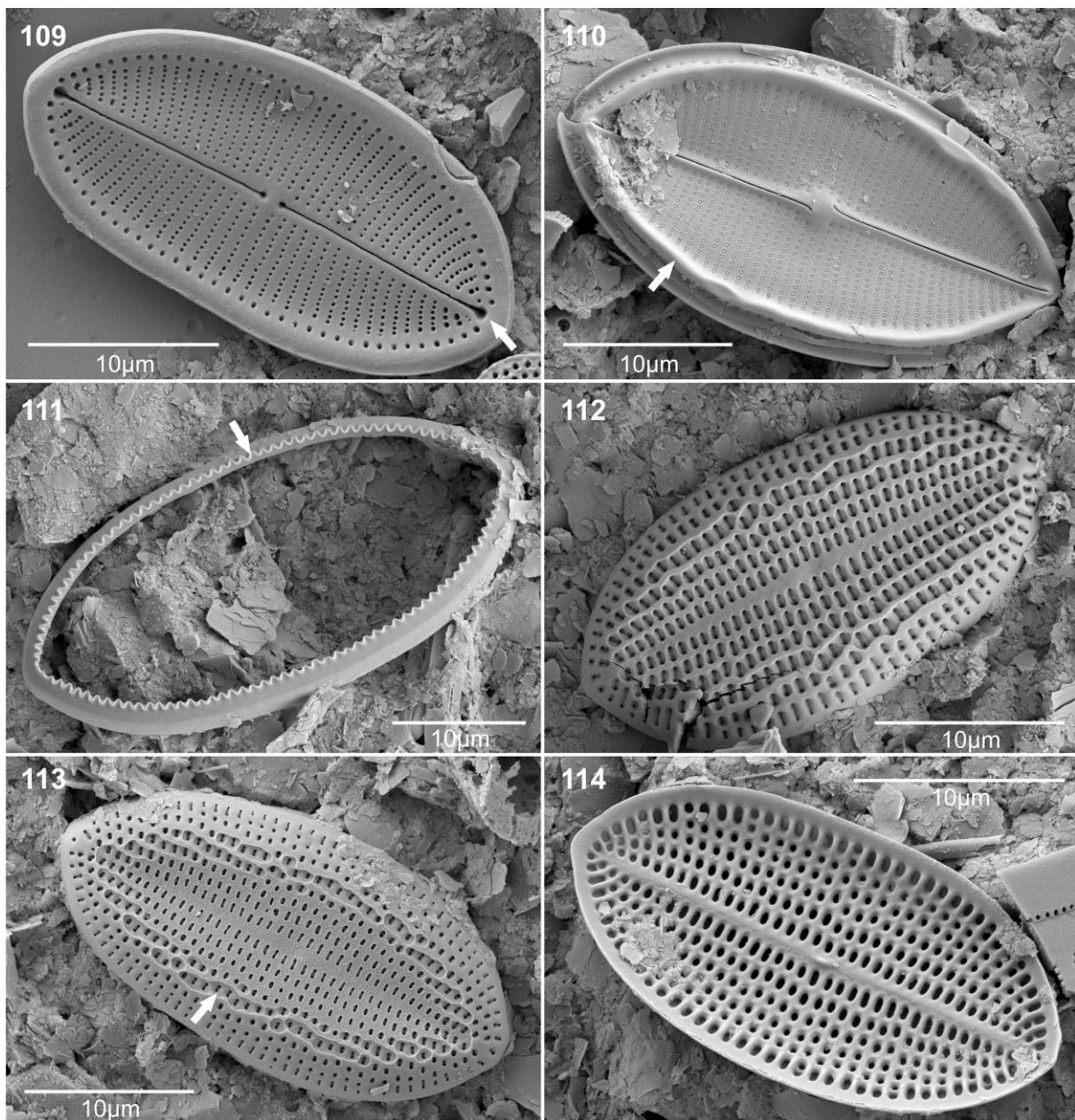
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Figures 99–108. *Cocconeis tropicoacuta* nom. nov., stat. nov., valve diminution series showing the size variability and shape outline in LM. **99–103.** RSV showing the characteristics cuneate apices. **103–108.** SV showing the elliptical areolae. Scale bar = 10  $\mu\text{m}$ .



Figures 109–114. *Cocconeis tropicoacuta* nom. nov., stat. nov., SEM. **109.** External view showing the narrow inner hyaline ring, the terminal raphe ends forming a great drop-like expansion and interrupting the last row of areolae (arrow). **110.** Internal view showing the hyaline ring very silicified and prominent (arrow). **111.** RV valvocopula margin with undulating regular fimbriae (arrow). **112, 113.** External view showing some of the areolae arranged in depressions on the valve face (113, arrow). **114.** Internal view showing the round to elliptic areolae.

## CAPÍTULO 2

New *Achnanthidium* species (Bacillariophyta, Achnanthidiaceae) from southeastern

Brazil

Lívia F. COSTA<sup>1,\*</sup>, Carlos E. WETZEL<sup>2</sup>, Jaques E. ZANON<sup>3</sup>,

Luc ECTOR<sup>4</sup> & Denise C. BICUDO<sup>5</sup>

<sup>1,3,5</sup> Instituto de Botânica, Ecology Department, Av. Miguel Estéfano 3687, 04301-012 São Paulo, SP, Brazil.

<sup>2,4</sup> Luxembourg Institute of Science and Technology (LIST), Environmental Research and Innovation Department (ERIN), 41 rue du Brill, 4422 Belvaux, Luxembourg.

\* Corresponding author: [livia.fcosta@hotmail.com](mailto:livia.fcosta@hotmail.com)

<sup>2</sup> [carlos.wetzel@list.lu](mailto:carlos.wetzel@list.lu)

<sup>3</sup> [jaques.zanon@gmail.com](mailto:jaques.zanon@gmail.com)

<sup>4</sup> [luc.ector@list.lu](mailto:luc.ector@list.lu)

<sup>5</sup> [denisecbicudo@gmail.com](mailto:denisecbicudo@gmail.com)

<sup>1</sup> [orcid.org/0000-0001-7148-9964](https://orcid.org/0000-0001-7148-9964)

<sup>2</sup> [orcid.org/0000-0001-5330-0494](https://orcid.org/0000-0001-5330-0494)

<sup>3</sup> [orcid.org/0000-0002-9619-4393](https://orcid.org/0000-0002-9619-4393)

<sup>4</sup> [orcid.org/0000-0002-4573-9445](https://orcid.org/0000-0002-4573-9445)

<sup>5</sup> [orcid.org/0000-0002-4248-3261](https://orcid.org/0000-0002-4248-3261)

***Achnanthidium* from southeastern Brazil**

**Abstract.** *Achnanthidium* presents the largest number of taxa in the family Achnanthidiaceae, including more than 200 species. It has always received great attention from taxonomists due to the high abundance of individuals living in periphytic diatom communities worldwide, allowing several new descriptions every year, especially in temperate regions. In South America, taxonomic studies regarding the genus are extremely scarce. However, its representatives are widely cited in diatom surveys and ecological studies conducted on the continent. The present study aims to expand the knowledge about *Achnanthidium* species occurring in tropical areas of southern Brazil. For this purpose, 345 samples from 179 sampling sites distributed throughout the state of São Paulo were analyzed for 'monoraphid' diatom diversity; this paper focuses on samples with a high abundance of *Achnanthidium* species. The permanent slides and stubs were observed using light and scanning electron microscopy. The ecological optimum was calculated for taxa that presented more than 10% of occurrence per habitat, and morphometric geometric analyses were applied to separate species within the *A. catenatum* group. In total, 13 *Achnanthidium* species were observed. Eight are described as new, four were described previously (*A. minutissimum*, *A. peetersianum*, *A. peruvianum* and *A. tropicocatenatum*) and one, due to its rarity and subsequent lack of SEM information, could not be clearly identified. The morphometric geometric analyses helped separate *Achnanthidium* sp. nov. 6 from *A. tropicocatenatum*. Ten species had their ecological optimum calculated. In general, these species prefer slightly acidic to neutral pH, medium electrolyte conductivity and mesotrophic water conditions.

**Keywords.** *Achnanthidium catenatum* group, ecological optimum, monoraphid, morphometric geometrics, São Paulo state.

## Introduction

*Achnanthidium* Kützing belongs to a paraphyletic group of 'monoraphid' diatoms composed of three families (Kulikovskiy *et al.* 2016). Among the genera included in these families, *Achnanthidium* presents the largest number of taxa within Achnanthidiaceae, with more than 200 species, varieties and taxonomic forms (Fourtanier & Kocielek 2011). It is a widespread genus found in brackish and freshwater environments, constituting one of the main components of the benthic community (e.g., Ponader & Potapova 2007; Novais *et al.* 2011); however, it also has planktonic representatives such as *A. catenatum* (Bílý & Marvan) Lange-Bertalot common in inland freshwater reservoirs in Europe. The cell adhesion to the substrate is due to the secretion of mucilage restricted to one of the valves (i.e., the raphe valve) of the organism. It is possible that this restriction led to the loss of the raphe in the other valve (araphid), which ceased to function (Cox 2006).

The genus has undergone many changes since its proposition, hindering the taxonomy of the group. Described by Kützing (1844), *Achnanthidium* was considered as a subgenus in Patrick & Reimer (1966) and later reestablished as a genus, included in its current family (Round *et al.* 1990). Posteriorly, a redefinition of the genus was proposed to delimit the concept of *Achnanthidium* only to the taxa around the '*Achnanthes minutissima*' complex (Round & Bukhtiyorova 1996). Thus, some taxa were relocated into other modern and recently described genera (e.g., *Gogorevia*, *Karayevia*, *Planothidium*, *Psammothidium* and *Rossithidium* in freshwater ecosystems).

In addition to the unstable and ever-changing classification, other problems in *Achnanthidium* taxonomy include its small size ranges, making it challenging to visualize structures with optical microscopy (i.e., striae, areolae), and the lack of morphological criteria that define the limits of species (Hlúbková *et al.* 2011). Currently, the circumscription of the species is based on morphological characteristics (i.e., valve outline, central area, striae density) of both valves, raphid and araphid, whose visualization often requires the use of SEM.

Notably, the incipient and growing use of molecular data to classify 'monoraphid' diatoms has revealed some ideas about the group taxonomy and its further split at the generic level (e.g., Kulikovskiy *et al.* 2016, 2020). Moreover, Pinseel *et al.* (2017) showed information at the species level, where *rbcL*, 28S and 18S markers were used to support the separation of one new species from the *Achnanthidium minutissimum* (Kützing) Czarnecki complex.

Indeed, *Achnanthidium* has been receiving attention with several descriptions of new species, in temperate (e.g., Novais *et al.* 2011; Van de Vijver *et al.* 2011; Wetzel *et al.* 2019), tropical, subtropical (e.g., Morales *et al.* 2011; Marquardt *et al.* 2017; Krahn *et al.* 2018; Yu *et al.* 2018, 2019; You *et al.* 2019; Miao *et al.* 2020) and polar (Taylor *et al.* 2014) regions, either using classic approaches or combining molecular tools with morphological data. Altogether, more than 50 taxa have been proposed as new in the last ten years, illustrating the growing knowledge about this genus once the type materials from "old names" are more narrowly circumscribed.

In South America, taxonomic studies published about the genus *Achnanthidium* are scarce. For example, there are only three publications known for Bolivia and Brazil (Morales *et al.* 2009, 2011; Marquardt *et al.* 2017), which describe three new species: *A. cadimae* E.Morales, E.Fernández & Ector in Morales *et al.*, *A. sehuencoensis* E.Morales in Morales *et al.* and *A. tropicocatenatum* Marquardt, C.E.Wetzel & Ector in Marquardt *et al.*. However, representatives of the genus are widely cited in diatom surveys (e.g., Morales & Vis 2007; Alvial *et al.* 2008; Sala *et al.* 2008; García & Maidana 2015; Bartožek *et al.* 2018) and ecological studies investigating the use of diatoms as bioindicators have been conducted (e.g., Lobo *et al.* 2004; Tapia 2008; Bere & Tundisi 2011).

In Brazil, studies with the *Achnanthidium* species have mentioned around 15 taxa located in the South and Southeast regions of the country. It is important to point out that these regions also have the largest number of researchers studying diatoms in the country. In the state of São Paulo (Southeast), 12 taxa are currently known (Bicudo *et al.* 1993, 2009; Fontana &

Bicudo 2012; Faustino *et al.* 2016; Costa *et al.* 2017; Marquardt *et al.* 2017, 2018; Bartozek *et al.* 2018). Only one study (Marquardt *et al.* 2017) presents quality SEM illustrations and information about the ultrastructure of the valves, an indispensable condition for precise identification.

The present study sought to expand the knowledge of *Achnanthidium* species in the state of São Paulo, Brazil, clarifying differences among species belonging to distinct so-called species-complex (i.e., *A. minutissimum* and *A. catenatum*). Consequently, this work provides autecological information for species with a broad distribution, SEM information, illustrations of the populations and descriptions of newly identified species.

## Material and methods

### Sampling

The material analyzed in this study comes from two distinct sampling campaigns in the state of São Paulo. As shown in Figure 1, the Biota project (grey dots) covered the entire state, and the AcquaSed project (red dots; process nº 2009/53898–9) focused on the metropolitan area and neighboring basins, including 32 reservoirs. In total, 345 samples were collected from 179 sampling sites (Figure 1). Samples included planktonic, periphytic (epilithic, epiphytic, and unknown substrate) and surface sediment materials from ultraoligotrophic to hypertrophic water conditions. Each sample was then analyzed for 'monoraphid' diatom diversity.

Samples from the AcquaSed project are listed in Table 1 with information about the sampling sites and reservoirs (i.e., geographic coordinates, drainage basin).

### Material preparation and microscopy

The raw samples were digested with concentrated 35% H<sub>2</sub>O<sub>2</sub> and 37% HCl, cleaned and diluted with deionized water (ECS 2003). Part of the prepared material was mounted on slides using Naphrax® mounting medium. Another portion of the oxidized material was filtered through a polycarbonate membrane filter (3 µm), washed with deionized water, mounted on

aluminum stubs and coated with platinum. The permanent slides were analyzed under a Leica® DMR microscope equipped with a Leica-DFC 500 high-resolution digital camera using Leica Application Suite software (v.3.7.0, Leica Microsystems<sup>©</sup>). The SEM analyses were performed with a Hitachi SU-70. Plates were created using CorelDraw X8.

### Type material

The entire population/slides will be chosen as the holotypes of the new taxa due to the different morphology of the two valves in one frustule of the monoraphid diatoms. Permanent slides, including holotypes, will be deposited in the Herbário Científico do Estado Maria Eneyda P. Kauffmann Fidalgo, Instituto de Botânica, São Paulo, Brazil (**SP**). Isotype slides will be deposited at the Meise Botanic Garden collection (**BR**).

Detailed descriptions were provided for all the taxa found. The terminology used followed Barber & Haworth (1981), Round *et al.* (1990) and other current studies (ex. Hlúbková *et al.* 2011, Novais *et al.* 2015).

### Morphometric analysis

A shape analysis was applied to access the group separation of the different species within the *A. catenatum* group based on the valve outline and central area shape. The utilized photomicrographs focused only on the valve view of the *Achnanthidium* raphe valves. Photomicrographs of four populations were converted in TPS files with the aid of the *TpsUtil* software (Rohlf 2007, v. 1.76): *a*) seventeen raphe valves from lectotype material of *A. catenatum* (Hlúbková *et al.* 2011, figs 1–15; Marquardt *et al.* 2017, fig. 6AG–AM); *b*) sixteen raphe valves of *A. tropicocatenatum* from Cachoeira do França reservoir (type locality, Fig. 14); *c*) eight raphe valves of *A. tropicocatenatum* from Santa Helena reservoir (Fig. 15) and *d*) eleven raphe valves of *Achnanthidium* sp. nov. 6 from Garças Lake, São Paulo (SP469486) (Fig. 11).

Using the *TpsDig* software v. 1.4 (Rohlf 2007), 40 landmarks were digitized along the valve outline of each specimen and ten along the central area (Figure 2). On the valve outline, four

of the landmarks were located at fixed positions, two on the valve poles (landmarks 1 and 21) and another two were present on the widest portion of the valve that is considered the midpoint between the proximal raphe ends (landmarks 11 and 31). To eliminate all non-shape information, we superimposed all of the landmark configurations using the Generalized Procrustes Analysis (GPA). These analyses employed the *shapes* package (Dryden 2018) and R software (R Core Team 2019), which showed that the final position of the shape coordinates was achieved by relating the superimposed landmarks with the consensus (average) shape (Peng *et al.* 2014). Finally, to summarize the morphometric analysis results we applied a Principal components analysis (PCA). By doing this we were able to access (via principal components) relevant information about size and shape of diatom's individuals, with first component summarizing mainly the size information and the second the shape information. After that, we used a multivariate analysis of variance (PERMANOVA) to test for significant difference between groups of taxa. For that we used the vegan (Oksanen *et al.* 2019) library in R (R Core Team 2019)

### **Quantitative and ecological analysis**

Due to the lack of environmental data from the Biota project samples, the quantitative and ecological analyses were only performed with the AcquaSed samples (n=224). The quantitative analysis was carried out by counting at least 500 diatom valves per slide at 1000× magnification. Species that exhibited a frequency of occurrence equal to or greater than 10% per habitat were included in the ecological analysis. The ecological optimum and tolerance were calculated for four variables (pH, conductivity, total phosphorus and total nitrogen) based on species relative abundances. Species optima were calculated using the weighted-averaging method (ter Braak & van Dam 1989; Wetzel & Ector 2014) performed in the R software v. 3.5.2 with the *wa( )* function in the *analogue* package (Simpson 2007).

## Results

In this present study, we identified 13 taxa belonging to *Achnanthidium*. Eight species are new descriptions, four were previously known, and one taxon could not be identified due to its rarity in the material. Ten species had their ecological optimum calculated for one, two or the three habitats (i.e., periphyton, plankton and surface sediments), depending on the occurrence in samples (>10%) of each species in each habitat. Ecological optima of the species for each habitat are presented in Table 2.

Class Bacillariophyceae Haeckel emend. Medlin & Kaczmarska (2004)

Subclass Bacillariophycidae D.G.Mann in Round *et al.* (1990)

Order Achnanthales P.C.Silva (Silva 1962)

Family Achnanthidiaceae D.G.Mann in Round *et al.* (1990)

Genus *Achnanthidium* Kützing (Kützing 1844)

***Achnanthidium minutissimum*** (Kützing) Czarnecki 1994

Fig. 3A–S

Basionym: *Achnanthes minutissima* Kützing 1833, p. 578, fig. 54

Synonyms: *Cocconeis minutissima* (Kützing) Schönfeldt 1907, p. 124; pl. 13, fig. 234

*Microneis minutissima* (Kützing) F.Meister 1912, p. 9

## Description

Valves linear-lanceolate, with protracted, rostrate to subcapitate apices; 12.3–17.7 µm long, 2.5–2.9 µm wide. Raphe valve: axial area linear, very narrow; central area small rounded, almost absent, sometimes forming a rectangular fascia reaching one or both sides. Raphe filiform, straight, ending after the last stria in the border between the valve face and mantle. Proximal raphe endings close, straight externally and slightly expanded, internally bent in opposite directions. Distal raphe endings externally straight and drop-like expanded,

internally forming small helictoglossae. Transapical striae slightly radiate throughout the entire valve, becoming denser and more strongly radiate towards the apices; 26–29 in 10 µm. Striae mainly composed of 3–4 rounded to elongated areolae, sometimes slit-like shaped near the valve margin. Mantle with one row of slit-like areolae. Rapheless valve: axial area narrow and linear, slightly widening towards the central area; central area absent or narrow lanceolate. Transapical striae slightly radiate throughout the entire valve, more spaced in the central area and sometimes lacking one stria unilaterally. Striae become denser towards the apices; 26–28 in 10 µm. Striae mainly composed of four rounded to elongated areolae. Mantle with one row of slit-like areolae.

### **Taxonomical remarks**

*Achnanthidium minutissimum* is a widely used name and the main species within a complex of morphologically similar species worldwide. The morphometric characteristics of all the species that belong to this complex and were observed in this study are summarized in Table 3. Its type material has been reevaluated several times (Krammer & Lange-Bertalot 1991; Potapova & Hamilton 2007; Novais *et al.* 2015; Marquardt *et al.* 2017) to clarify the circumscription of this widespread name. In the past, the high morphological similarity with some related species [i.e., *Achnanthidium microcephalum* Kützing and *Achnanthidium neocryptocephalum* (Grunow in Van Heurck) Novais & Van de Vijver, ( $\equiv$  *A. minutissima* var. *cryptocephala* Grunow)] made their separation difficult, and the synonymization with other species was accepted until recently (Patrick & Reimer 1966; Lange-Bertalot & Krammer 1989; Krammer & Lange-Bertalot 1991).

Novais *et al.* (2015) reanalyzed their type materials and differentiated *A. microcephalum* by its wider axial area than *A. minutissimum* in some rapheless valves. Moreover, the authors showed that *A. neocryptocephalum* always presents a rectangular fascia in the raphe valve, a rare feature in *A. minutissimum* or *A. microcephalum*.

*Achnanthidium jackii* Rabenhorst, described in 1861 (Rabenhorst 1861–1862) from "Quellwasser bei Salem" (Municipality of Baden-Württemberg State, southern Germany) was studied in full detail by Van de Vijver *et al.* (2018), has priority over *A. neocryptocephalum* (Grunow) Novais & Van de Vijver in Novais *et al.* (2015), a new name for *Achnanthes minutissima* var. *cryptocephala* Grunow in Van Heurck (1880), thus also a taxonomic synonym of *A. jackii*.

*Achnanthidium barbei* Le Cohu & Pérès in Pérès *et al.* also belongs to the *A. minutissimum*-complex and presents a similar morphology. It never forms a fascia in the central area of the raphe valve and has more protracted apices (Pérès *et al.* 2014).

In other *A. minutissimum* populations found in our studied material, the width range is wider, as in Hedberg reservoir population: 2.0–3.6 µm.

### Distribution and ecological information

This taxon distribution is confusing due to its problematic taxonomic drift over time, leading to erroneous identifications in several parts of the globe. Despite this, it is still a name often cited worldwide, including in South America (e.g., Morales *et al.* 2011; Wojtal *et al.* 2011; Cantonati *et al.* 2014; Bartožek *et al.* 2018). In our dataset, *A. minutissimum* was the most frequent species (63% of occurrence) observed in periphytic, planktonic and surface sediment assemblages. In all these habitats, the ecological preference of the species was for neutral waters (pH optimum of 6.8–7.2), low to medium conductivity (optimum of 64.3–148.7 µS cm<sup>-1</sup>) and mesotrophic conditions (TP optimum of 25.5–46.4 µg L<sup>-1</sup> and TN optimum of 596–1303.8 µg L<sup>-1</sup>); however, it also occurred in enriched waters (TP >50 µg L<sup>-1</sup>).

### *Achnanthidium peetersianum* C.E.Wetzel, Jüttner & Ector in Wetzel *et al.* 2019

Fig. 4A–V

#### Description

Valves small linear-elliptic, with subcapitate to small capitate apices; 7.6–12.3 µm long, 2.0–2.7 µm wide. Raphe valve: axial area narrow and linear; central area absent or small rounded, rarely forming a bilateral fascia; 1–2 central shortened striae on both sides of the valve. Raphe filiform straight, ending in the last stria. Proximal raphe endings externally straight and expanded, internally slightly bent in opposite directions. Distal raphe endings externally bent to the same side and slightly expanded, internally forming small helictoglossae. Transapical striae slightly radiate throughout the entire valve, more spaced in the central area, becoming denser and more strongly radiate towards the apices, 29–30 in 10 µm. Striae mainly composed of 3–4 rounded, almost squared or elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae. Rapheless valve: axial area narrow and linear, slightly widening towards the central area; central area absent or narrow lanceolate; 1–2 central shortened striae on both sides of the valve. Transapical striae slightly radiate throughout the entire valve, more spaced in the central area, and becoming denser towards the apices, 32–34 in 10 µm. Striae mainly composed of four rounded, almost squared to elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae.

### **Taxonomical remarks**

This species has narrow valves with subcapitate to small capitate apices, similar to *A. macrocephalum* (Hustedt) Round & Bukhtiyarova, although the latter taxon presents broadly capitate apices. Despite being difficult to separate, a reevaluation of *A. macrocephalum* type material was recently published in the same study as *A. peetersianum* was firstly described (Wetzel *et al.* 2019). Using SEM micrographs, the authors demonstrated that *A. peetersianum* presented predominantly rounded areolae and had a higher density per stria, while *A. macrocephalum* stria is composed of 1–2 elongated areaole of different lengths. Moreover, the axial area of the rapheless valve of the latter taxon is expanded, widening towards the central area. Recently, Yu *et al.* (2019) described two small species that resemble ours: *A. subtilissimum* P.Yu, Q.-M.You & Q.-X.Wang in Yu *et al.* and *A. limosua* P.Yu, Q.-M.You &

Q.-X.Wang in Yu *et al.* The first species is likely a synonym of *A. macrocephalum*, and the second presents visibly larger valves (3.2–4.0 µm) than *A. peetersianum*.

*Achnanthidium indistinctum* Van de Vijver & Kopalová also bears several similarities with *A. peetersianum*. They can be distinguished in girdle view by the apparently thickened striae in the first taxon, which is not visible in ours (see Fig. 4O–P). Concerning the valve view, *A. indistinctum* presents rostrate apices and an almost nonexistent central area that never forms a fascia. On the other hand, *A. peetersianum* exhibits subcapitate to capitate apices and a small rounded central area that rarely forms a fascia. Notably, SEM analysis can also help separate both taxa due to the distal raphe ends straight in *A. indistinctum* and bent in our specimens. Despite the slightly narrower valves in the latter taxon (1.8–2.2 µm, Van de Vijver & Kopalová 2014), the metric features of these two species overlap.

*Achnanthidium reimperi* (Camburn) Ponader & Potapova has a similar valve outline with our population; however, it presents longer and wider valves and a rhomboid central area on the rapheless valve (Ponader & Potapova 2007).

### Distribution and ecological information

Recently described from Le Sauvigny River in France, it was one of the most well-distributed species found in this study (40% of occurrence). It is plausible that this taxon was previously identified in previous Brazilian publications as *A. macrocephalum*. In this study, *A. peetersianum* occurred in all of the sampled habitats. Its ecological preference was for slightly acidic (pH optimum of 6.6–6.7), electrolytic-poor (optimum of 28.2–49.1 µS cm<sup>-1</sup>) and oligo- to mesotrophic waters (TP optimum of 14.9–27.2 µg L<sup>-1</sup>, TN optimum of 374.4–519.9 µg L<sup>-1</sup>). It should be pointed out that we also observed this species in enriched waters (TP >50 µg L<sup>-1</sup>).

*Achnanthidium peruvianum* E.Morales & Ector in Morales *et al.* 2011

Fig. 5A–AB

Synonym: *Achnanthes kryophiloides* M.H.Hohn in Patrick *et al.* 1966, p. 468; pl. 1, fig. 5, 6

## Description

Valves linear-elliptic, with broadly rounded, slightly tapering apices; 8.0–20.2 µm long, 2.3–4.3 µm wide. Raphe valve: axial area narrow and linear; central area usually forming a rectangular or acute-angled fascia, reaching both margins. Raphe filiform straight, prolonged after the striae, ending on the border between the valve face and mantle. Proximal raphe endings close, externally straight and slightly drop-like expanded. Distal raphe endings externally straight and drop-like expanded. Transapical striae radiate throughout the entire valve; 28–30 in 10 µm. Striae mainly composed of 4–5 rounded areolae, sometimes elongated near the valve margin. Mantle with one row of slit-like areolae. Rapheless valve: axial area linear, very narrow; central area absent. Transapical striae slightly radiate throughout the entire valve, becoming more strongly radiate towards the apices, sometimes missing one stria in the central area; 28–34 in 10 µm. Striae mainly composed of 4–5 rounded areolae, sometimes elongated near the valve margin. Mantle with one row of slit-like areolae. Girdle view rectangularly arched to the raphe valve, showing the areolae on the mantle.

## Taxonomical remarks

The species presents similar morphological features to another South American species called *Achnanthidium sehuencoensis*, which has almost the same valve outline. However, SEM analysis separated the two due to the deflected distal raphe endings, the non-expanded proximal raphe endings and lower striae density (20–24/10 µm, Morales *et al.* 2009) compared to *A. peruvianum*.

*Achnanthidium petersenii* (Hustedt) C.E.Wetzel, Ector & Jüttner has wider fascia on the raphe valve. However, the rapheless valves in the axial and central areas are distinct. The axial ones are moderately broad, and the central area broadly rhombic-lanceolate (Jüttner *et al.* 2019). In *A. peruvianum*, the axial area is narrow and linear, while the central area is undifferentiated with short striae (Morales *et al.* 2011).

## Distribution and ecological information

*Achnanthidium peruvianum* was described from the Rondos River in Peru, South America (Hohn in Patrick *et al.* 1966). In our samples, the species was rare (abundance <3%), occurring in less than 5% of the counted samples in planktonic and surface sediment assemblages. In the surface sediment, it showed ecological preferences for neutral (pH optimum of 6.9), electrolyte-poor (optimum of 30.8 µS cm<sup>-1</sup>), and oligotrophic environmental water conditions (TP optimum of 18.4 µg L<sup>-1</sup> and TN optimum of 372.5 µg L<sup>-1</sup>).

***Achnanthidium tropicocatenatum*** Marquardt, C.E.Wetzel & Ector in Marquardt *et al.* 2017

Figs 6A–V, 7A–P

**Description**

Valves linear-lanceolate, slightly inflated in the central portion, with subcapitate to capitate apices; 6.7–18.1 µm long, 2.3–3.1 µm wide. Raphe valve: axial area narrow and linear; central area almost absent to small rounded, with 2–3 shortened striae on both sides of the valve. Raphe filiform straight, ending right after the last stria on the valve face. Proximal raphe endings close, externally straight, internally slightly bent in opposite directions. Distal raphe endings externally straight and slightly drop-like expanded, internally forming small helictoglossae. Transapical striae slightly radiate throughout the entire valve, becoming denser towards the apices; 39–42 in 10 µm (indiscernible in LM). Striae mainly composed of 4–6 rounded to elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae. Rapheless valve: axial area narrow and linear, widening towards the central area; central area small rounded, with 1–3 shortened striae on both sides of the valve. Transapical striae slightly radiate throughout the entire valve, becoming denser towards the apices; 36–40 in 10 µm (indiscernible in LM). Striae mainly composed of 4–6 rounded to elongated, sometimes being slit-like near the valve margins. Mantle with one row of elongated or slit-like areolae. Girdle view rectangularly arched, with pointed apices slightly curved towards the rapheless valve.

### Taxonomical remarks

*Achnanthidium tropicocatenatum* and *A. catenatum* are similar. In the protologue of the first species, many characters were taken into account to provide the differences (e.g., SEM, LM, geometric morphometry analysis, and ecological preferences) between them. Nevertheless, it is possible to distinguish both taxa by the less inflated central portion, fewer capitate apices and higher striae density in *A. tropicocatenatum* (28–30 striae/10 µm in *A. catenatum*, Bílý & Marvan 1959). Also, in *A. catenatum*, the girdle view is distinct, with the valve being strongly curved with the apices sharply pointed. In contrast, in *A. tropicocatenatum*, the valve is less arched with apices slightly curved (Marquardt *et al.* 2017).

*Achnanthidium* sp. nov. 6 also resembles *A. tropicocatenatum*. Both taxa differ by the slightly distinct valve outlines, striae densities and girdle views (see above: *Achnanthidium* sp. nov. 6).

Finally, Marquardt *et al.* (2017) commented about the confusion made with two different populations in Novais *et al.* (2015), probably joining two different species in one taxon (*A. lusitanicum* Novais & M.Morais in Novais *et al.*). The first population from Janeiro de Baixo, Zêzere River (Tejo basin, Portugal), is considered the type material of the species and differs from *A. tropicatenatum* by its rostrate apices, or broadly rounded ones in smaller specimens. The SEM analyses revealed that *A. tropicocatenatum* shows the distal raphe ends always straight, while *A. lusitanicum* from the type locality presents distal raphe ends unilaterally deflected in the images of the protologue.

### Distribution and ecological information

The taxon was recently described despite its presence in Brazilian literature being misidentified as *A. catenatum*. Indeed, in our samples, the species had the highest frequency of occurrence (61%). Its ecological optimum was calculated for all of the habitats. The species showed an ecological preference for slightly acid to neutral (pH optimum of 6.7–6.9), medium conductivity (optimum of 53.7–94.3 µS cm<sup>-1</sup>) and mesotrophic waters (TP optimum

of 19.8–37.3 µg L<sup>-1</sup> and TN optimum of 504.2–665.8 µg L<sup>-1</sup>). *Achnanthidium tropicocatenatum* was previously described from oligotrophic conditions in São Paulo state (Marquardt *et al.* 2017); however, it presented greater tolerance in our study area and was identified from oligo- to eutrophic conditions.

### ***Achnanthidium* sp. nov. 1**

Fig. 8A–X

Holotype, isotype and type locality will be designated.

#### **Description**

Valves narrowly linear-elliptic, with acutely rounded apices; 9.3–21.2 µm long, 2.2–3.2 µm wide. Raphe valve: axial area narrow and linear; central area rounded, sometimes forming a rectangular fascia, with 0–1 shortened striae on both sides of the valve. Raphe filiform straight, ending after the last stria. Proximal raphe endings close, internally slightly bent to opposite directions. Distal raphe endings externally straight, internally forming small helictoglossae. Transapical striae slightly radiate throughout the entire valve, more spaced in the central area, becoming denser and more strongly radiate towards the apices; 27–30 in 10 µm. Striae mainly composed of 4–5 rounded to elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae. Rapheless valve: axial area narrow and linear; central area absent, with 1–2 shortened stria on both sides of the valve. Transapical striae slightly radiate throughout the entire valve, more spaced in the central area, and becoming denser and more strongly radiate towards the apices; 28–30 in 10 µm. Striae mainly composed of 4–5 rounded to elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae.

#### **Taxonomical remarks**

Concerning the characteristic linear valve shape, *Achnanthidium lineare* W.Smith resembles *Achnanthidium* sp. nov. 1. However, *Achnanthidium lineare* differs from Brazilian

populations mostly by the always-rectangular fascia in the central area of the raphe valve and broadly rounded apices. Van de Vijver *et al.* (2011) analyzed *A. lineare* type material and described two new species (*A. pseudolineare* Van de Vijver, Novais & Ector in Van de Vijver *et al.* and *A. sublineare* Van de Vijver, Jarlman & Ector in Van de Vijver *et al.*). *Achnanthidium sublineare* possess the same central area formed by a rectangular fascia in the raphe valve, a wider and lanceolate axial area in the rapheless valve, 1–2 areolae per striae and narrower valves (1.5–2.1 µm wide).

*Achnanthidium petuniabuktianum* Pinseel, Van de Vijver & Kopalová also presents linear valves with rounded apices, but in SEM images, it shows a distinct wide axial area and striae with just 1–2 areolae, differing from *Achnanthidium* sp. nov. 1. Also, the distal raphe endings are bent to the same side in *A. petuniabuktianum* while straight in our species. *Achnanthidium ovatum* Tosh.Watanabe & Tuji (Watanabe *et al.* 2008) also have a similar valve outline and striation pattern; however, this species is heteropolar, differing from *Achnanthidium* sp. nov. 1. In Brazilian waters, *A. minutissimum* can co-occur with *Achnanthidium* sp. nov. 1 but can be distinguished by its lanceolate valves with rostrate apices and the linear valves with acutely rounded apices of *Achnanthidium* sp. nov. 1.

#### Distribution and ecological information

*Achnanthidium* sp. nov. 1 was found in all three habitats (i.e., periphyton, sediment and plankton) and present in 23% of the counted samples. Due to its wide distribution, the ecological optimum could be calculated: the ecological preference of the species was for neutral pH (optimum of 6.8–7.1), medium to high conductivity (optimum of 76.2–167.4 µS cm<sup>-1</sup>), and meso- to eutrophic waters (TP optimum of 38.9–78.0 µg L<sup>-1</sup> and TN optimum of 639.3–1632.0 µg L<sup>-1</sup>).

#### *Achnanthidium* sp. nov. 2

Figs 9A–AA, 10A–AB

Holotype, isotype and type locality will be designated.

### Description

Valves rhombic-lanceolate to linear-lanceolate with rounded, subrostrate to rostrate apices; 6.5–16.5 µm long, 2.6–3.4 µm wide. Raphe valve: axial area narrow and linear; central area small rounded, with 1–4 shortened striae on both sides of the valve. Raphe filiform ending right after the last stria. Proximal raphe endings close, externally straight, internally slightly bent to opposite directions. Distal raphe endings externally straight, internally forming small helictoglossae. Transapical striae radiate throughout the entire valve, more spaced in the central area; 30–34 in 10 µm. Striae mainly composed of 5–6 rounded to elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae. Rapheless valve: axial area narrow and linear, slightly widening towards the central area; central area absent to small rounded, with 1–3 shortened striae on both sides of the valve. Transapical striae radiate throughout the entire valve; 32–34 in 10 µm. Striae mainly composed of 5–6 rounded to elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae or elongated.

### Taxonomical remarks

*Achnanthidium* sp. nov. 2 share morphological similarities with *A. eutrophilum* (Lange-Bertalot) Lange-Bertalot, mainly due to its rhombic-lanceolate valve and striation pattern.

Despite this, our species has slender valves narrower than the latter taxon and presents higher striae density (*A. eutrophilum*: 3.2–5.8 µm wide, striae 25–30/10 µm, Hlúbková *et al.* 2011).

*Achnanthidium ennediense* (Compère) Compère & Van de Vijver has similar valve outline and width; however, it presents longer valves than ours (15.5–26.5 µm, Compère & Van de Vijver 2011) and a clearly rounded central area in the raphe valve. Additionally, *A. eutrophilum* and *A. ennediense* can be distinguished from *Achnanthidium* sp. nov. 2 by their broadly rounded apices.

*Achnanthidium caravelense* Novais & Ector in Novais *et al.* is similar to *Achnanthidium* sp. nov. 2 when analyzed by LM and SEM. However, it differs from *Achnanthidium* sp. nov. 2 mainly by the elliptic raphe valve with almost linear margins. The SEM images show that *A. caravelense* presents a lower number of areolae per striae (2–4, Novais *et al.* 2011). The valve outline of *Achnanthidium palmeti* Gassiole, Le Cohu & M.Coste is very similar to *Achnanthidium* sp. nov. 2, and the cell metrics overlap. However, *A. palmeti* specimens have wider valves (2.7–4.6 µm, Gassiole *et al.* 2013). The main character differing both species is the curvature of the raphe ending and the strong apical curvature on the valve face of *A. palmeti*. Indeed, *Achnanthidium* sp. nov. 2 has straight raphe endings and an almost flat valve face.

A similar Brazilian population was cited as *A. jackii* Rabenhorst by Bartozek *et al.* (2018). However, the cited species had linear-lanceolate valves with broadly rounded and rostrate apices, a central area always forming fascia and lower striae density (28–30/10 µm, Van de Vijver *et al.* 2018). Notably, the central area of *Achnanthidium* sp. nov. 2 never produces a fascia, being often absent or small rounded with some shortened striae.

#### Distribution and ecological information

The taxon was found in all three habitats (occurrence of 10%), but it was more often found in surface sediments from reservoirs. In the sediment assemblages, the ecological preferences of the taxa were for slightly alkaline (pH optimum of 7.3), medium conductivity (optimum of 100.3 µS cm<sup>-1</sup>) and eutrophic waters (TP optimum of 56.7 µg L<sup>-1</sup> and TN optimum of 1016.5 µg L<sup>-1</sup>). It is worth mentioning that it also occurred in oligo- and mesotrophic waters.

#### *Achnanthidium* sp. nov. 3

Fig. 11A–W

Holotype, isotype and type locality will be designated.

#### Description

Valves small linear-lanceolate, with rostrate to subcapitate apices; 6.0–15.3 µm long, 2.5–3.2 µm wide. Raphe valve: axial area narrow and linear; central area absent to small rounded, with 1–2 shortened striae on both sides of the valve. Raphe filiform straight, ending after the last stria, on the border of the valve face. Proximal and distal raphe endings externally straight and very slightly expanded. Transapical striae slightly radiate throughout the entire valve, becoming slightly denser and more strongly radiate towards the apices, 26–30 in 10 µm. Striae mainly composed of four rounded or squared areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae. Rapheless valve: axial area narrow and linear, slightly widening towards the central area; central area absent or small rounded, with up to 2 shortened striae on both sides of the valve (it can also lack a stria). Transapical striae slightly radiate throughout the entire valve, becoming denser towards the apices, 30–32 in 10 µm. Striae mainly composed of 4–5 rounded areolae, elongated or slit-like near the valve margin. Mantle with one row of slit-like areolae.

### **Taxonomical remarks**

The species can be confused with smaller specimens of *A. tropicocatenatum*; however, they differ by the subcapitate to capitate apices and higher striae density (38–42/10 µm) in *A. tropicocatenatum*. *Achnanthidium peetersianum* presents small capitate apices and has narrower valves (2.0–2.7 µm) than *Achnanthidium* sp. nov. 3. Despite displaying different raphid valves, the rapheless valves of *Achnanthidium* sp. nov. 8 present almost the same valve outline as *Achnanthidium* sp. nov. 3. However, the valves in *Achnanthidium* sp. nov. 8 are slightly wider and longer.

Considering metric features and the areolae ultrastructure, *Achnanthidium saprophilum* (H.Kobayasi & Mayama) Round & Bukhtiyarova resembles *Achnanthidium* sp. nov. 3. However, the central area of *A. saprophilum* species has a characteristic rhombic shape, and it is small rounded or almost absent in *Achnanthidium* sp. nov. 3. As shown in Fig. 11T, the areolae morphology of the valves from our population is variable, including apically

elongated areolae, as described for *A. saprophilum* (Kobayasi & Mayama 1982). Upon reexamining the type material of *A. saprophilum*, this feature was not observed and was considered a slight valve ornamentation deformity due to the heavily polluted sampling site (Hlúbková *et al.* 2011). It is also worth mentioning that the same pattern was observed in populations from a reservoir treated with algaecides (cf. Moschini-Carlos *et al.* 2010).

### **Distribution and ecological information**

The new species occurred in planktonic, periphytic and surface sediment assemblages at a low occurrence (<5%). In one surface sediment sample, it occurred with a high relative abundance (25%). The ecological preferences are characterized by neutral waters (pH 7.1), with relatively high conductivity  $225.6 \mu\text{S cm}^{-1}$ , and mesotrophic conditions (TP  $20 \mu\text{g L}^{-1}$ , TN  $699.9 \mu\text{g L}^{-1}$ ).

### ***Achnanthidium* sp. nov. 4**

Fig. 12A–Q

Holotype, isotype and type locality will be designated.

### **Description**

Valves linear-lanceolate, with subtle shoulders and subcapitate to small capitate apices; 18.2–22.7  $\mu\text{m}$  long, 2.7–3.0  $\mu\text{m}$  wide. Raphe valve: axial area narrow and linear; central area rounded, with 2–4 shortened striae on both sides of the valve, rarely forming a rectangular fascia. Raphe filiform straight, prolonged after the striae, ending on the border between the valve face and mantle. Proximal raphe endings close, externally straight, internally slightly bent in the opposite directions. Distal raphe endings externally straight, internally forming small helictoglossae. Transapical striae slightly radiate throughout the entire valve, more spaced in the central area, and becoming denser and more strongly radiate towards the apices; 29–33 in 10  $\mu\text{m}$ . Striae mainly composed of 3–4 rounded to elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae. Rapheless valve: axial

area narrow and linear, widening towards the central area; central area absent or narrow lanceolate, with 1–2 shortened striae on both sides of the valve, sometimes with the presence of two small depressions rounded or dash-like, spaced and apically arranged. Transapical striae slightly radiate throughout the entire valve, more spaced in the central area, and becoming denser towards the apices; 28–32 in 10 µm. Striae mainly composed of four rounded to elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae. Girdle view rectangularly arched, with pointed apices recurved to the rapheless valve.

### **Taxonomical remarks**

*Achnanthidium caledonicum* (Lange-Bertalot) Lange-Bertalot can be confused with *Achnanthidium* sp. nov. 4. The main difference between these two species is the broadly capitate apices in *A. caledonicum*, while the Brazilian species present small capitate to subcapitate specimens. Another difference is that the valves of *A. caledonicum* are linear with parallel margins, while the one in our population is linear-lanceolate and narrower. Previously, one Austrian specimen, in a mixed population, closely resembling *Achnanthidium* sp. nov. 4 was illustrated in Lange-Bertalot & Krammer (1989, pl. 55, fig. 4) and identified as *Achnanthes minutissima* var. *scotica* (J.R.Carter) Lange-Bertalot (currently considered a taxonomic synonym of *Achnanthidium caledonicum*). Furthermore, similar German specimens were also identified as *Achnanthidium* cf. *caledonicum* (pl. 24, figs 58–60 in Lange-Bertalot *et al.* 2017).

*Achnanthidium sieminskae* Witkowski, Kulikovskiy & Riaux-Gobin described from marine environments of the Kerguelen Archipelago, Austral Islands (Witkowski *et al.* 2012) resemble our population. However, it is rare for the central area to form a rectangular fascia in the Brazilian specimens. The presence of two point-like depressions in the central portion of the axial area is also not observed in *A. sieminskae*.

*Achnanthidium neomicrocephalum* Lange-Bertalot & F.Staab bears some similarities with *Achnanthidium* sp. nov. 4, such as linear-lanceolate valves with capitate to subcapitate apices. Its slender valve shape with longer, narrower valves (22–38 µm long, 2.5–2.8 µm wide, Krammer & Lange-Bertalot 2004) allows their separation, even though there are some overlaps in size ranges reported (21.6–25.3 µm long, 2.5–2.8 µm wide, by Silva-Lehmkuhl *et al.* 2019). Another distinction between the taxa is the subtle shoulders observed in the Brazilian populations not present in *A. neomicrocephalum*.

Recently described from China, *Achnanthidium longissimum* P.Yu, Q.-M.You & Kocielek was compared in its protologue to *A. caledonicum* and *A. neomicrocephalum* (Yu *et al.* 2018). Despite the similar valve outline to *Achnanthidium* sp. nov. 4, *A. longissimum* has capitate apices, and the raphe terminal fissures are strongly bent towards the same side of the valve. This feature is clearly visible in the LM images. *A. longissimum* also has larger valves and a reduced striae density (36–48 µm long, 4.0–4.5 µm wide, 22–25 striae in 10 µm, Yu *et al.* 2018) when compared to the new Brazilian taxon.

Another similar species to *Achnanthidium* sp. nov. 4 is *Achnanthidium digitatum* Pinseel, Vanormelingen, P.B.Hamilton & Van de Vijver. However, *A. digitatum* has smaller, narrower valves and a higher striae density (8.6–19.1 long, 1.8–2.3 wide, 31–36 striae in 10 µm, Pinseel *et al.* 2017). Additionally, the valves of *A. digitatum* are more linear, with margins almost parallel, and the striae are mostly composed of two areolae, an observation that is different from the population described here.

In our study area, *Achnanthidium* sp. nov. 4 co-occurred with two taxa that are sometimes difficult to separate using LM: *A. minutissimum* and *A. tropicocatenatum*. In general, smaller specimens are difficult to discern; however, *Achnanthidium* sp. nov. 4 is usually slender and has less lanceolate valve margins than *A. minutissimum*. They also have slightly distinct apices, while the latter taxon presents more rostrate than subcapitate apices. Concerning

*Achnanthidium tropicocatenatum*, it is smaller and presents visibly inflated valves in the median portion.

### Distribution and ecological information

*Achnanthidium* sp. nov. 4 was distributed in all the habitats, occurring in 20% of the counted samples. In all the communities, the species showed ecological preferences for slightly acid (pH optimum of 6.6–6.8), medium conductivity (optimum of 56–74  $\mu\text{S cm}^{-1}$ ), and oligo- to mesotrophic water conditions (TP optimum of 14.1–22.5  $\mu\text{g L}^{-1}$  and TN optimum of 321.6–468.2  $\mu\text{g L}^{-1}$ ).

### *Achnanthidium* sp. nov. 5

Fig. 13A-AA

Holotype, isotype and type locality will be designated.

### Description

Valves linear to narrowly lanceolate, with subrostrate to rostrate apices; 8.4–15.0  $\mu\text{m}$  long, 2.1–2.6  $\mu\text{m}$  wide. Raphe valve: axial area narrow and linear; central area small rounded, almost absent, with 1–3 shortened striae on both sides of the valve, and never forming a fascia. Raphe filiform straight, ending right after the last stria. Proximal raphe endings close, externally straight and slightly expanded, internally slightly bent in opposite directions. Distal raphe endings externally deflected to the same side, internally forming small helictoglossae. Transapical striae slightly radiate throughout the entire valve, becoming denser and more strongly radiate towards the apices, 30–31 in 10  $\mu\text{m}$ . Striae mainly composed of 3–5 rounded to elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae. Rapheless valve: axial area narrow and linear, slightly widening towards the central area; central area very small rounded or absent, with 1–2 shortened striae on both sides of the valve. Transapical striae slightly radiate throughout the entire valve, becoming denser towards the apices, 30–32 in 10  $\mu\text{m}$ . Striae mainly composed of 4–5 rounded to elongated

areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae. Girdle view rectangularly arched, with apices recurved to the rapheless valve.

### **Taxonomical remarks**

*Achnanthidium* sp. nov. 5 is differentiated by a more linear or narrowly lanceolate valve shape, with subrostrate apices. In contrast, *A. minutissimum* has linear-lanceolate valves with protracted and rostrate apices. Considering our populations, *Achnanthidium* sp. nov. 5 presented a higher striae density (30–32/10 µm), despite our *A. minutissimum* presenting a lower striae density than the German type material (26–28 vs. 30–35/10 µm, Novais *et al.* 2015). Notably, the slightly deflected distal raphe endings are always present in *Achnanthidium* sp. nov. 5 valves, a feature that was not observed in the *A. minutissimum* specimens observed here. Still, the central area never forms a fascia and is almost absent in the new species.

Due to its linear shape, *Achnanthidium* sp. nov. 1 is morphologically close to this taxon, but *Achnanthidium* sp. nov. 5 can be narrowly lanceolate. These species also differ with respect to the apices, which are acutely rounded in the first and subrostrate in the latter. Nevertheless, the slightly deflected distal raphe endings and the higher striae density in *Achnanthidium* sp. nov. 5 can be used to separate both taxa.

### **Distribution and ecological information**

The taxon was rare and occurred just in one sample (epiphytic).

### ***Achnanthidium* sp. nov. 6**

Fig. 14A–AB

Holotype, isotype and type locality will be designated.

### **Description**

Valves linear-lanceolate, inflated central portion, with subcapitate to broadly capitate apices; 9.0–17.5 µm long, 2.6–3.4 µm wide. Raphe valve: axial area linear; central area rounded, with

1–3 shortened striae on both sides of the valve. Raphe filiform straight, ending before the last stria on the valve face. Proximal raphes endings close, externally straight, internally slightly bent in opposite directions. Distal raphe endings externally straight and internally forming small helictoglossae. Transapical striae radiate throughout the entire valve, becoming denser towards the apices; 30–35 in 10 µm (indiscernible in LM). Striae mainly composed of 4–6 rounded to elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae. Rapheless valve: axial area narrow and linear, widening towards the central area; central area rounded. Transapical striae slightly radiate throughout the entire valve, becoming denser towards the apices; 31–36 in 10 µm (indiscernible in LM). Striae mainly composed of 4–5 rounded to elongated areolae, sometimes slit-like near the valve margins. Mantle with one row of elongated or slit-like areolae. Girdle view rectangularly arched (C-shaped), with apices sharply pointed and curved to the rapheless valve.

### **Taxonomical remarks**

The distinction of the new species and the others belonging to the *A. catenatum* group (*A. catenatum* and *A. tropicocatenatum*) can be made by a set of features since many of them overlap. *Achnanthidium* sp. nov. 6 has broadly capitate apices and valves marked by central inflation, while *A. tropicocatenatum* has valves with subcapitate apices and subtle central inflation. Both central areas are rounded, but it is mostly larger in the first species. Nevertheless, the separation of them was mainly established by the striae density, which is lower in *Achnanthidium* sp. nov. 6 and the morphology of the girdle view, which can be arched. *Achnanthidium* sp. nov. 6 has valves forming a C-shaped girdle view with apices sharply pointed, and *A. tropicocatenatum* presents valves that are less curved only at the apices. Due to the valve curvature of the new species, changing the focus on the microscope is required to visualize both apices and the central portion. This particularity is also more apparent in *A. catenatum*. However, the latter taxon is mainly distinguished from ours by its metric parameters, such as slightly wider valves and lower striae density (3-3.5 wide, 28-30

striae in 10  $\mu\text{m}$ , Bílý & Marvan 1959). Consequently, the striae density range in *Achnanthidium* sp. nov. 6 is intermediate when compared to the other two.

### **Shape analysis**

The PCA analyses considering the morphology of the raphe valves (i.e., valve outline and central area) can segregate the three taxa (Fig. 19). For example, when considering both axes, *Achnanthidium catenatum* (lectotype) was differentiated from the other two by the shape, as evidenced by the clear gap between them. Populations of *A. tropicocatenatum* were also separated from *Achnanthidium* sp. nov. 6 by a morphological gap, though smaller.

Similarity tests (PERMANOVA) performed on the shape coordinates of the groups revealed statistically significant differences ( $p$  values:  $< 0.001$ ,  $< 0.01$ ,  $< 0.05$ ) between *A. tropicocatenatum* (FR - type locality) and *A. catenatum* type compared with *Achnanthidium* sp. nov. 6 from Garças Lake (SP469486) as shown in Table 4.

### **Distribution and ecological information**

The species was distributed in all the habitats (20% of occurrence), reaching high abundances in the samples (highest: 89.5%). The ecological preference of *Achnanthidium* sp. nov. 6 was for neutral to slightly alkaline (pH optimum of 7.0–7.4), high conductivity (optimum of 150.2–254.1  $\mu\text{S cm}^{-1}$ ) and eutrophic waters conditions (TP optimum of 69.0–92.0  $\mu\text{g L}^{-1}$ , TN optimum of 1448.3–1911.1  $\mu\text{g L}^{-1}$ ). Interestingly, this species exhibits excellent tolerance, also occurring in oligo to supereutrophic waters.

### ***Achnanthidium* sp. nov. 7**

Fig. 15A–U

Holotype, isotype and type locality will be designated.

### **Description**

Valves linear-elliptic, with non-protracted, broadly rounded apices; 5.3–10.3  $\mu\text{m}$  long, 2.5–2.9  $\mu\text{m}$  wide. Raphe valve: axial area narrow and linear; central area absent or small rounded,

sometimes with one shortened stria on both sides of the valve. Raphe filiform straight, prolonged, ending after the last stria. Proximal raphe endings close, externally straight and drop-like expanded, internally bent in opposite directions. Distal raphe endings externally straight, internally forming small helictoglossae. Transapical striae slightly radiate throughout the entire valve, more spaced in the central area; 28–30 in 10 µm. Striae mainly composed of 5–6 almost squared or elongated areolae. Mantle with one row of slit-like areolae. Rapheless valve: axial area narrow and linear, slightly widening towards the central area; central area absent or narrow lanceolate. Transapical striae slightly radiate throughout the entire valve, more spaced in the central area; 26–28 in 10 µm. Striae mainly composed of 4–5 rounded to almost squared areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae.

### Taxonomical remarks

*Achnanthidium* sp. nov. 7 bears a strong resemblance to *A. straubianum* (Lange-Bertalot) Lange-Bertalot but has been regarded as a separate species based on the differences of the areolae shape in the rapheless valve and mantle. The areolae of *A. straubianum* vary in shape, slit-like, narrowly linear, large rounded or rectangular, depending on the development stage of the valve (see isotype Figs 176–179, Hlúbiková *et al.* 2011). Furthermore, it can be slit-like or narrowly elliptic in the mantle. In *Achnanthidium* sp. nov. 7, the areolae are always slit-like in the mantle and predominantly squared in the valve face. Concerning the metric features, *A. straubianum* presented wider valves in the protologue (3.5–4 µm wide, Lange-Bertalot & Metzeltin 1996), compared to the isotype that has narrower valves (2.6–3.5 µm, Hlúbiková *et al.* 2011). However, both are slightly wider than the *Achnanthidium* sp. nov. 7 described herein.

Another taxon resembling *Achnanthidium* sp. nov. 7 is *A. nanum* (F.Meister) Novais & Jüttner, a species described from Switzerland (Meister 1935). *Achnanthidium nanum* presents a different valve outline in the illustrations of the protologue and type material reevaluated in

Novais *et al.* (2015). It also has linear-lanceolate valves differing from the linear-elliptic valves of *Achnanthidium* sp. nov. 7. Also, the species present different areolae shapes and the central area of our specimens is often absent while it is clearly visible in *A. nanum*.

Two species recently described from Korea (*Achnanthidium ovale* M.Miao & B.-H.Kim and *Achnanthidium cavitatum* M.Miao & B.-H.Kim) also can be compared to the Brazilian species. *Achnanthidium ovale* has a more elliptic valve outline, wider valves (3.8–4.1 µm) and a greater striae density (30–35 in 10 µm). The ultrastructural differences, such as the terminal raphe fissures strongly bent to the same side in the Korean taxon, are another notable features (Miao *et al.* 2020). *Achnanthidium cavitatum* is similar in that it has linear-elliptic valves; however, the main difference from *Achnanthidium* sp. nov. 7 is the wider axial area observed in the rapheless valve (Miao *et al.* 2020). *Achnanthidium cavitatum* also has slightly wider valves (3.0–3.5 µm, Miao *et al.* 2020) than our population.

Furthermore, *Achnanthidium rivulare* Potapova & Ponader and *A. crassum* (Hustedt) Ponader & Potapova differ from *Achnanthidium* sp. nov. 7 in their linear-lanceolate axial area in the raphe valve, unilaterally deflected distal raphe ends and wider valves (2.6–4.4 µm and 3–4.5 µm respectively, Potapova & Ponader 2004).

#### **Distribution and ecological information**

*Achnanthidium* sp. nov. 7 was found in 8% of the counted samples, mainly occurring in periphytic samples with an ecological preference for alkaline (pH optimum of 7.9), high conductivity (optimum of 182.7 µS cm<sup>-1</sup>) and mesotrophic water conditions (TP optimum of 41.1 µg L<sup>-1</sup> and TN optimum 1307.8 µg L<sup>-1</sup>). It also presented a wide tolerance and was found to occur in oligo- to eutrophic waters.

#### ***Achnanthidium* sp. nov. 8**

Figs 16A–AI, 17A–F

Holotype, isotype and type locality will be designated.

## Description

Valves 7.8–16.5  $\mu\text{m}$  long, 3.0–3.8  $\mu\text{m}$  wide. Raphe valve: valves linear-lanceolate to rhombic-lanceolate, with subrostrate to rostrate apices. Axial area narrow and linear; central area absent or small rounded. Raphe filiform straight, prolonged, ending after the last stria on the border between the valve face and mantle. Proximal raphe endings close, externally straight, internally slightly bent in opposite directions. Distal raphe endings externally deflected to the same side, internally forming small helictoglossae. Transapical striae slightly radiate throughout the entire valve, more spaced in the central area, and becoming denser and more strongly radiate towards the apices; 27–34 in 10  $\mu\text{m}$ . Striae mainly composed of 3–5 rounded to elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae. Rapheless valve: valves linear-lanceolate, with rostrate to subcapitate apices. Axial area narrow and linear, slightly widening towards the central area; central area absent to very small rounded. Transapical striae slightly radiate throughout the entire valve, becoming denser towards the apices; 30–32 in 10  $\mu\text{m}$ . Striae mainly composed of 4–5 rounded to elongated areolae, sometimes slit-like near the valve margin. Mantle with one row of slit-like areolae.

## Taxonomical remarks

The different valve outline between the raphe and rapheless valves make the taxon easily distinguishable from one another. The rhombic-lanceolate outline of the raphe valve resembles the taxon to *A. eutrophilum* (Lange-Bertalot) Lange-Bertalot. Besides the different rapheless valve outline, *A. eutrophilum* presents wider valves and slightly lower striae density (3.2–5.8  $\mu\text{m}$  wide, striae density 25–30/10  $\mu\text{m}$ , Hlúbková *et al.* 2011). Using SEM is possible to see other differences such as the prolonged distal raphe ends after the last stria and the almost never slit-like areolae shape. In contrast, in *Achnanthidium* sp. nov. 8, the distal raphe endings terminate right after the last stria, and the areolae are often slit-like near the margins of the valve.

From our study area, the rapheless valve of *Achnanthidium* sp. nov. 8 is similar to valves of *Achnanthidium* sp. nov. 2. The latter taxon differs mainly by the apices format in the raphe valve (see below). While the rapheless valve has overlapping metric features, they can be distinguished by the visibly rounded central area in *Achnanthidium* sp. nov. 2 and the absence or presence of very small rounded ones in *Achnanthidium* sp. nov. 8.

### **Distribution and ecological information**

*Achnanthidium* sp. nov. 8 was restricted to samples from Garças Lake, a eutrophic urban lake (Bicudo *et al.* 2007, 2020) in the metropolitan area of São Paulo. It occurred in plankton, but mainly in periphyton (highest abundance: 15%). The limnological conditions of the sampling site varied from acid to alkaline (pH 5.8–8.6), conductivity was high ( $307\text{--}378 \mu\text{S cm}^{-1}$ ), as the nutrients concentration (TP  $97\text{--}107.1 \mu\text{g L}^{-1}$ , TN  $1734.2\text{--}2757.9 \mu\text{g L}^{-1}$ ) of the environment.

### ***Achnanthidium* sp. 9**

Fig. 18A–K

### **Description**

Valves linear-elliptic to linear-lanceolate, with broadly rounded apices to subrostrate; 7.4–11.6  $\mu\text{m}$  long, 3.5–4.1  $\mu\text{m}$  wide. Raphe valve: axial area narrow and linear; central area small rounded, with 1–2 shortened striae on both sides of the valve. Raphe filiform straight. Transapical striae slightly radiate throughout the entire valve, becoming denser and strongly radiate towards the apices; 24–28 in 10  $\mu\text{m}$ . Rapheless valve: axial area narrow and linear, widening towards the central area; central area absent to lanceolate. Transapical striae radiate throughout the entire valve, becoming denser and strongly radiate towards the apices; 23–27 in 10  $\mu\text{m}$ .

### **Taxonomical remarks**

*Achnanthidium modestiformis* (Lange-Bertalot) Van de Vijver presents similar morphometric features (12–17 µm long, 3.5–4 µm wide, striae density 24–25 /10 µm, Bourrelly & Manguin 1954, as *Achnanthes modesta* Manguin). However, the Brazilian population has different apices with broadly rounded to rostrate, whereas it is more capitate in *A. modestiformis*. Moreover, *Achnanthidium* sp. 9 has shorter valves.

Another similar specimen cited in Brazilian literature is *Achnanthes acares* M.H.Hohn & Hellerman (Schneck *et al.* 2008). The illustrations presented by Hohn & Hellerman (1963) concerning this taxon demonstrates a different central area, elliptical in the raphe valve and coarse striae resembling *Planothidium*. Krammer & Lange-Bertalot (1991) illustrated the holotype of this species using LM; however, they only showed the raphe valves of the specimens, which probably do not correspond to a monoraphid taxon.

Additionally, another Brazilian population resembled ours, but it was not identified at the species level (Santos *et al.* 2011, figs 21–24: *Achnanthidium* sp. 9). More studies are necessary concerning the identity of this taxon.

### Distribution and ecological information

*Achnanthidium* sp. 9 was rare (occurrence of <5%), occurring in the three habitats in low abundance ( $\leq 0.6\%$ ). In the surface sediment, it occurred in 10% of the samples, showing ecological preferences for neutral pH (optimum of 7.2), medium conductivity (optimum of  $82.9 \mu\text{S cm}^{-1}$ ), and hypereutrophic environments (TP optimum of  $425.3 \mu\text{g L}^{-1}$  and TN optimum of  $8469.1 \mu\text{g L}^{-1}$ ). The taxon presented the highest range of tolerance, occurring from oligotrophic to hypereutrophic water conditions.

### Discussion

During the last thirty years, approximately sixteen *Achnanthidium* taxa have been cited in taxonomic studies from Brazilian environments (Bicudo *et al.* 1993; Moutinho *et al.* 2007; Fontana & Bicudo 2012; Marra *et al.* 2016; Costa *et al.* 2017; Marquardt *et al.* 2017, 2018;

Silva-Lehmkuhl *et al.* 2019). Over the past decade, this increase is probably due to the development of group knowledge and improvements in sampling, oxidation and microscopy techniques. Based on the literature's revision, it is plausible that five taxa correspond to *A. microcephalum*, *A. minutissimum*, *A. saprophilum*, *A. tropicocatenatum*, *Achnanthidium* sp. 9. Moreover, *A. biasolettianum* could not be evaluated, and *A. blancheanum*, *A. catenatum*, *A. caledonicum*, *A. eutrophilum*, *A. jackii*, *A. lineare*, *A. macrocephalum*, *Achnanthidium* cf. *macrocephalum*, *A. neomicrocephalum* and *Achnanthidium* sp. were probably misidentified. *Achnanthidium jackii*, *A. lineare*, *Achnanthidium* cf. *macrocephalum* and *Achnanthidium* sp. were recently found in samples from ten reservoirs in the state of São Paulo (Bartozek *et al.* 2018); however, after reevaluating some of the samples and taxonomic information available, they likely correspond to *Achnanthidium* sp. nov. 2, *Achnanthidium* sp. nov. 1, *A. peetersianum* and *A. minutissimum*, respectively. Furthermore, three taxa were observed in the South of Brazil (Paraná state): *A. caledonicum*, *A. eutrophilum* and *A. macrocephalum* (Marra *et al.* 2016), which appear to be conspecifics of *Achnanthidium* sp. nov. 4, *Achnanthidium* sp. nov. 2 and *A. peetersianum*. *Achnanthidium neomicrocephalum* was also found in the South (Silva-Lehmkuhl *et al.* 2019), but its valve outline is consistent with *Achnanthidium* sp. nov. 4. It is worth mentioning that all of the taxa were morphologically distinguished from each other according to the 'taxonomical remarks' presented in the results section. Notably, before the proposition of *A. tropicocatenatum*, populations with its morphology were called *A. catenatum* (e.g., Fontana & Bicudo 2012; Marra *et al.* 2016) or confused with *A. blancheanum* (Bicudo *et al.* 2009).

The most taxonomically difficult complex of *Achnanthidium* species found in the state of São Paulo includes the *A. minutissimum* group and, more specifically, the *A. catenatum-tropicocatenatum* species. These taxa were identified in the majority of the samples analyzed and presented large populations that allowed for better definition and separation of each one. However, the co-occurrence and morphologic similarities made it challenging to identify

these populations with confidence. *Achnanthidium tropicocatenatum*, which exhibits the most widespread morphology among the samples, was described from one of the samples studied here (Cachoeira do França reservoir). However, populations similar to *A. catenatum* were also found and are described here as a new species (*Achnanthidium* sp. nov. 6). Due to the challenge of visualizing the sometimes overlapping set of morphological features that distinguish these taxa, a landmark-based geometric morphometric analysis was employed to clarify the boundaries of the different populations found.

Apart from the *Achnanthidium* species, this methodology was previously performed with different groups of diatoms and successfully distinguished species based on their valve outlines (Potapova & Hamilton 2007; Fránková *et al.* 2009; Peng *et al.* 2014; Rusanov *et al.* 2018). The geometric morphometric analysis was also performed with the protologue of *A. tropicocatenatum* and demonstrated that this is a new species among populations from *A. catenatum* and *A. minutissimum* type materials (Marquardt *et al.* 2017).

In our study, the separation afforded by the shape analysis agrees with the morphological observations of two different species with similar morphologies occurring in the samples analyzed, supporting the description of *Achnanthidium* sp. nov. 6 as a new species. Notably, the increased use of molecular data with diatoms has become a powerful tool for supporting the differentiation of taxa (Pinseel *et al.* 2017) and could be utilized for investigating the *A. catenatum* group of species. Such an approach could also differentiate *A. minutissimum* and *A. microcephalum*, which are now separated based on the axial area of the rapheless valve (Novais *et al.* 2015) and a proportionally shorter length for *A. microcephalum*.

Overall, the identified taxa preferred slightly acid to neutral waters, medium electrolyte conductivity and mesotrophic water conditions. The exceptions to these conditions were *Achnanthidium* sp. nov. 6, *Achnanthidium* sp. nov. 7, *Achnanthidium* sp. nov. 2 and *Achnanthidium* sp. 9. *Achnanthidium* sp. nov. 6 preferred eutrophic environments with high conductivity. Additionally, *A. tropicocatenatum* was previously described as an

oligotraphentic species (Marquardt *et al.* 2017). However, considering the broad range of trophic conditions in our dataset, *A. tropicocatenatum* showed a preference for mesotrophic waters with medium electrolyte content.

Due to the constant changes and development of these organisms, the revision and taxonomic updates are of particular relevance. Herein, we observed 13 taxa in the studied locations distributed throughout the state of São Paulo, with two additional occurrences from literature being confirmed (*A. microcephalum* and *A. saprophilum*). Thus, a total of 15 *Achnanthidium* sensu stricto species are now known to occur in Brazil. While most of those identified so far are localized to the southern hemisphere, *A. minutissimum*, *Achnanthidium* sp. nov. 4, and *A. peetersianum* were found to occur in the northern hemisphere.

Another interesting fact is the complete absence of species in this region with strongly deflected raphe ends (i.e., *Achnanthidium pyrenaicum*, *A. rivulare*, etc.). This feature is often dominant in assemblages collected in the northern hemisphere and represents a biogeographic observation that needs to be further explored.

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Table 1. Sampling sites and its respective general information, including herbarium number of the samples collected and analyzed for each one. Legends: A – Acquased Project; B – Biota Project; Basins: 1 – Mantiqueira; 2 – Paraíba do Sul; 3 – Litoral Norte; 4 – Pardo; 5 – Piracicaba/Capivari/Jundiaí; 6 – Alto Tietê; 7 – Baixada Santista; 8 – Sapucaí/Grande; 9 – Mogi Guaçu; 10 – Médio Tietê/Sorocaba; 11 – Ribeira de Iguape e Litoral Sul; 12 – Baixo Pardo/Grande; 13 – Tietê/Jacaré; 14 – Alto Paranapanema; 15 – Turvo/Grande; 16 – Tietê/Batalha; 17 – Médio Paranapanema; 18 – São José dos Dourados; 19 – Baixo Tietê; 20 – Aguapeí; 21 – Peixe; 22 – Pontal do Paranapanema.

| <b>Project</b> | <b>Basin</b> | <b>Reservoir (A) or Municipality (B)</b> | <b>Site</b> | <b>Latitude (S)</b> | <b>Longitude (W)</b> | <b>Herbarium Number (SP)</b>           |
|----------------|--------------|--|-------------|---------------------|----------------------|--|
| A              | 5            | Atibainha                                | AT1         | 23°08'50"           | 46°18'49"            | 469253, 469275, 469297                 |
| A              | 5            | Atibainha                                | AT3         | 23°11'12"           | 46°22'51"            | 469255, 469277, 469299                 |
| A              | 5            | Cachoeira                                | CA2         | 23°00'37"           | 46°17'11"            | 469249, 469271, 469293                 |
| A              | 5            | Cachoeira                                | CA3         | 23°01'56"           | 46°17'20"            | 469250, 469272, 469294                 |
| A              | 5            | Jacareí                                  | JC6         | 22°58'33.37"        | 46°20'39.26"         | 428846, 428855, 428864                 |
| A              | 5            | Jacareí                                  | JC7         | 22°57'16.03"        | 46°18'52.24"         | 428847, 428856, 428865                 |
| A              | 5            | Jaguari                                  | JA1         | 22°54'57.18"        | 46°24'19.76"         | 428839, 428848, 428857                 |
| A              | 5            | Salto Grande                             | SG1         | 22°43'43"           | 47°13'56"            | 469263, 469285, 469307, 469372, 469381 |
| A              | 5            | Salto Grande                             | SG4         | 22°41'59"           | 47°16'33"            | 469266, 469288, 469310, 469375, 469384 |
| A              | 5            | Tatu                                     | TU1         | 22°38'42"           | 47°17'10"            | 469267, 469289, 469311, 469376, 469385 |
| A              | 6            | Billings - Rio Grande                    | RG2         | 23°45'59.46"        | 46°30'35.7"          | 401560, 401584, 427898                 |
| A              | 6            | Billings - Rio Grande                    | RG4         | 23°43'43.74"        | 46°26'31.62"         | 401562, 401574, 401586, 427900, 427907 |
| A              | 6            | Billings - Rio Pequeno                   | RP6         | 23°47'41.34"        | 46°28'21.66"         | 401564, 401576, 401588                 |
| A              | 6            | Billings - Rio Pequeno                   | RP7         | 23°47'1.62"         | 46°26'11.28"         | 401565, 401577, 401589, 427902, 427909 |
| A              | 6            | Billings - Corpo Central                 | CC9         | 23°45'40.92"        | 46°38'54.06"         | 401567, 401579, 401591, 427903, 427910 |
| A              | 6            | Billings - Taquacetuba                   | TQ10        | 23°48'44.1"         | 46°37'51.24"         | 401568, 401580, 401592, 427904, 427911 |
| A              | 6            | Cabuçu                                   | CB1         | 23°23'25.6"         | 46°31'42.8"          | 428921, 428936, 428938, 428939, 428942 |
| A              | 6            | Cabuçu                                   | CB3         | 23°24'0.6"          | 46°31'56.6"          | 428923, 428941                         |
| A              | 6            | Cachoeira da Graça                       | CG2         | 23°39'17.68"        | 46°57'57.75"         | 427584, 427591, 427598, 469558         |
| A              | 6            | Guarapiranga                             | GU1         | 23°46'29.76"        | 46°47'13.2"          | 469455, 469469, 428507                 |
| A              | 6            | Guarapiranga                             | GU2         | 23°45'17.7"         | 46°46'11.22"         | 469456, 469470, 428508                 |
| A              | 6            | Guarapiranga                             | GU6         | 23°45'0.72"         | 46°43'36.9"          | 469460, 469474, 428512                 |
| A              | 6            | Guarapiranga                             | GU7         | 23°43'38.82"        | 46°43'25.38"         | 469461, 469475, 428513                 |
| A              | 6            | Guarapiranga                             | GU8         | 23°42'58.14"        | 46°43'36.72"         | 469462, 469476, 428514                 |
| A              | 6            | Guarapiranga                             | GU12        | 23°41'53.1"         | 46°44'40.38"         | 469466, 469480, 428518                 |
| A              | 6            | Jundiaí                                  | JU1         | 23°38'50.2"         | 46°09'48.1"          | 427988, 427989, 427996, 468830, 468849 |
| A              | 6            | Jundiaí                                  | JU2         | 23°39'07.2"         | 46°11'34.3"          | 427997, 468831, 468850                 |
| A              | 6            | Lago das Garças                          | LG1         | 23°38'44.4"         | 46°37'29.9"          | 469483, 469484, 469485, 469486         |

|   |    |                      |      |              |              |   |
|---|----|----------------------|------|--------------|--------------|---|
| A | 6  | Ninféias             | NI1  | 23°38'19.9"  | 46°37'20.3"  | 469515, 469516, 469517,<br>469518, 469519 |
| A | 6  | Paiva Castro         | PC1  | 23°19'39"    | 46°36'42"    | 469259, 469281, 469303,<br>469369, 469379 |
| A | 6  | Paiva Castro         | PC4  | 23°19'49"    | 46°40'37"    | 469262, 469284, 469306,<br>469551, 469552 |
| A | 6  | Paraitinga           | PA1  | 23°31'22.0"  | 45°54'17.8"  | 427984, 427985, 427992,<br>427994, 468847 |
| A | 6  | Pedro Beicht         | PB3  | 23°42'58.07" | 46°57'46.16" | 427580, 427587, 427594                    |
| A | 6  | Pedro Beicht         | PB4  | 23°43'44.06" | 46°58'5.10"  | 427581, 427588, 427595,<br>469559         |
| A | 6  | Pedro Beicht         | PB5  | 23°42'55.07" | 46°58'19.28" | 427582, 427589, 427596,<br>469560         |
| A | 6  | Ponte Nova           | PN2  | 23°36'04.3"  | 45°55'44.04" | 427923, 427926, 427983,<br>468845         |
| A | 6  | Rasgão               | RA2  | 23°23'28.59" | 47°01'19.03" | 427990, 427991, 468860                    |
| A | 6  | Ribeirão do Campo    | RC1  | 23°39'31.8"  | 45°49'23.22" | 427916, 427919, 468841                    |
| A | 6  | Ribeirão do Campo    | RC3  | 23°38'15.2"  | 45°50'0.78"  | 427921, 427982, 468843                    |
| A | 6  | Taiaçupeba           | TA1  | 23°36'25.9"  | 46°15'59.5"  | 427986, 427987, 468833,<br>468835, 468857 |
| A | 6  | Taiaçupeba           | TA2  | 23°34'40.9"  | 46°16'50.3"  | 468858                                    |
| A | 6  | Tanque Grande        | TG3  | 23°22'29"    | 46°27'31"    | 428920, 428926, 428929,<br>428932, 428935 |
| A | 10 | Barra Bonita         | BB2  | 22°36' 42"   | 48°19'14.9"  | 469244, 469515, 469520,<br>469545, 469554 |
| A | 10 | Barra Bonita         | BB5  | 22°31'56.04" | 48°27'37.3"  | 469247, 469518, 469523,<br>469553, 469557 |
| A | 10 | Hedberg              | HB1  | 23°25'55.86" | 47°35'33.06" | 469240, 469508, 469511,<br>469534, 469539 |
| A | 10 | Ipaneminha           | IP3  | 23°32'34.62" | 47°31'8.88"  | 469239, 469504, 469507,<br>469530         |
| A | 10 | Itupararanga         | IT1  | 23°37'11.58" | 47°13'59.34" | 469232, 469492, 469497,<br>469526         |
| A | 10 | Itupararanga         | IT5  | 23°36'53.1"  | 47°23'34.7"  | 469236, 469496, 469501                    |
| A | 10 | Santa Helena         | SH2  | 23°34'58.56" | 47°25'50.52" | 469230, 469454, 469490,<br>469524, 469525 |
| A | 11 | Cachoeira da Fumaça  | FU2  | 24°00'17.1"  | 47°15'44.8"  | 469200, 469393, 469413                    |
| A | 11 | Cachoeira do França  | FR3  | 23°55'53.4"  | 47°10'34.4"  | 469197, 469390, 469410,<br>469430, 469443 |
| A | 11 | Jurupará             | JP1  | 23°56'00"    | 47°22'18.0"  | 469208, 469401, 469421,<br>469435, 469446 |
| A | 11 | Jurupará             | JP4  | 23°57'36.7"  | 47°23'43.6"  | 469211, 469404, 469424,<br>469438, 469449 |
| A | 11 | Salto do Iporanga    | SI3  | 24°05'54.5"  | 47°43'25.8"  | 469207, 469400, 469420                    |
| A | 11 | Serraria             | SE3  | 24°08'28.8"  | 47°32'32.3"  | 469204, 469397, 469417                    |
| A | 14 | Paineiras            | PI1  | 23°51'13.8"  | 47°36'54.8"  | 469212, 469405, 469425,<br>469439, 469450 |
| A | 14 | Paineiras            | PI3  | 23°50'34.8"  | 47°38'26.2"  | 469214, 469407, 469427,<br>469441, 469452 |
| B | 1  | Campos do Jordão     | 1–3  | -            | -            | 239041, 255736, 255737                    |
| B | 2  | Piquete              | 1    | 22°37'24.2"  | 45°09'40.1"  | 355362                                    |
| B | 2  | São José do Barreiro | 1    | -            | -            | 188322                                    |
| B | 2  | São José dos Campos  | 1    | -            | -            | 188210                                    |
| B | 3  | Ubatuba              | 1    | -            | -            | 188344                                    |
| B | 4  | Caconde              | 1, 2 | 21°34'39.9"  | 46°37'31.0"  | 355356, 355357                            |
| B | 5  | Bragança Paulista    | 1    | -            | -            | 188208                                    |
| B | 5  | Capivari             | 1, 2 | -            | -            | 239044, 255723                            |
| B | 5  | Piracicaba           | 1    | -            | -            | 239095                                    |
| B | 5  | Rio Claro            | 1, 2 | -            | -            | 188219, 255726                            |
| B | 5  | São Pedro            | 1, 2 | -            | -            | 188436, 255724                            |

|   |    |                         |      |             |             |  |
|---|----|-------------------------|------|-------------|-------------|--|
| B | 6  | Mogi das Cruzes         | 1    | -           | -           | 188211<br>255749, 294906, 294908,<br>294909, 427342, 427343,<br>427344 |
| B | 6  | São Paulo               | 1–7  | -           | -           | 188434<br>239096, 255761   |
| B | 7  | Itanhaém                | 1    | -           | -           | 188434   |
| B | 8  | Batatais                | 1, 2 | -           | -           | 255738   |
| B | 8  | Pedregulho              | 1    | -           | -           | 239038, 255739   |
| B | 8  | Guará                   | 1, 2 | -           | -           | 371175   |
| B | 8  | Rifaína                 | 1    | -           | -           | 164898   |
| B | 9  | Águas da Prata          | 1    | -           | -           | 188327<br>255732, 255733, 255734,<br>255735                            |
| B | 4  | Casa Branca             | 1    | -           | -           | 255732, 255733, 255734,<br>255735                                      |
| B | 9  | Mogi Guaçu              | 1–4  | -           | -           | 371178   |
| B | 13 | São Carlos              | 1, 2 | -           | -           | 188212, 255727   |
| B | 10 | Itu                     | 1, 2 | -           | -           | 188435, 255725   |
| B | 10 | Piedade                 | 1, 2 | -           | -           | 255766, 255767   |
| B | 10 | Porangaba               | 1    | -           | -           | 188207   |
| B | 10 | Porto Feliz             | 1    | 23°12'46.9" | 47°30'3.6"  | 371182   |
| B | 10 | Sarapuí                 | 1    | 23°34'1.4"  | 47°52'55.4" | 371173   |
| B | 11 | Cananéia                | 1    | -           | -           | 130812   |
| B | 11 | Eldorado                | 1–3  | -           | -           | 239137, 239171, 255765   |
| B | 11 | Iporanga                | 1    | 24°35'40.5" | 48°37'12.4" | 355393   |
| B | 11 | Juquiá                  | 1    | 24°18'23.6" | 47°37'21.8" | 365692   |
| B | 11 | Miracatu                | 1    | -           | -           | 255763   |
| B | 11 | Pedro de Toledo         | 1    | 24°16'48.8" | 47°12'6.2"  | 371181   |
| B | 12 | Barretos                | 1    | -           | -           | 255772   |
| B | 12 | Colina                  | 1    | 20°45'41.1" | 48°30'3'.6" | 371174   |
| B | 12 | Viradouro               | 1    | 20°53'47.7" | 48°17'1.9"  | 371184   |
| B | 13 | Barra Bonita            | 1    | -           | -           | 255742   |
| B | 13 | Itajú                   | 1, 2 | -           | -           | 255771, 239143   |
| B | 13 | Itirapina               | 1    | -           | -           | 163994   |
| B | 13 | Jaú/Bariri              | 1, 2 | -           | -           | 239142, 255768   |
| B | 13 | Lençóis Paulista        | 1    | -           | -           | 239236   |
| B | 14 | Capão Bonito            | 1    | 23°53'37.3" | 48°15'21.3" | 365693   |
| B | 14 | Guareí                  | 1    | 23°19'54.8" | 48°11'11.8" | 371176   |
| B | 14 | Itaberá                 | 1    | 23°51'11.3" | 49°09'10.8" | 355392   |
| B | 14 | Itaí                    | 1, 2 | -           | -           | 239094, 255760   |
| B | 14 | Itapetininga            | 1–3  | -           | -           | 239042, 255740, 255741   |
| B | 14 | Itapeva                 | 1    | 23°58'28.0" | 48°55'02.5" | 371180   |
| B | 14 | Itaporanga              | 1, 2 | -           | -           | 355358, 355359   |
| B | 14 | Piraju                  | 1    | -           | -           | 239140   |
| B | 14 | Ribeirão Branco         | 1    | -           | -           | 239244   |
| B | 15 | Cosmorama               | 1, 2 | 20°30'18.4" | 49°46'14.4" | 355389, 355404   |
| B | 15 | Macedônia               | 1    | 20°08'19.5" | 50°11'56.4" | 355366   |
| B | 15 | Monte Alto              | 1    | -           | -           | 239233   |
| B | 18 | Monte Aprazível         | 1, 2 | 20°45'18.0" | 49°42'13.9" | 355374, 355375   |
| B | 15 | Olímpia                 | 1    | 20°44'18.3" | 48°51'7.0"  | 371177   |
| B | 15 | Santa Albertina         | 1    | 20°3'20.1"  | 50°46'01"   | 355385   |
| B | 15 | Uchoa                   | 1, 2 | -           | -           | 239043, 255729   |
| B | 15 | Urânia                  | 1    | -           | -           | 239237   |
| B | 16 | Matão                   | 1, 2 | -           | -           | 188433, 255722   |
| B | 16 | Reginópolis             | 1–3  | -           | -           | 239144, 255769, 255770   |
| B | 15 | Santa Adélia            | 1    | 21°11'48.8" | 48°47'59.4" | 371179   |
| B | 17 | Assis                   | 1, 2 | -           | -           | 239090, 255756   |
| B | 17 | Avaré                   | 1, 2 | -           | -           | 255759, 255773   |
| B | 17 | Paraguaçu Paulista      | 1    | -           | -           | 239085   |
| B | 17 | Santa Cruz do Rio Pardo | 1    | 22°45'24.8" | 49°29'07.7" | 355370   |
| B | 18 | General Salgado         | 1    | -           | -           | 239241   |
| B | 19 | Itapura                 | 1, 2 | 22°16'41.0" | 51°48'16.5" | 355388, 370964   |

|   |    |                            |      |             |             |                |
|---|----|----------------------------|------|-------------|-------------|----------------|
| B | 15 | Mirassol                   | 1    | -           | -           | 255728         |
| B | 19 | Andradina                  | 1    | -           | -           | 239234         |
| B | 19 | Araçatuba                  | 1    | -           | -           | 239239         |
| B | 19 | Santo Antônio do Arancangá | 1    | 20°50'30.2" | 50°27'14.4" | 355386         |
| B | 21 | Inubia Paulista            | 1    | -           | -           | 239091         |
| B | 21 | Marília                    | 1, 2 | -           | -           | 239086, 255753 |
| B | 20 | Nova Independência         | 1    | 21°03'32.0" | 51°26'30.1" | 370963         |
| B | 20 | Pacaembu                   | 1    | 21°32'50.2" | 51°18'25.1" | 370962         |
| B | 20 | Panorama                   | 1    | 21°21'35.1" | 51°52'08.7" | 371185         |
| B | 20 | Tupã                       | 1, 2 | -           | -           | 239088, 255755 |
| B | 20 | Dracena                    | 1    | -           | -           | 255758         |
| B | 20 | Parapuã                    | 1    | 21°56'31.5" | 50°56'24.7" | 371186         |
| B | 17 | Rancharia                  | 1, 2 | -           | -           | 239087, 255754 |
| B | 22 | Teodoro Sampaio            | 1    | -           | -           | 239136         |

Table 2. Ecological optima of *Achnanthidium* species with more than 10% of occurrence in each habitat. Cond.: conductivity ( $\mu\text{S cm}^{-1}$ ); TN: total nitrogen ( $\mu\text{g L}^{-1}$ ); TP: total phosphorus ( $\mu\text{g L}^{-1}$ ).

Table 3. Morphometric data comparing similar taxa with a linear-lanceolate valve outline belonging to the *A. minutissimum*-complex. ND: No data.

|                      | <i>Achnanthidium</i><br>sp. nov. 1 | <i>Achnanthidium</i><br>sp. nov. 3 | <i>Achnanthidium</i><br><i>minutissimum</i> | <i>Achnanthidium</i><br>sp. nov. 4      | <i>Achnanthidium</i><br>sp. nov. 5              | <i>Achnanthidium</i><br><i>peetersianum</i> | <i>Achnanthidium</i> sp.<br>nov. 6                     | <i>Achnanthidium</i><br><i>tropicocatenatum</i>         |
|----------------------|------------------------------------|------------------------------------|---|---|---|---|--|---|
| <b>Valve outline</b> | Narrowly linear-elliptic           | Small linear-lanceolate            | Linear-lanceolate                           | Linear-lanceolate                       | Linear to narrowly lanceolate                   | Small linear-elliptic                       | Linear-lanceolate, inflated central portion            | Linear-lanceolate, slightly inflated central portion    |
| <b>Valve apices</b>  | Acutely rounded                    | Rostrate to subcapitate            | Protracted, rostrate to subcapitate         | Subcapitate to small capitate           | Subrostrate to rostrate                         | Subcapitate to small capitate               | Subcapitate to broadly capitate                        | Subcapitate to capitate                                 |
| <b>Length (µm)</b>   | 9.3–21.2                           | 6.0–15.3                           | 12.3–17.7                                   | 18.2–22.7                               | 8.4–15.0  | 7.6.4–12.3                                  | 9.0–17.5   | 6.7–18.1  |
| <b>Width (µm)</b>    | 2.2–3.2                            | 2.5–3.2                            | 2.5–2.9                                     | 2.7–3.0                                 | 2.1–2.6   | 2.0–2.7                                     | 2.6–3.4  | 2.3–3.1   |
| <b>Girdle view</b>   | ND                                 | ND                                 | ND  | Rectangular arched, with pointed apices | Rectangular arched, with apices slightly curved | ND  | Rectangular arched, (C-shaped), apices sharply pointed | Rectangular arched, with pointed apices slightly curved |

|                                       |   |   |  |  |   |  |  |  |
|---------------------------------------|---|---|--|--|---|--|--|--|
| <b>Axial area</b>                     | Narrow and linear   | Narrow and linear   | Linear, very narrow  | Narrow and linear  | Narrow and linear   | Narrow and linear  | Linear   | Narrow and linear  |
| <b>Central area</b>                   | Rounded, sometimes forming fascia                               | Absent to very small rounded  | Small rounded, almost absent, sometimes forming fascia     | Rounded, rarely forming fascia                                   | Small rounded, almost absent  | Absent or small rounded, rarely forming fascia                               | Rounded  | Almost absent to small rounded                                   |
| <b>Raphe distal ends (externally)</b> | Straight  | Straight and slightly expanded  | Straight and drop-like expanded                            | Straight   | Bent to the same side   | Bent to the same side and slightly expanded                                  | Straight   | Straight and slightly drop-like expanded                         |
| <b>Striae density (in 10 µm)</b>      | 27–30   | 26–30   | 26–29  | 29–33  | 30–31   | 29–30  | 30–35  | 39–42  |
| <b>Areolae opening</b>                | Rounded to elongated, sometimes being slit-like near the margin | Rounded to almost square or elongated, sometimes being slit-like near margins | Rounded to elongated, sometimes slit-like near the margins | Rounded to elongated, sometimes being slit-like near the margins | Rounded to elongated, sometimes being slit-like near the valve margin | Rounded, almost squared or elongated, sometimes being slit-like near margins | Rounded to elongated, sometimes being slit-like near the margins | Rounded to elongated, sometimes being slit-like near the margins |

**Areolae number  
(at the center)**

4–5

4

3–4

3–4

3–5

3–4

4–6

4–6

**Rapheless valve**

|                                  |   |   |   |  |   |  |  |
|----------------------------------|---|---|---|--|---|--|--|
| <b>Axial area</b>                | Narrow and linear   | Narrow and linear, slightly widening towards the central area | Narrow and linear, slightly widening towards the central area | Narrow and linear, widening towards the central area     | Narrow and linear, slightly widening towards the central area | Narrow and linear, slightly widening towards the central area            | Narrow and linear, widening towards the central area     |
|                                  |   |   |   |  | Very small  |  |  |
| <b>Central area</b>              | Absent  | Absent or small rounded                                       | Absent  | Absent   | rounded or absent   | Absent   | Absent   |
| <b>Striae density (in 10 µm)</b> | 28–30   | 30–32   | 26–28   | 28–32  | 30–32   | 32–34  | 31–36  |
| <b>Areolae opening</b>           | Rounded to elongated, sometimes being slit-like near the slit-like near the | Rounded, being elongated or slit-like near the margins        | Rounded to elongated  | Rounded to elongated, sometimes being slit-like near the | Rounded to elongated, sometimes being slit-like near the      | Rounded, almost squared to elongated, sometimes being slit-like near the | Rounded to elongated, sometimes being slit-like near the |

|   |         |         |                           |         |         |   |     |     |
|---|---------|---------|---------------------------|---------|---------|---|-----|-----|
| margins                                   | margins | margins | slit-like near<br>margins | margins | margins |   |     |     |
| <hr/>                                     |         |         |                           |         |         |   |     |     |
| <b>Areolae number<br/>(at the center)</b> | 4–5     | 4–5     | 4                         | 4       | 4–5     | 4 | 4–5 | 4–6 |

Table 4. PERMANOVA results from similarity tests between groups.

| <b>Pairs</b>   | <b>F.Model</b> | <b>R2</b> | <b>p.adj</b> |
|--|----------------|-----------|--------------|
| <i>A. catenatum</i> vs <i>A. tropicocatenatum</i> (FR)             | 9.361          | 0.232     | 0.012        |
| <i>A. catenatum</i> vs <i>Achnanthidium</i> sp. nov. 6             | 38.831         | 0.599     | 0.006        |
| <i>A. catenatum</i> vs <i>A. tropicocatenatum</i> (SH)             | 11.570         | 0.335     | 0.024        |
| <i>A. tropicocatenatum</i> (FR) vs <i>Achnanthidium</i> sp. nov. 6 | 12.559         | 0.334     | 0.018        |
| <i>A. tropicocatenatum</i> (FR) vs <i>A. tropicocatenatum</i> (SH) | 1.903          | 0.080     | 1.000        |
| <i>Achnanthidium</i> sp. nov. 6 vs <i>A. tropicocatenatum</i> (SH) | 1.312          | 0.072     | 1.000        |

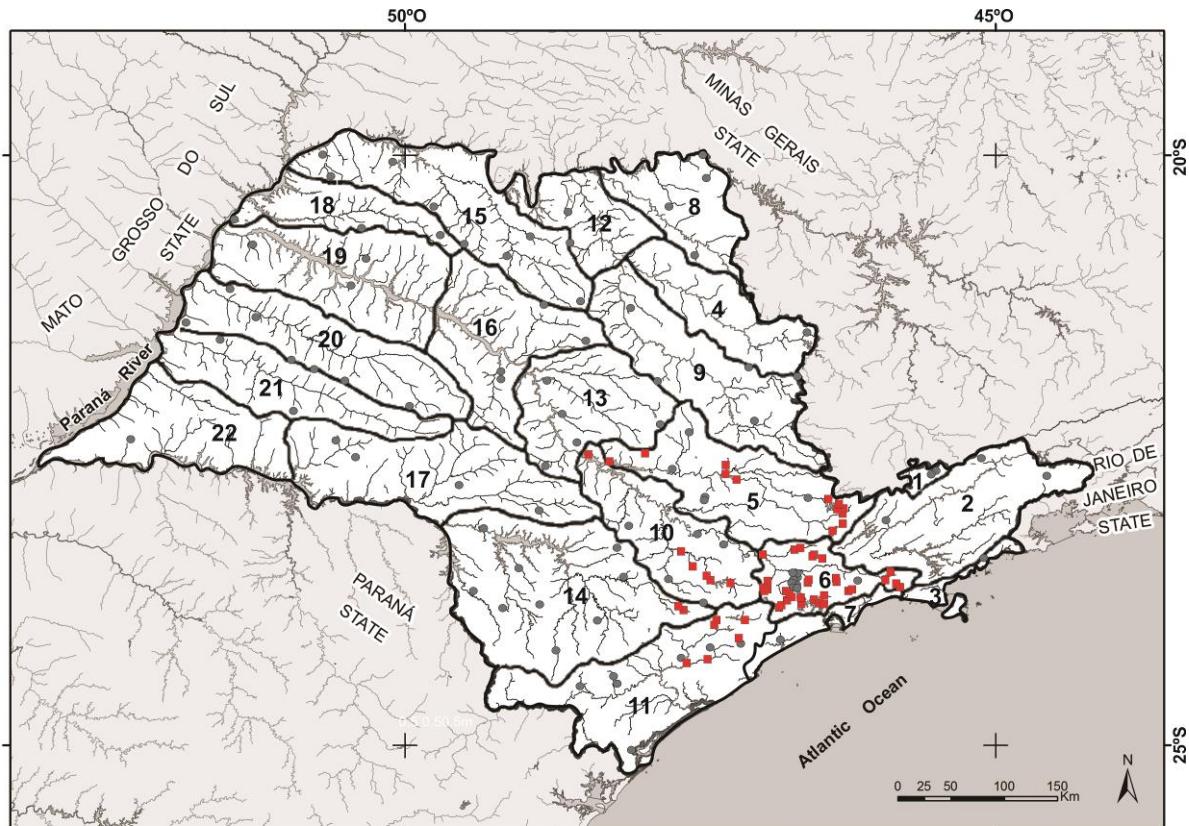


Figure 1. Sampling sites distributed in 22 basins in the state of São Paulo, Brazil. Red squares: Acquased project sampling sites. Grey circles: Biota project sampling sites.

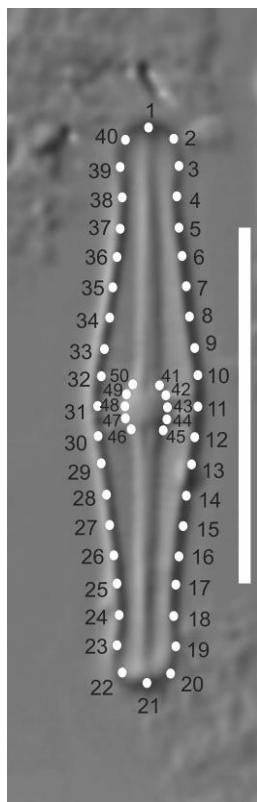


Figure 2. Landmarks used for GPA analysis arranged on the valve outline and central area of the raphe valve.

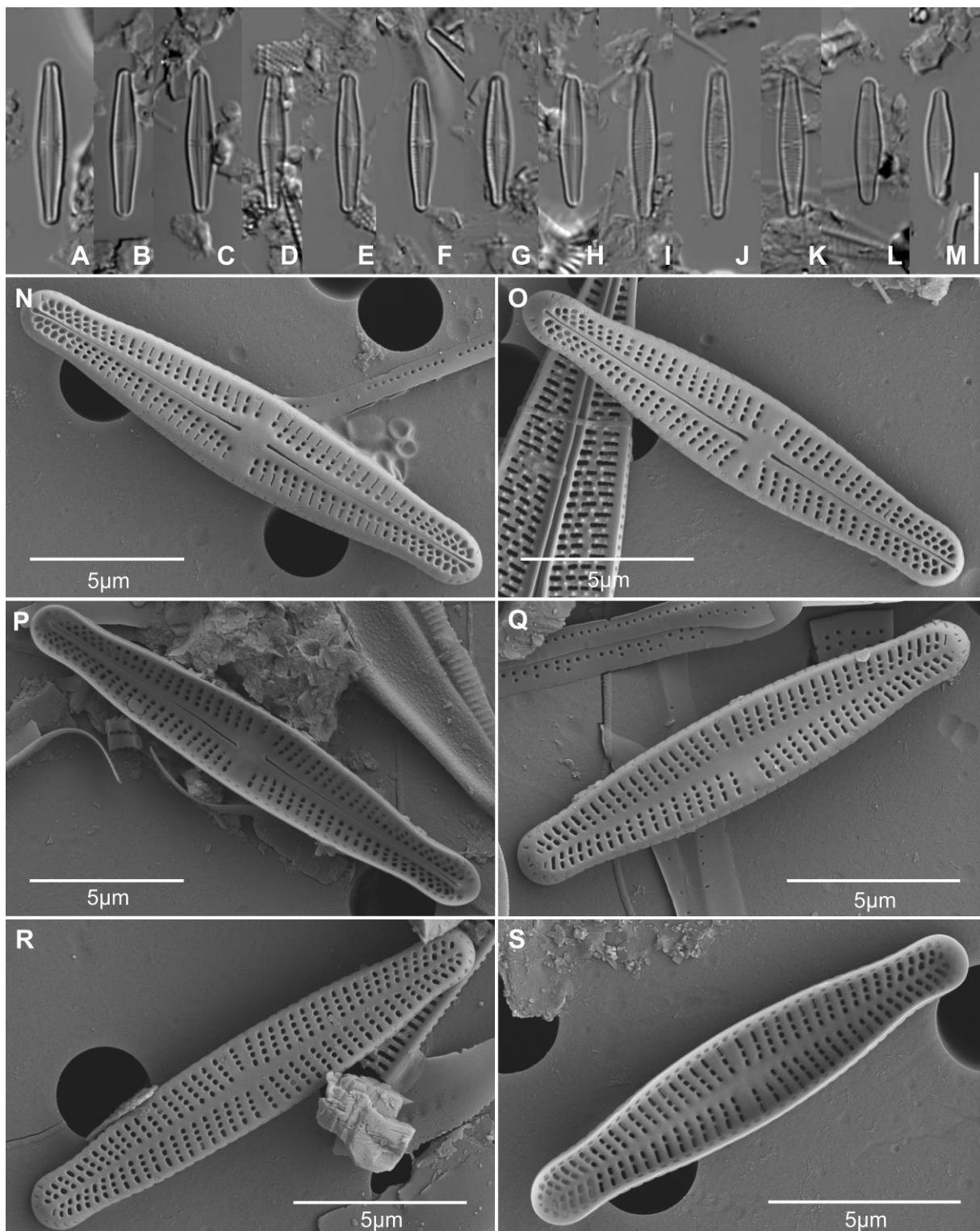


Figure 3 (A–S). *Achnanthidium minutissimum* (Kützing) Czarnecki from Tanque Grande reservoir (SP428935). (A–H). LM views of raphe valves. (I–M). LM views of rapheless valves. (N, O). SEM external views of raphe valves. (P). SEM internal view of the raphe valve. (Q, R). SEM external views of rapheless valves. (S). SEM internal view of the rapheless valve. LM scale 10  $\mu\text{m}$ .

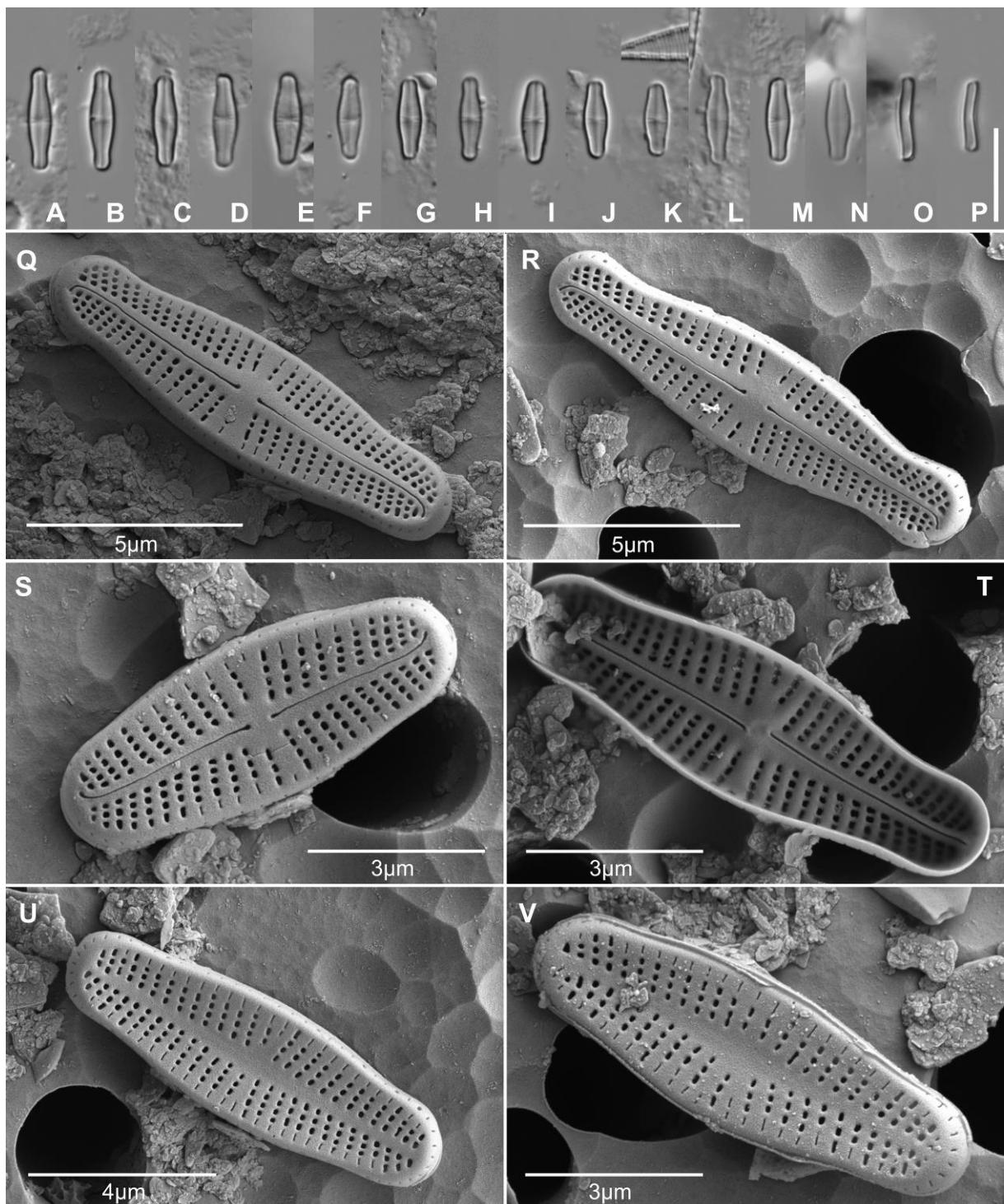


Figure 4 (A–V). *Achnanthidium peetersianum* C.E.Wetzel, Jüttner & Ector in Wetzel *et al.* (SP371175). (A–K). LM views of raphe valves. (L–N). LM views of rapheless valves. (O, P). LM views of girdle views. (Q–S). SEM external views of raphe valves. (T). SEM internal view of the raphe valve. (U, V). SEM external views of rapheless valves. LM scale 10 µm.

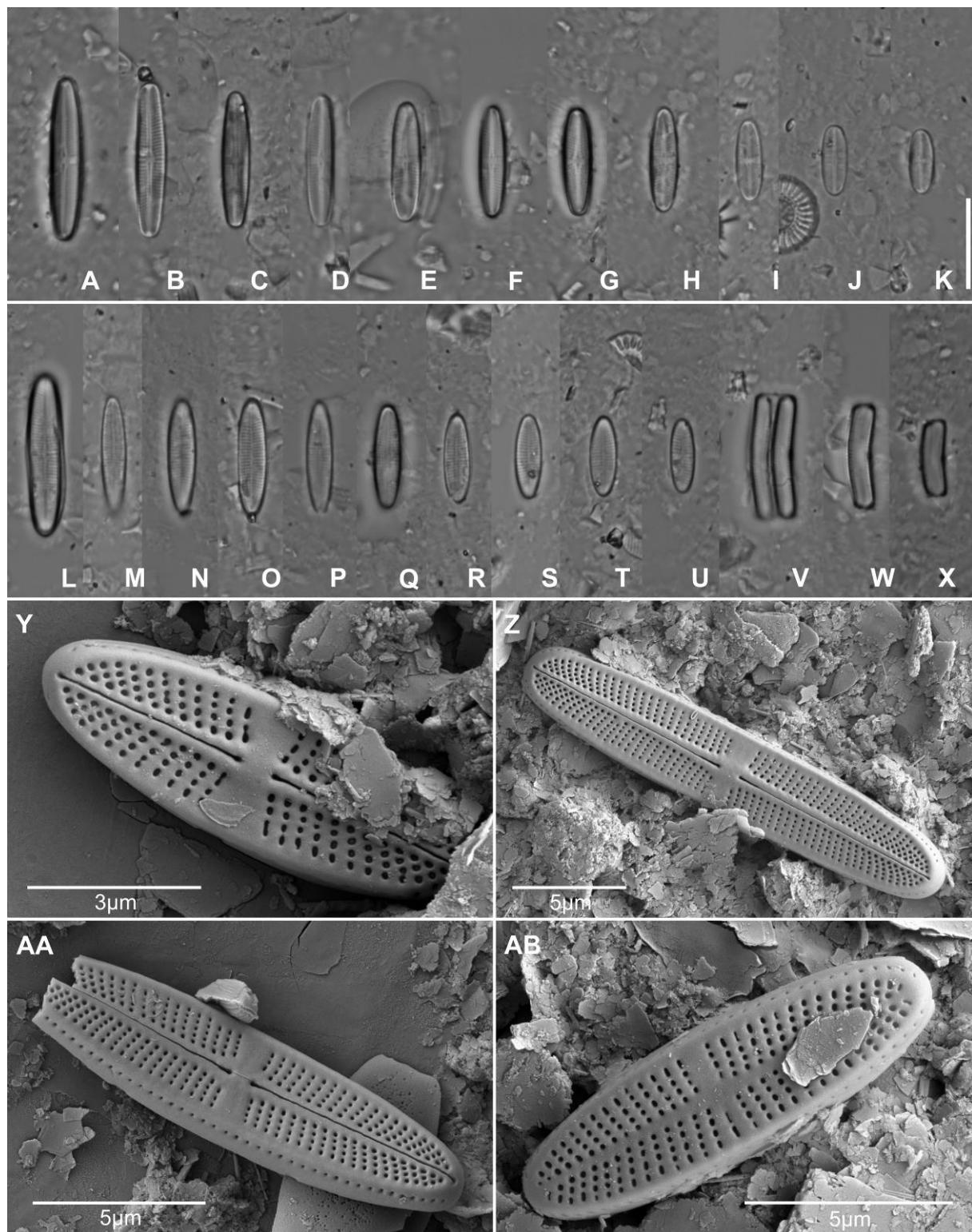


Figure 5 (A–AB). *Achnanthidium peruvianum* E.Morales & Ector in Morales *et al.* from Salto do Iporanga reservoir (SP469207). (A–K). LM views of raphe valves. (L–U). LM views of rapheless valves. (V–X). LM views of girdle views. (Y–AA). SEM external views of raphe valves. (AB). SEM external view of the rapheless valve. LM scale 10  $\mu\text{m}$ .

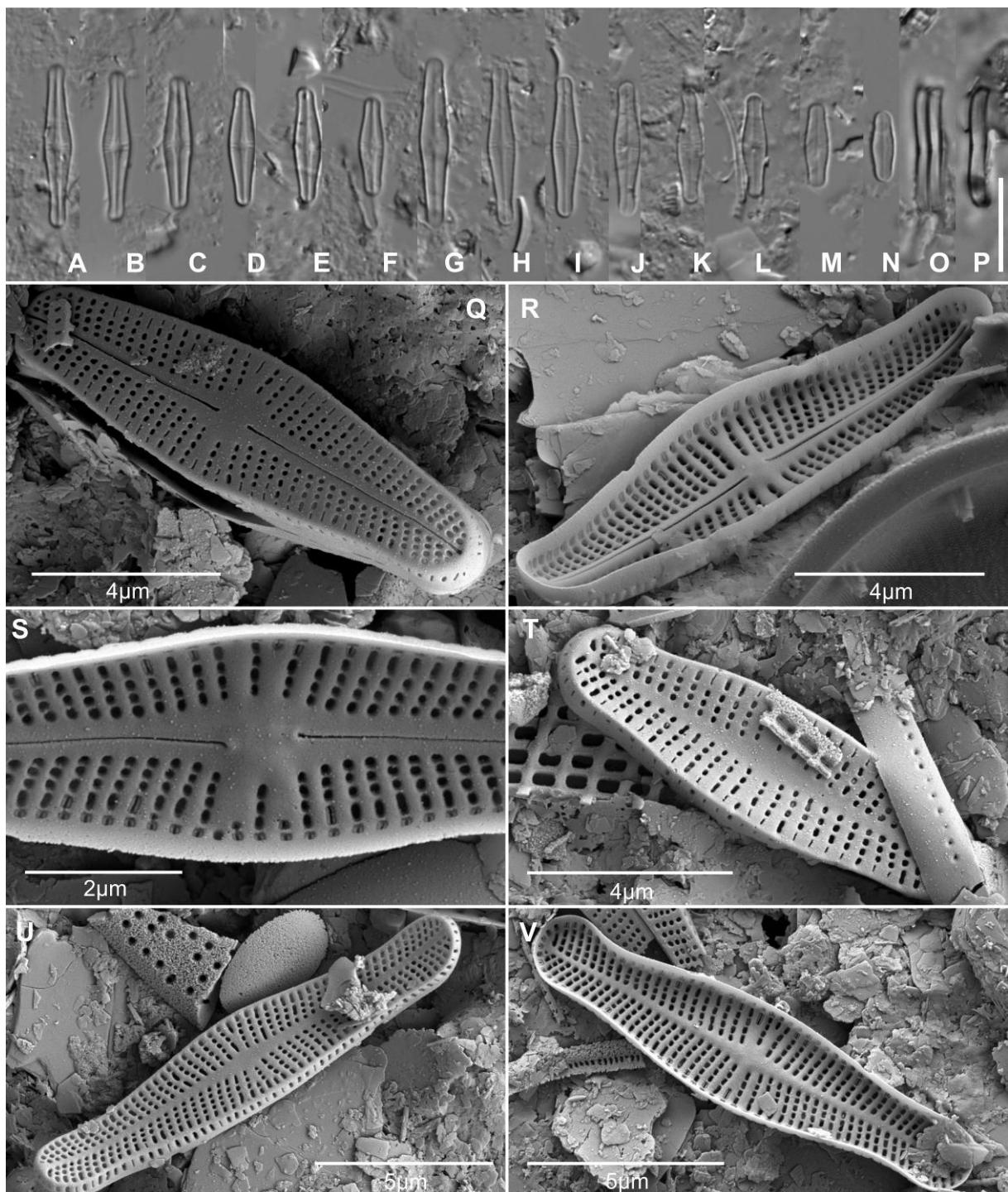


Figure 6 (A–V). *Achnanthidium tropicocatenatum* Marquardt, C.E.Wetzel & Ector in Marquardt *et al.* from Cachoeira do França reservoir (type locality, SP469430). (A–F). LM views of raphe valves. (G–N). LM views of rapheless valves. (O, P). LM views of girdle views. (Q). SEM external view of raphe valve. (R, S). SEM internal views of raphe valves. (T, U). SEM external views of rapheless valves. (V). SEM internal view of the rapheless valve. LM scale 10 µm.

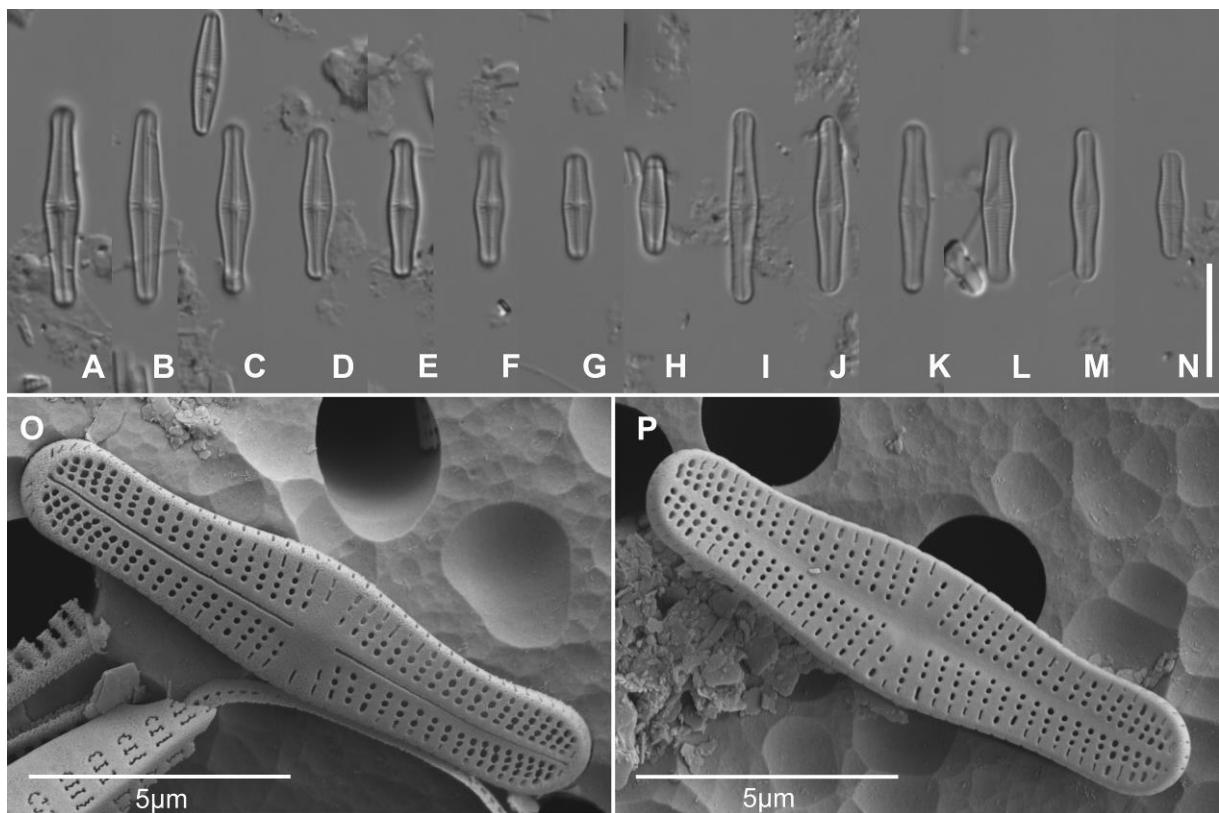


Figure 7 (A–P). *Achnanthidium tropicocatenatum* Marquardt, C.E.Wetzel & Ector in Marquardt *et al.* from Santa Helena reservoir (SP469524). (A–H). LM views of raphe valves. (I–N). LM views of rapheless valves. (O). SEM external view of raphe valve. SEM external view of the rapheless valve. LM scale 10 µm.

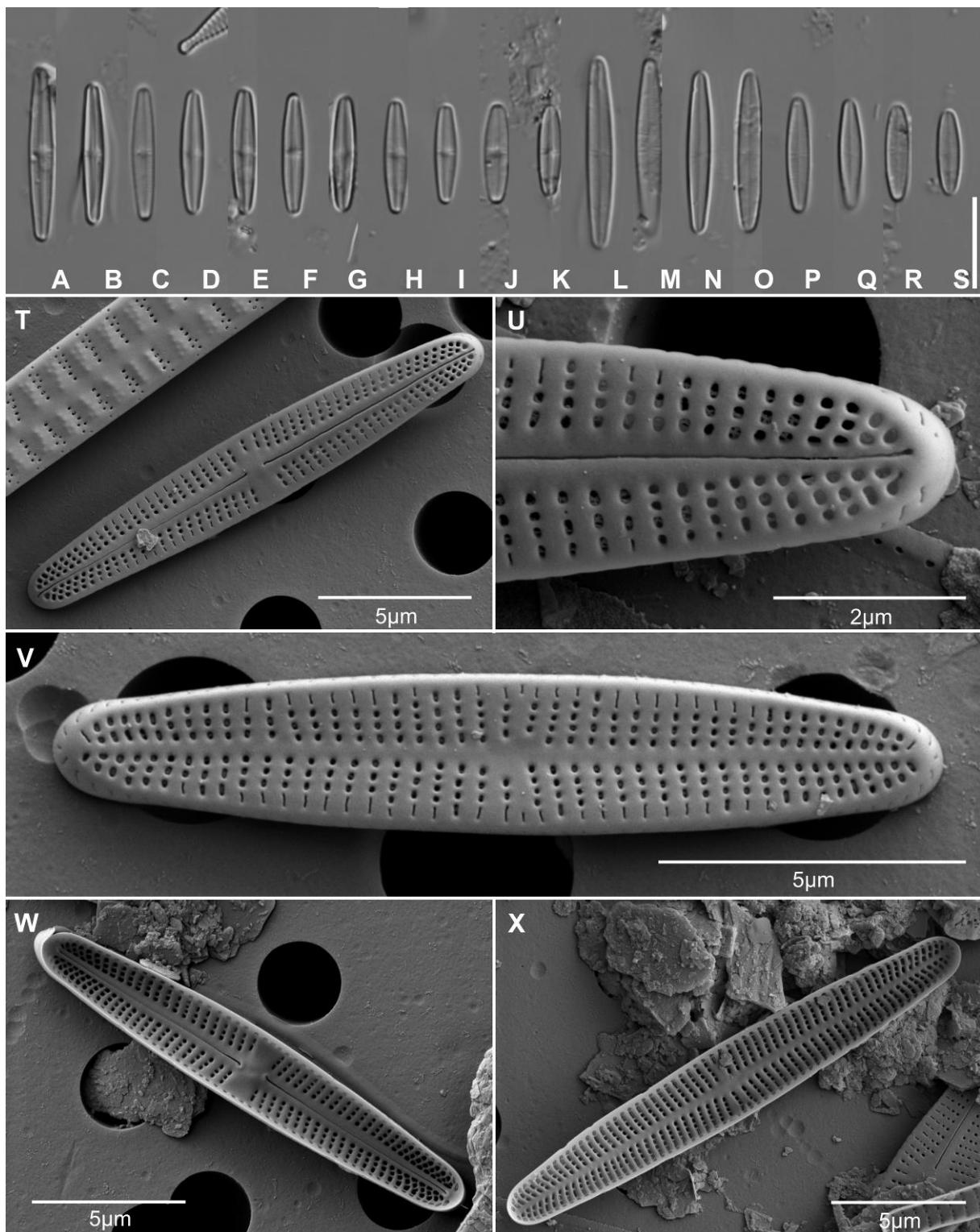


Figure 8 (A–X). *Achnanthidium* sp. nov. 1 from the Rio Grande reservoir (SP427898). (A–K). LM views of raphe valves. (L–S). LM views of rapheless valves. (T). SEM external view of raphe valve. (U). SEM external detail of the distal raphe end. (V). SEM external view of the rapheless valve. (W). SEM internal view of the raphe valve. (X). SEM internal view of the rapheless valve. LM scale 10  $\mu\text{m}$ .

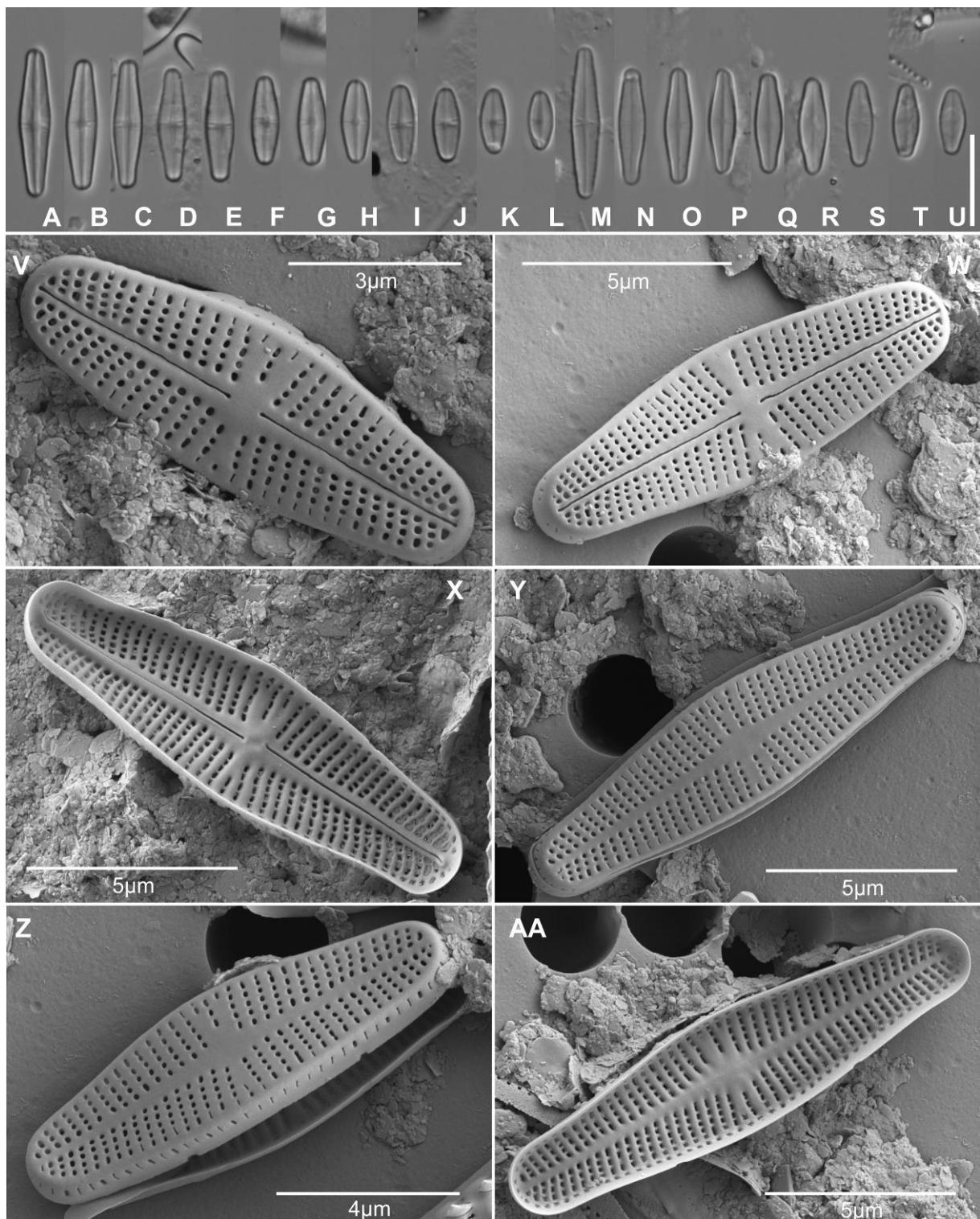


Figure 9 (A–AA). *Achnanthidium* sp. nov. 2 from the Hedberg reservoir (SP469539). (A–L). LM views of raphe valves. (M–U). LM views of rapheless valves. (V, W). SEM external views of raphe valves. (X). SEM internal view of the raphe valve. (Y, Z). SEM external views of rapheless valves. (AA). SEM internal view of the rapheless valve. LM scale 10  $\mu\text{m}$ .

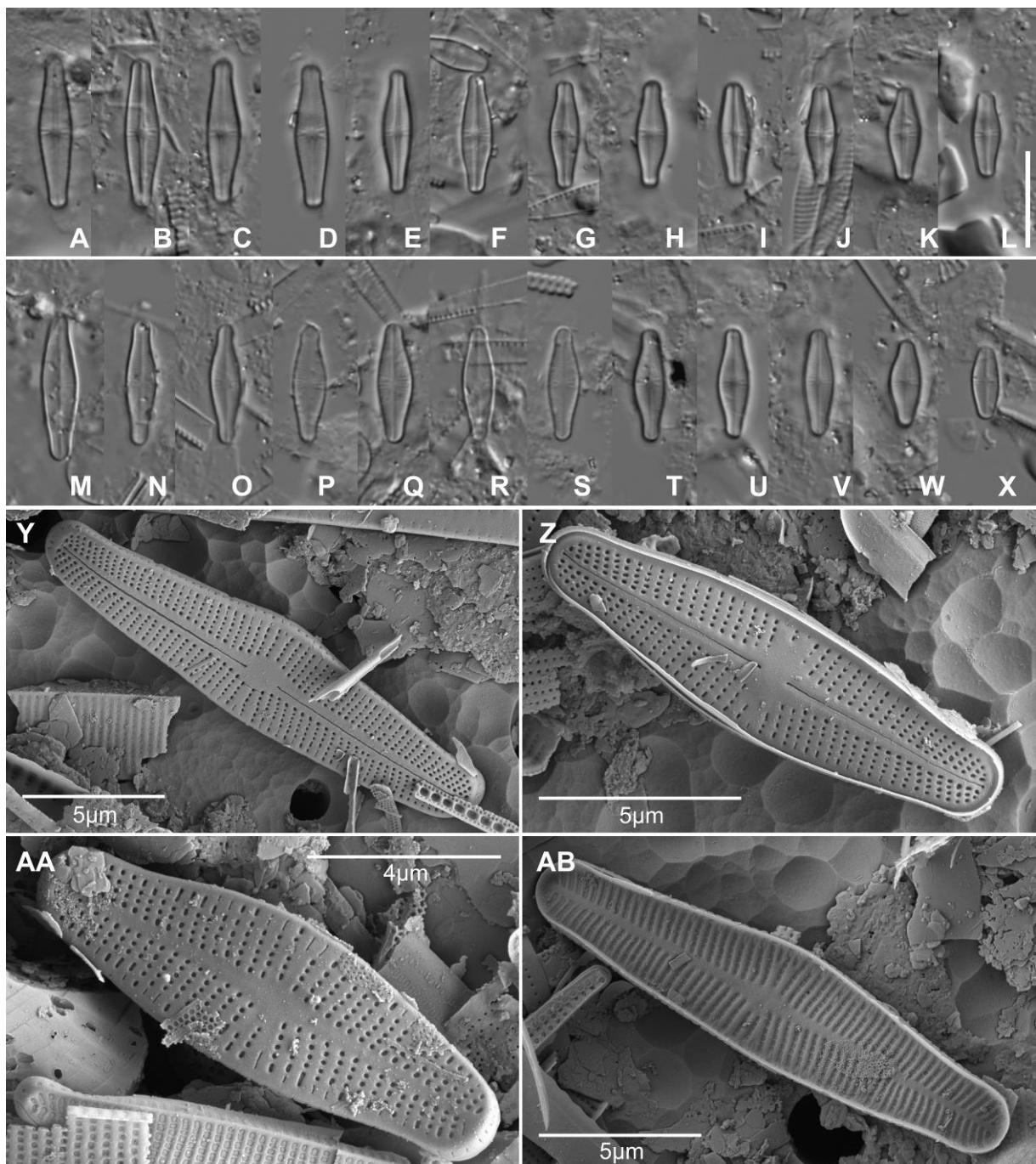


Figure 10 (A–AB). *Achnanthidium* sp. nov. 2 from the Guarapiranga reservoir (SP428513). (A–L). LM views of raphe valves. (M–X). LM views of rapheless valves. (Y, Z). SEM external views of raphe valves. (AA). SEM external view of the rapheless valve. (AB). SEM internal view of the rapheless valve. LM scale 10  $\mu\text{m}$ .

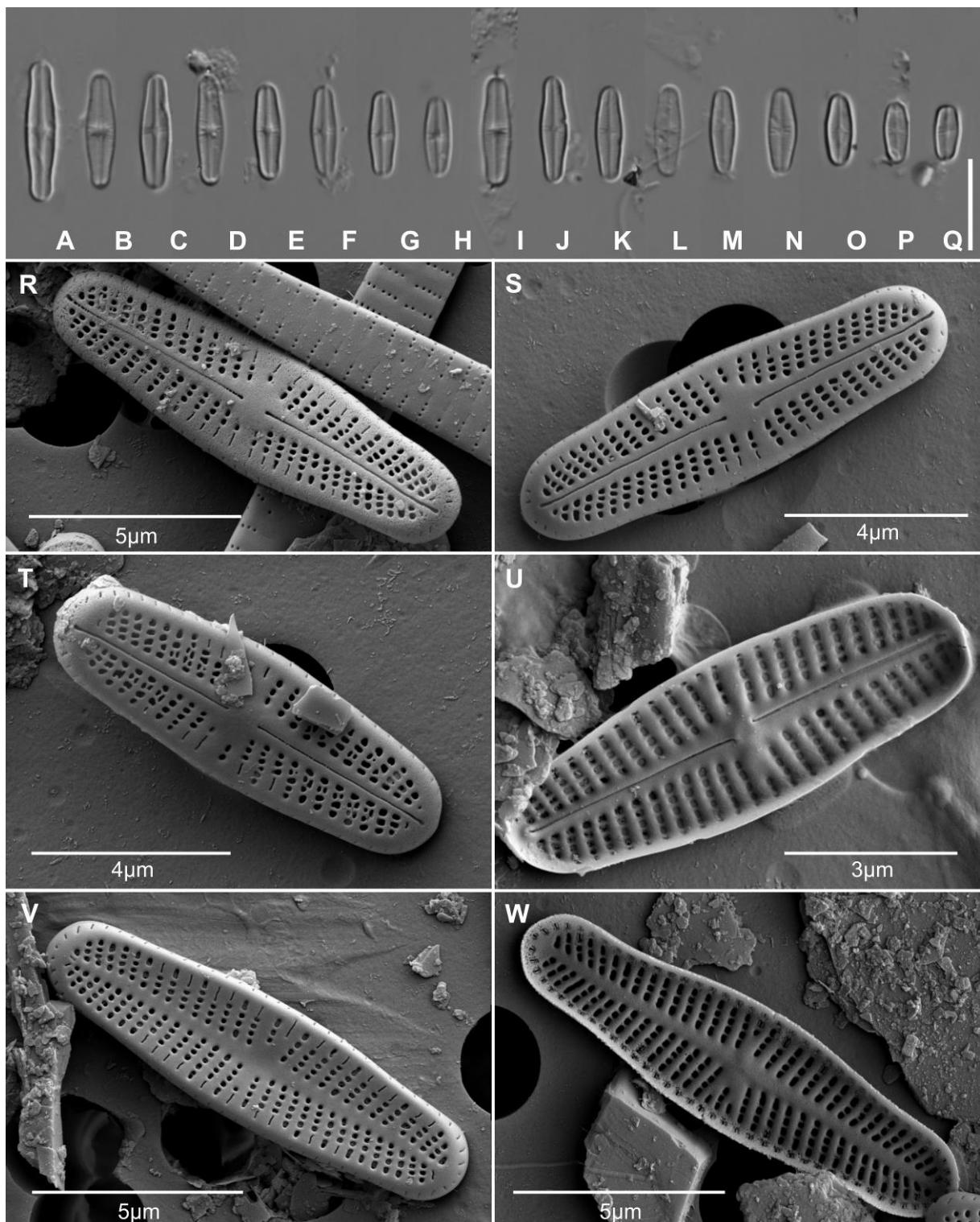


Figure 11 (A–W). *Achnanthidium* sp. nov. 3 from the Rio Grande reservoir (SP427898). (A–H). LM views of raphe valves. (I–Q). LM views of rapheless valves. (R–T). SEM external views of raphe valves. (U). SEM internal view of raphe valves. (V). SEM external view of the rapheless valve. (W). SEM internal view of the rapheless valve. LM scale 10 µm.

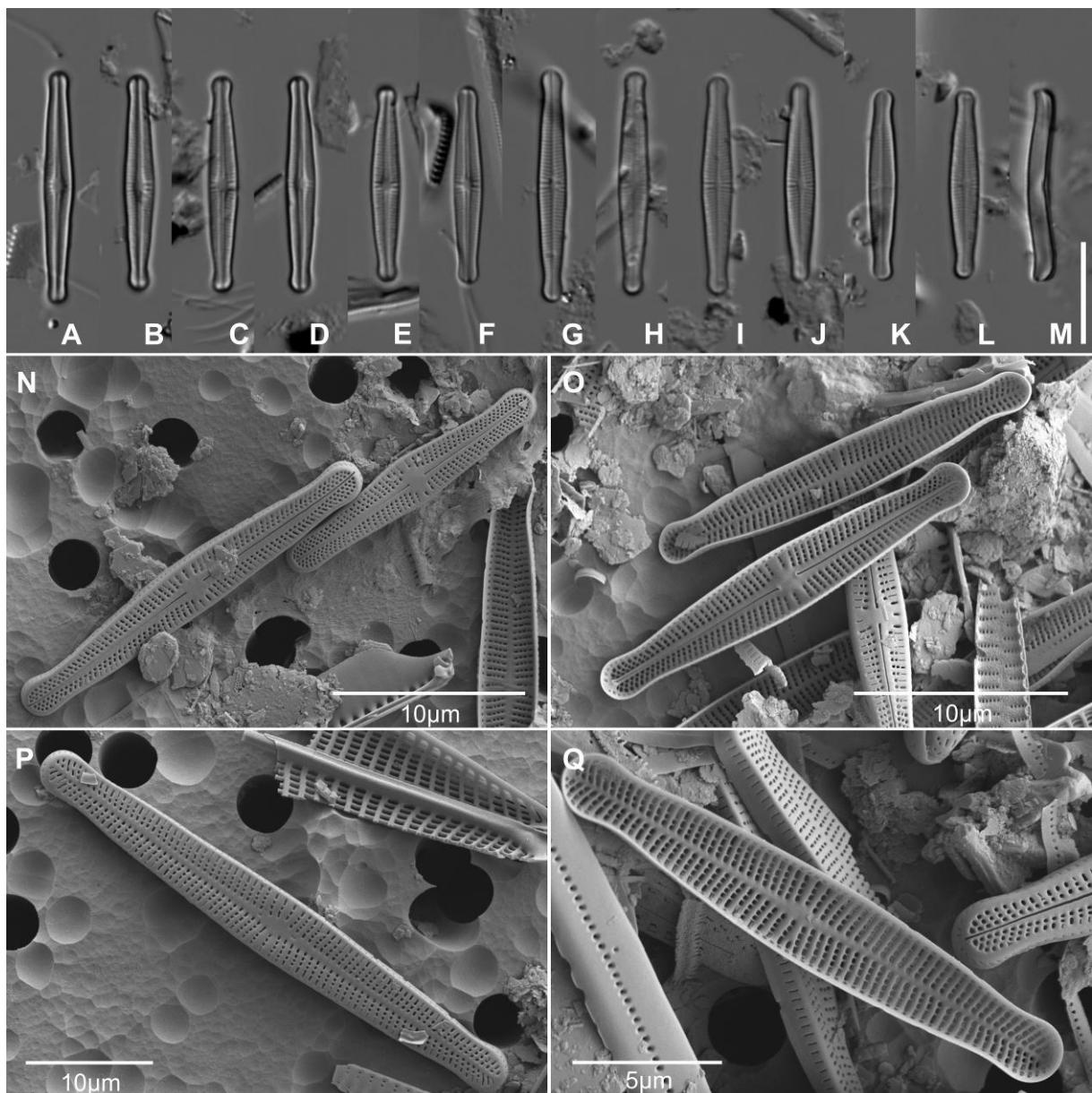


Figure 12 (A–Q). *Achnanthidium* sp. nov. 4 from the Santa Helena reservoir (SP469524). (A–F). LM views of raphe valves. (G–L). LM views of rapheless valves. (M). LM view of girdle view. (N). SEM external view of raphe valve. (O). SEM internal view of the raphe valve. (P). SEM external view of the rapheless valve. (Q). SEM internal view of the rapheless valve. LM scale 10 µm.

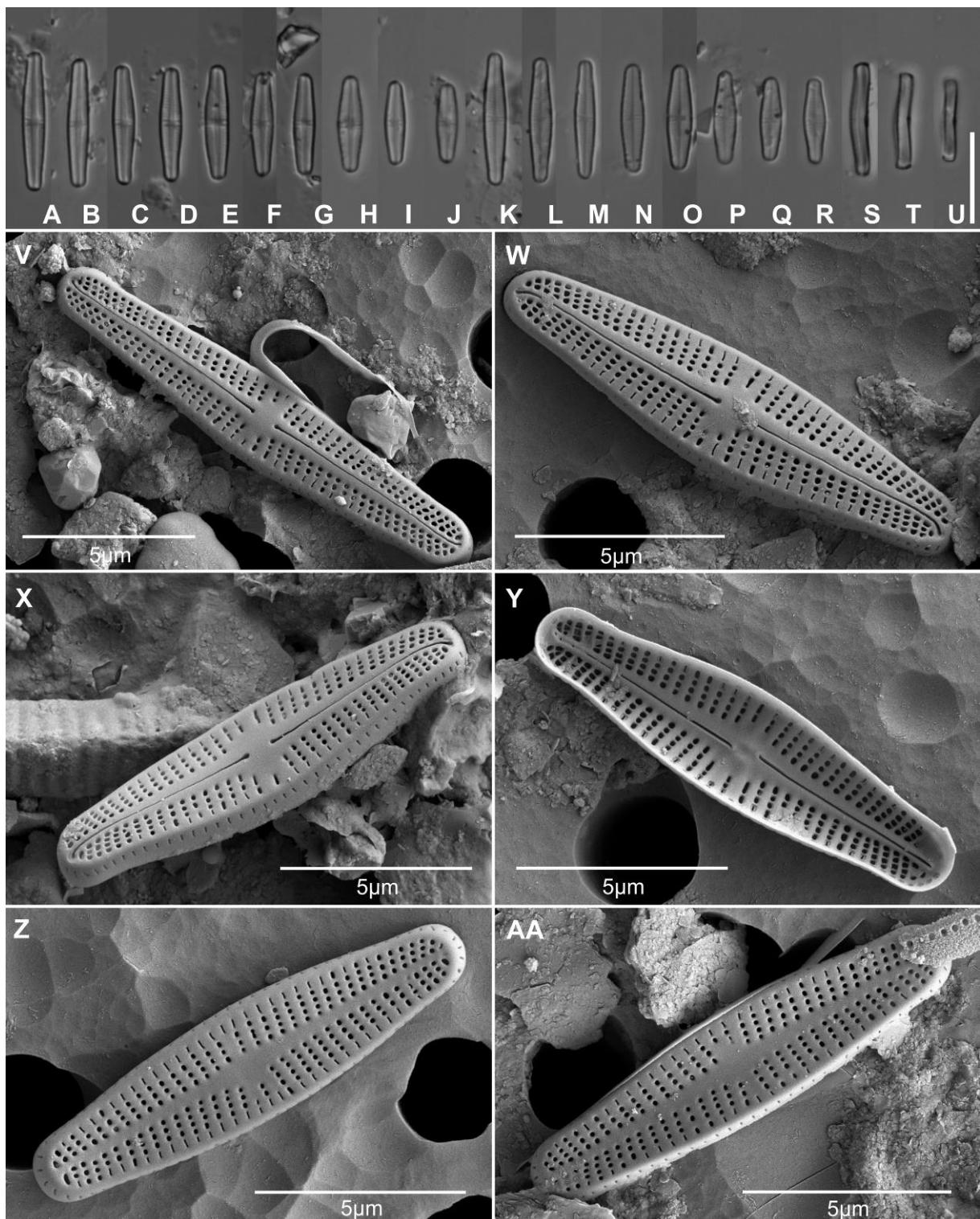


Figure 13 (A–AA). *Achnanthidium* sp. nov. 5 from São Pedro municipality (SP255724). (A–J). LM views of raphe valves. (K–R). LM views of rapheless valves. (S–U). LM views of girdle views. (V–X). SEM external views of raphe valves. (Y). SEM internal view of the raphe valve. (Z, AA). SEM external views of rapheless valves. LM scale 10  $\mu\text{m}$ .

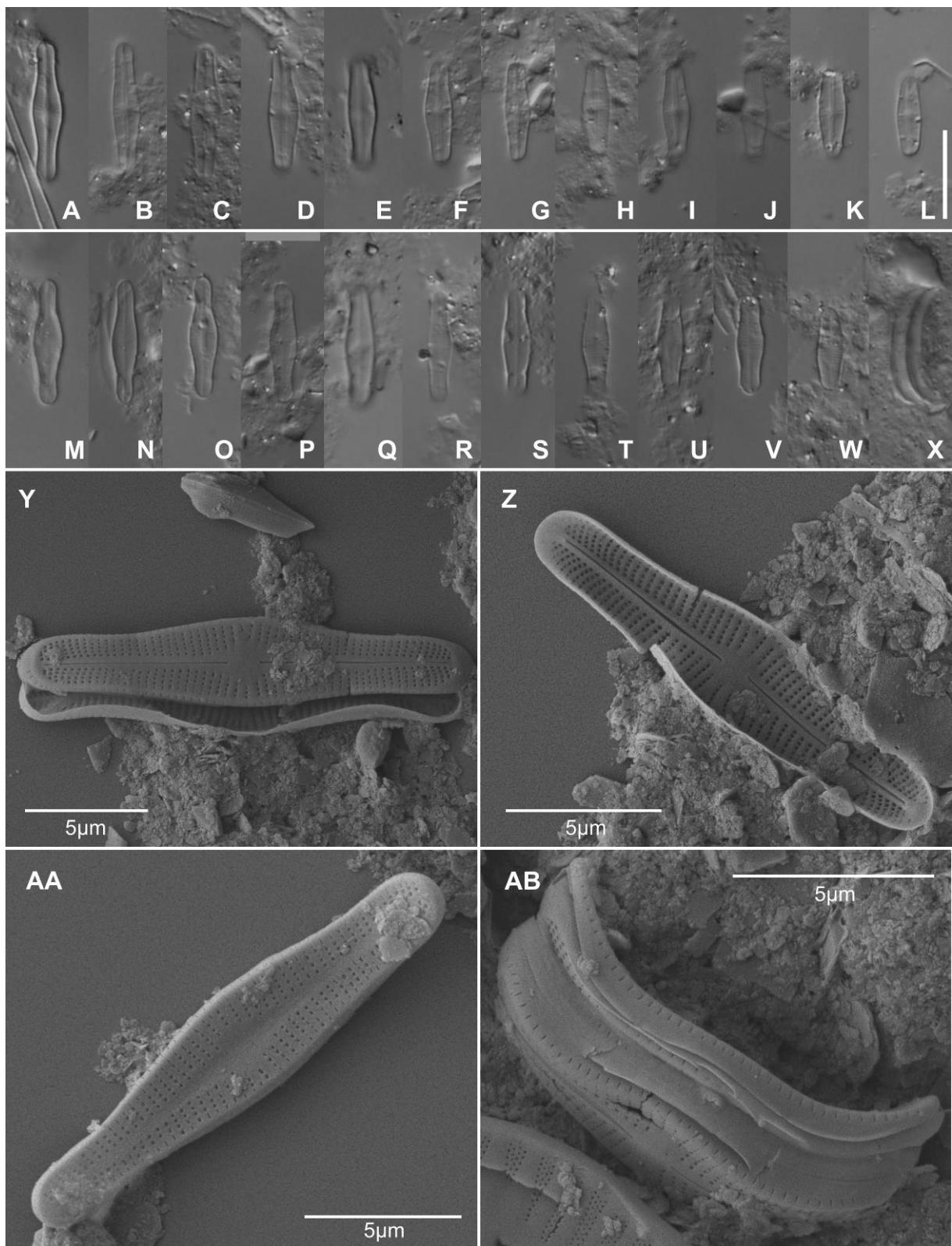


Figure 14 (A–AB) *Achnanthidium* sp. nov. 6 from the Garças Lake (SP469486). (A–L). LM views of raphe valves. (M–W). LM views of rapheless valves. (X). LM view of girdle view. (Y). SEM external view of raphe valve. (Z). SEM internal view of the raphe valve. (AA). (AB).

SEM external view of the rapheless valve. (AB). SEM girdle views of three valves. LM scale 10  $\mu\text{m}$ .

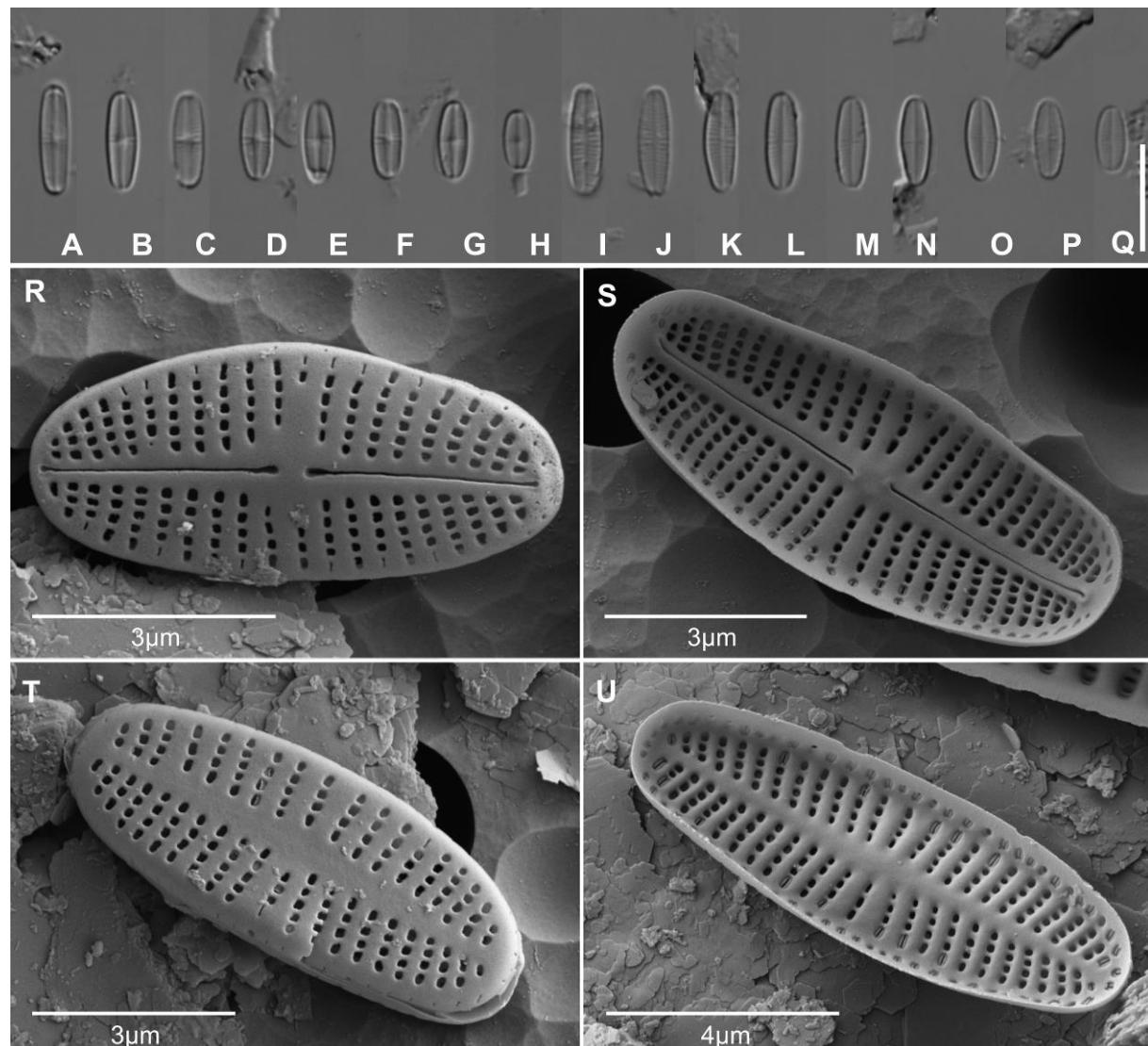


Figure 15 (A–U). *Achnanthidium* sp. nov. 7 from the Taquacetuba branch of Billings Complex (SP427904). (A–H). LM views of raphe valves. (I–Q). LM views of rapheless valves. (R). SEM external view of raphe valve. (S). SEM internal view of the raphe valve. (T). SEM external view of the rapheless valve. (U). SEM internal view of the rapheless valve. LM scale 10  $\mu\text{m}$ .

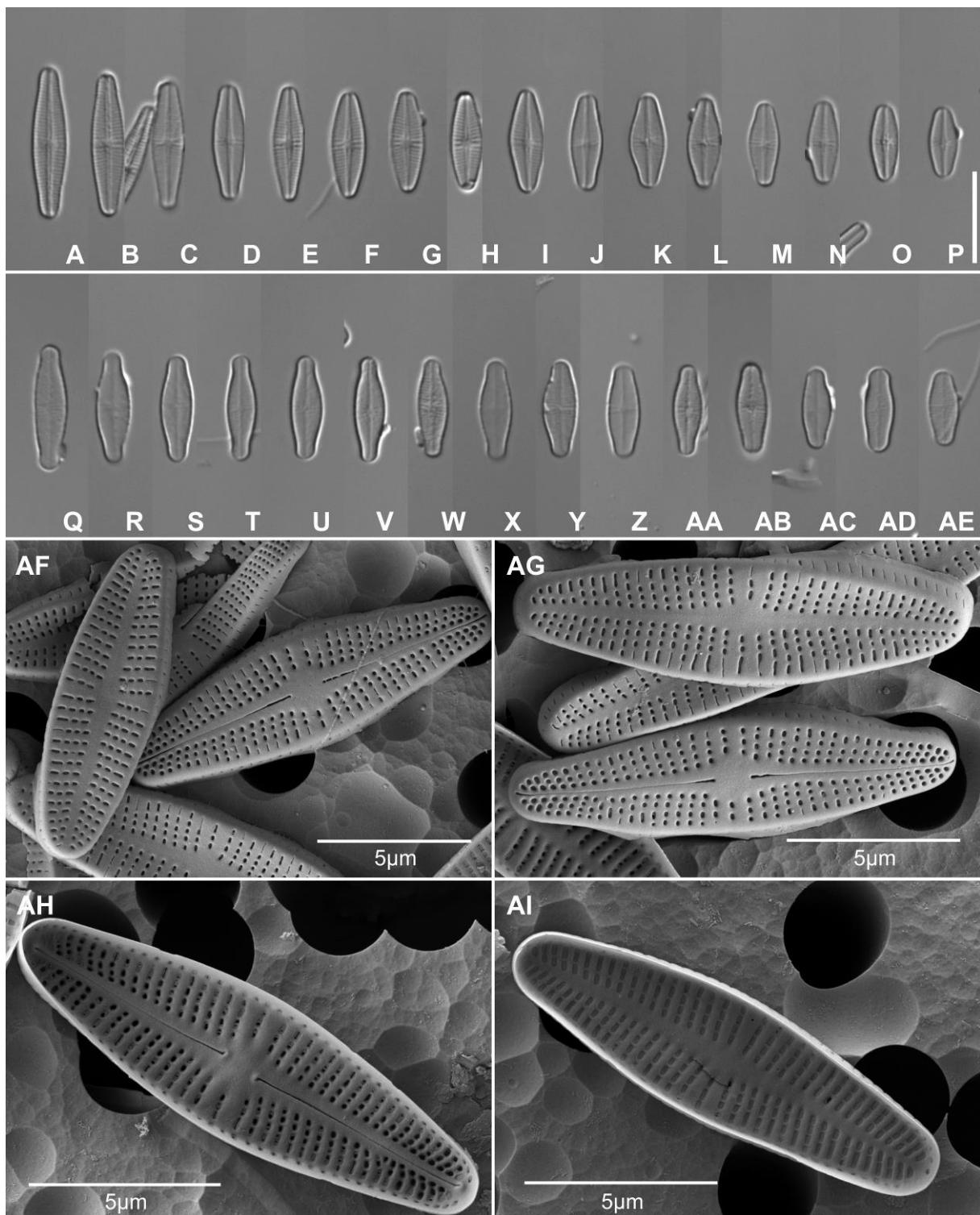


Figure 16 (A–AI). *Achnanthidium* sp. nov. 8 from the Garças Lake (SP469485). (A–P). LM views of raphe valves. (Q–AE). LM views of rapheless valves. (AF, AG). SEM external views of both raphe and rapheless valves. (AH). SEM internal view of the raphe valve. (AI). SEM internal view of the rapheless valve. LM scale 10  $\mu\text{m}$ .

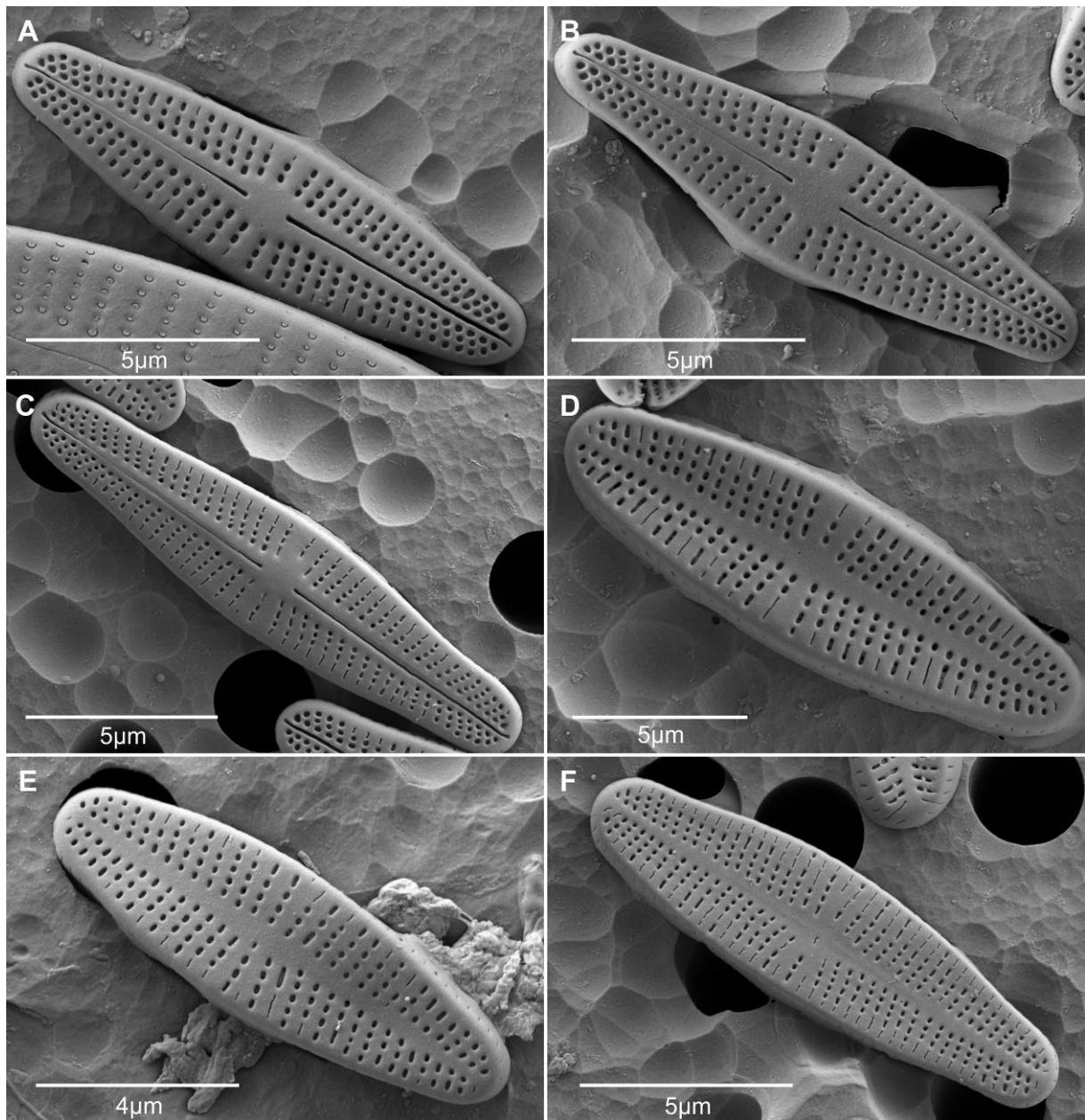


Figure 17 (A–F). *Achnanthidium* sp. nov. 8 from the Garças Lake (SP469485). (A–C). SEM external views of raphe valves. (D–F). SEM external views of rapheless valves.

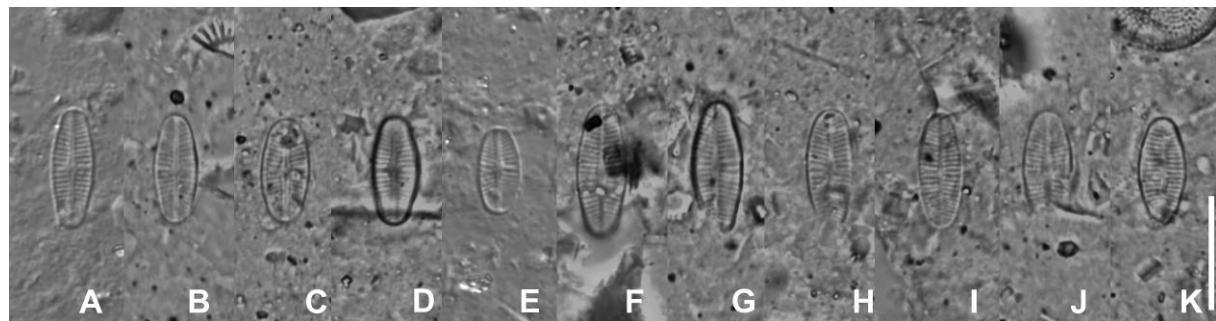


Figure 18 (A–K). *Achnanthidium* sp. from the Serraria reservoir (SP469204). (A–E). LM views of raphe valves. (F–K). LM views of the rapheless valves. Scale 10  $\mu\text{m}$ .

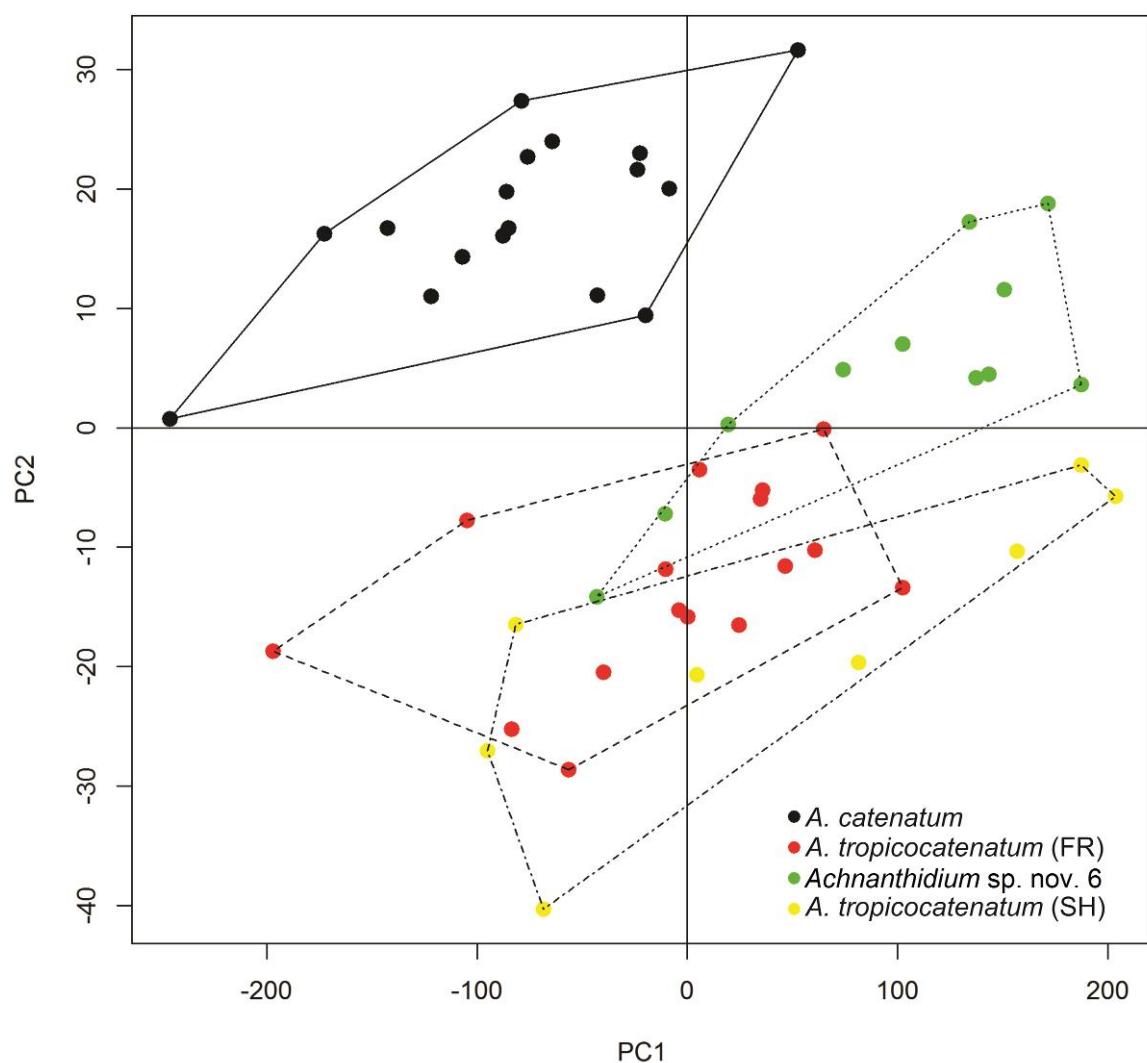


Figure 19. PCA plot of Procrustes-transformed digitized landmarks coordinates among four populations: *A. catenatum* (lectotype), *Achnanthidium* sp. nov. 6 from Garças Lake (SP469486) and *A. tropicocatenatum* from Cachoeira do França reservoir (FR, type locality) and Santa Helena reservoir (SH).

## CAPÍTULO 3

**Criptógamos do Parque Estadual das Fontes do Ipiranga, São Paulo, SP. Algae, 47:**

**Bacillariophyceae (Achnanthaceae, Cocconeidaceae Achnanthidiaceae)<sup>3</sup>**

Criptógamos do PEFI: Diatomáceas Monorrafídeas

**ABSTRACT – (Cryptogams of the Parque Estadual das Fontes do Ipiranga, São Paulo, SP.**

**Algae, 47: Bacillariophyceae (Achnanthaceae, Cocconeidaceae and Achnanthidiaceae)).** The monoraphid diatoms was for long time part of the Achnanthales order, however phylogenetic studies have shown they are not a monophyletic group. Among the monoraphid, most taxa are predominantly marine but at least 16 genera occur in freshwater. As part of the Algae series of the Parque Estadual das Fontes do Ipiranga (PEFI), this study aims to enhance the knowledge about the diversity of this group in this region and São Paulo state. In this way, 25 permanent slides made from PEFI materials were analysed from the collection of Herbário Científico do Estado “Maria Eneyda P. Kauffmann Fidalgo” (SP), Instituto de Botânica, Secretaria de Infraestrutura e Meio Ambiente do Estado de São Paulo. Nineteen slides presented at least one representative of the group. In total, 18 taxa were observed: one of Achnanthaceae, three of Cocconeidaceae and 14 of Achnanthidiaceae, being *Achnanthidium* the most diverse and well distributed genus. Finally, two new records for the State of São Paulo and Brazil were presented (*Platessa hustedtii* and *Planothidium victori*, respectively).

**Keywords:** Achnanthales, *Achnanthidium*, freshwater, monoraphid

**RESUMO – (Criptógamos do Parque Estadual das Fontes do Ipiranga, São Paulo, SP. Algae,**

**47: Bacillariophyceae (Achnanthaceae, Cocconeidaceae e Achnanthidiaceae)).** As

diatomáceas monorrafídeas constituíram por muito tempo a ordem Achnanthales, contudo,

estudos filogenéticos têm mostrado que elas não compõem um único grupo monofilético.

Dentre as monorrafídeas, a grande maioria dos táxons é predominantemente marinho, mas

pelo menos 16 gêneros ocorrem em água doce. Como parte da série Algas do Parque Estadual das Fontes do Ipiranga (PEFI), esse trabalho tem como objetivo ampliar o conhecimento da diversidade taxonômica das diatomáceas monorrafídeas nesta região e no Estado de São Paulo. Foram analisadas 25 lâminas permanentes confeccionadas a partir de material do PEFI e provenientes do acervo do Herbário Científico do Estado “Maria Eneyda P. Kauffmann Fidalgo” (SP), Instituto de Botânica, Secretaria de Infraestrutura e Meio Ambiente do Estado de São Paulo. Dezenove lâminas apresentaram ocorrência de pelo menos um representante do grupo. No total, foram observados 18 táxons: um de Achnanthaceae, três de Cocconeidaceae e 14 de Achanthidiaceae, sendo *Achnanthidium* o gênero mais diverso e bem distribuído. Ainda, foram apresentadas novas citações para o Estado de São Paulo e Brasil (*Platessa hustedtii* e *Planothidium victori*, respectivamente).

Palavras-chave: Achnanthales, *Achnanthidium*, água doce, monorrafídeas

## Introdução

As diatomáceas monorrafídeas, compreenderam por muito tempo a ordem Achnanthales P.C. Silva, formada pelas famílias Achnanthaceae Kützing, Coccneidaceae Kützing e Achnanthidiaceae D.G. Mann (Round et al. 1990). Contudo, estudos filogenéticos têm mostrado que na verdade elas compõem mais de um grupo monofilético, estando dispersas na árvore filogenética (Medlin & Kaczmarska 2004, Kulikovskiy et al. 2016, 2019a).

Presentemente, a família Achnanthaceae é classificada na ordem Mastogloiales D.G. Mann, juntamente com outros táxons não-monorrafídeos. Por sua vez, as famílias Achnanthidiaceae e Coccneidaceae passaram, provisoriamente, a fazer parte da ordem Coccneidales E.J.Cox, enquanto novos estudos filogenéticos não sejam realizados (Cox 2015).

A grande maioria dos gêneros monorrafídeos é predominantemente encontrado em águas marinhas e salobras ou são exclusivos desses habitats, entretanto pelo menos 16 gêneros ocorrem em água doce. Em Achnanthaceae, *Achnanthes* Bory englobou a maioria das monorrafídeas descritas até passar por uma grande divisão a partir dos anos 90 (Tudesque et al. 2016). *Platessa* Lange-Bertalot e *Platebaikalia* Kulikovskiy, Glushchenko, Genkal & Kociolek também pertencem à mesma família. *Platebaikalia* é monoespecífico e foi descrito a partir de ambiente dulcícola, no Lago Baikal, Rússia (Kulikovskiy et al. 2019b).

Dentro da família Coccneidaceae, apenas dois gêneros, *Coccneis* Ehrenberg e *Anorthoneis* Grunow, ocorrem também em água doce apesar de serem preferencialmente marinhos (Kociolek et al. 2018a).

Em Achnanthidiaceae a maior representatividade é de táxons dulcícolas pertencentes aos gêneros *Achnanthidium* Kützing, *Eucoccneis* Cleve ex Meister, *Planothidium* Round & Buktiyarova, *Psammothidium* Buktiyarova & Round, *Rossithidium* Buktiyarova & Round, *Lemnicola* Round & Basson, *Kolbesia* Round & Buktiyarova ex Round, *Karayevia* Round & Buktiyarova ex Round, *Gliwiczia* Kulikovskiy, Lange-Bertalot & Witkowski, *Crenotia*

Wojtal, A.Witkowski & C.Riaux-Gobin, *Skabitschewskia* Kulikovskiy & Lange-Bertalot (Kociolek et al. 2018b) e *Gogorevia* Kulikovskiy, Glushchenko, Maltsev & Kociolek.

A respeito da filogenia dessa última família, *Achnanthidium*, *Psammothidium*, *Rossithidium* e também *Lemnicola* formam um clado monofilético, além de seu agrupamento ser morfologicamente consistente (Kulikovskiy et al. 2016). Contudo, *Karayevia* sensu Bukhtiyarova (2006) faz parte, juntamente com *Madinithidium* C.Desrosiers, A.Witkowski & C.Riaux-Gobin (monorrafídea marinha), de uma linhagem independente no clado da família Stauroneidaceae e não de Achnanthidiaceae (Kulikovskiy et al. 2019a). Já os outros táxons ainda demandam mais estudos a esse respeito.

Na última década no Brasil, foram propostas novas espécies de monorrafídeas para os gêneros *Achnanthidium*, *Planothidium* e *Coccneis* (Wetzel & Ector 2014a, Marquardt et al. 2017, Costa et al. 2019, Wetzel et al. 2019, Morais et al. 2020). Na região Sudeste, especificamente no Estado de São Paulo, a contribuição mais ampla de monorrafídeas foi um estudo não publicado de Carneiro (2003), o qual reportou 46 táxons observados em distintos ecossistemas e habitats a partir do levantamento florístico da ordem Achnanthales no Estado. Ademais, trabalhos publicados apresentaram 32 táxons como contribuições reais, ou seja, passíveis de revisão taxonômica para o Estado (Bicudo et al. 1993, Bicudo et al. 2009, Costa-Böddeker et al. 2012, Fontana & Bicudo 2012, Faustino et al. 2016, Bartozek et al. 2018, Costa et al. 2019, Marquardt et al. 2017, 2018). Dentre esses, nove táxons foram observados no epipélon da cachoeira da Reserva Biológica do Alto da Serra de Paranapiacaba (Bicudo et al. 2009) e outros nove em vários ambientes dulcícolas a partir do levantamento da diversidade de *Coccneis* no Estado (Costa et al. 2019).

A pequena representação do grupo em trabalhos publicados para o Estado de São Paulo muito provavelmente indica uma subestimativa de sua biodiversidade. No Parque Estadual das Fontes do Ipiranga (PEFI), o conhecimento aprofundado da biodiversidade de diatomáceas (Bacillariophyta) inclui até o momento táxons de Coscinodiscophyceae (diatomáceas

cêntricas), Cymbellales, Eunotiales, Surirellales, Pinnulariaceae, *Brachysira* e uma espécie de monorrafídea, *Lemnicola hungarica* (Grunow) Round & Basson (Bicudo et al. 1999, Morandi et al. 2006, Carneiro & Bicudo 2007, Rocha & Bicudo 2008, Marquardt & Bicudo 2014, Ferreira & Bicudo 2017, Morais et al. 2019 e Silva-Lehmkuhl et al. 2020), demonstrando uma lacuna no conhecimento deste grupo de diatomáceas para a área de estudo. Assim, de modo a complementar os esforços científicos no PEFI e Estado de São Paulo, esse estudo tem como objetivo ampliar o conhecimento da diversidade de diatomáceas monorrafídeas na região.

### **Material e métodos**

Área de estudo – O Parque Estadual das Fontes do Ipiranga, localizado na região sudeste do Município de São Paulo, abrange uma área de 526,38 ha de um dos mais importantes fragmentos de Mata Atlântica em área urbana do país (Godoy & Trufem 2007). O PEFI abriga instituições governamentais (ex. Instituto de Botânica, Fundação Parque Zoológico, Parque de Ciência e Tecnologia da USP) além de Reserva Biológica, com rica biodiversidade, dois aquíferos subterrâneos e 24 nascentes permanentes (Bicudo et al. 2002). Apesar disso, seu histórico inclui fragmentações e ocupação desordenada, com seu entorno marcado por bairros periféricos com alta densidade populacional, o que tem gerado ao longo do tempo conflitos socioambientais que impactam o Parque, como o despejo de esgotos clandestinos e lixo (Matheus et al. 2008, Cerati et al. 2011).

Material para análise – Foram analisadas 25 lâminas permanentes (Tabela 1) provenientes do acervo do Herbário Científico do Estado “Maria Eneyda P. Kauffmann Fidalgo” (SP) do Instituto de Botânica, Secretaria do Meio Ambiente do Estado de São Paulo. Elas foram confeccionadas a partir de amostras de diferentes habitats (perifítico, fitoplanctônico e metafítico) provenientes de sete ambientes aquáticos dentro do PEFI (Lago das Garças, Lago das Ninféias, Lago dos Bugios, Lago do Monjolo, Lago do Cientec, Hidrofitotério e córrego Pirarungáua), além de material terrestre incluindo amostras obtidas de musgos e troncos de

árvores. Técnicas de amostragem do material e confecção das lâminas permanentes estão descritas em estudos anteriores também realizados com o material do PEFI (Marquardt & Bicudo 2014, Ferreira & Bicudo 2017).

**Análise do material –** As lâminas permanentes foram observadas por meio de microscópio óptico binocular Zeiss, Axio Imager A2, com câmara acoplada (MRc5). O exame taxonômico foi baseado em análise populacional ( $n = 10$ , exceto táxons mais raros) de cada ambiente e habitat de forma a registrar a variabilidade das características morfológicas e métricas por unidade amostral. A delimitação das populações considerou o protólogo sempre que possível. Os táxons foram identificados com auxílio de periódicos especializados, obras clássicas, modernas e trabalhos específicos de monorrafídeas (ex. Patrick & Reimer 1966, Krammer & Lange-Bertalot 1991, Bukhtiyarova & Round 1996, Carneiro & Bicudo 2007, Potapova & Hamilton 2007, Hlúbková et al. 2011, Jüttner et al. 2011, Morales et al. 2011, Van de Vijver et al. 2011, Wojtal et al. 2011, Wetzel et al. 2013, 2017, Tofilovska et al. 2014, Novais et al. 2015, Costa et al. 2019). Além disso, a classificação dos táxons foi feita de acordo com Adl et al. (2019) e Cox (2015) e a terminologia descritiva seguiu Anonymous (1975) e Barber & Haworth (1981).

## **Resultados e discussão**

Dezenove lâminas (76%) apresentaram ocorrência de pelo menos um representante de diatomáceas monorrafídeas (Tabela 2). No total, foram observados 18 táxons: um de Achnanthaceae, três de Coccconeidaceae e 14 de Achnanthidiaceae. *Achnanthidium* foi o gênero mais diverso com oito táxons na área de estudo, enquanto *Coccconeis* e *Planothidium* apresentaram três representantes cada, *Gogorevia*, dois e *Lemnicola* e *Platessa* apenas um táxon cada. Em relação aos diferentes ambientes analisados, o córrego Pirarungáua foi o único em que não foi observada a ocorrência de diatomáceas monorrafídeas. Contrapondo, no hidrofitotério, em material coletado sobre musgo, foi encontrada a maior diversidade com a ocorrência de nove táxons de cinco dos gêneros inventariados nesse estudo, com exceção

apenas de *Lemnicola*. Duas amostras do Lago das Garças também apresentaram maior diversidade, tendo sido observados sete táxons.

Segue abaixo o enquadramento taxonômico dos táxons inventariados e respectivas informações taxonômicas e de distribuição.

Divisão Bacillariophyta Haeckel 1878

Subdivisão Bacillariophytina Medlin & Kaczmarzka 2004 emend. Adl et al. 2019

Classe Bacillariophyceae Haeckel 1878 emend. Adl et al. 2019

Subclasse Bacillariophycidae D.G.Mann in Round et al. 1990 emend. Adl et al. 2019

Ordem Mastogloiales D.G.Mann emend. E.J.Cox 2006

Família Achnanthaceae Kützing 1844

***Platessa*** Lange-Bertalot 2004

*Platessa* é caracterizada por possuir valvas pequenas, planas e elípticas, e estrias predominantemente bisseriadas com aréolas ocluídas por hymen (Romero 2016).

***Platessa hustedtii* (Krasske)** Lange-Bertalot in Krammer & Lange-Bertalot 2004, p. 445

Basiônimo: *Coccconeis hustedtii* Krasske 1923, p. 193, fig. 10a, b

Figura 1

Características métricas: 11,9 µm de comprimento; 5,2–5,3 µm de largura, 18–19 estrias em 10 µm.

Este táxon foi bastante raro e apenas duas valvas arrafídeas foram encontradas. Apesar disso, sua morfologia é bastante característica com a presença de uma área axial lanceolada larga e área central indistinta.

A espécie já foi observada em estudos realizados no Sul do país (Bes et al. 2012, Lobo et al. 2016, Silva et al. 2017) e na mesma região também foram encontrados registros de *Achnanthes rupestroides* Hohn, sinônimo de *P. hustedtii* (Oliveira et al. 2002, Ferrari &

Ludwig 2007, Moresco et al. 2011). No Estado de São Paulo, o táxon é citado pela primeira vez no presente estudo.

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, hidrofitotério, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S.Wengrat & B. Pellegrini (SP427342, SP427343).

Ordem Cocconeidales E.J.Cox 2015

Família Cocconeidaceae Kützing 1844

*Cocconeis* Ehrenberg 1837

Espécimes de *Cocconeis* apresentam valvas elípticas ou quase circulares, valva rafídea usualmente menos convexa do que a arrafídea e estrias usualmente unisseriadas com aréolas cobertas por hymen com perfurações lineares (Round et al. 1990).

*Cocconeis amerieuglypta* L.F.Costa, C.E.Wetzel & Ector in Costa et al. 2019, p. 16, figs 1–12, 25–30

Figuras 2–5

Características métricas: 15,2–28,9 µm de comprimento; 8,9–17,1 µm de largura, 22–27 estrias em 10 µm.

Encontrado em duas amostras, o táxon distingue-se dos similares principalmente pela morfologia da valva arrafídea, na configuração e densidade de estrias e também pela área axial (Costa et al. 2019).

O táxon foi descrito recentemente de amostra do Rio Tietê no Estado de São Paulo (Costa et al. 2019), mas já foi encontrado anteriormente no Estado do Paraná, citado como *C. placentula* var. *placentula* (Moro & Fürstenberger 1993).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, hidrofitotério, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427343); material sobre musgos e tronco de árvore, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427344).

*Cocconeis euglypta* Ehrenberg 1854, pl. 34, fig. 6A, fig. 2

Figura 6

Características métricas: 27,9 µm de comprimento; 12,9 µm de largura, 24 estrias em 10 µm.

Apesar de penas uma valva ter sido encontrada a identificação foi possível pois a valva arrafídea é a que mais diferencia o táxon de outros próximos, principalmente pelas aréolas dispostas apicalmente em zigzag, o que é um padrão da espécie (Romero & Jahn 2013).

*Cocconeis euglypta* já foi bastante citada no Brasil, também como *C. placentula* var. *euglypta* e principalmente na região Sul (Bertolli et al. 2010, Bes et al. 2012, Bartozek et al. 2013, Nardelli et al. 2016, Silva et al. 2017), mas foi observada também em São Paulo em estudo taxonômico específico do gênero (Costa et al. 2019).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, Lago das Ninféias, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255743).

*Cocconeis neodiminuta* Krammer 1990, p. 151, figs 1, 2 part, 8–20, 40–45

Figura 7

Características métricas: 13,9 µm de comprimento; 8,2 µm de largura, 15 estrias em 10 µm.

Uma única valva foi observada (arrafídea), mas a espécie é bastante diferenciada das demais pelas características métricas, sendo uma espécie pequena como o próprio nome indica. Além disso, na valva arrafídea as aréolas são bastante conspícuas e a área axial é estreita (Romero & Van de Vijver 2011).

Antes mencionada apenas em literatura sem informações taxonômicas e ilustrações, a espécie foi recentemente encontrada no Estado de São Paulo (Costa et al. 2019).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, Lago das Garças, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255751).

Família Achnanthidiaceae D.G. Mann 1990

***Achnanthidium*** Kützing 1844

*Achnanthidium* possui células pequenas usualmente menores do que 30 µm em comprimento e 5 µm em largura; valvas linear-lanceoladas a linear-elípticas; estrias unisseriadas, radiais ou quase transversas, levemente espaçadas no centro (às vezes ausentes ou reduzidas) e mais próximas em direção aos ápices (Round & Bukhtiyarova 1996).

***Achnanthidium minutissimum*** (Kützing) Czarnecki 1994, p. 157

Figuras 8–15

Características métricas: 9,1–18,3 µm de comprimento; 1,9–3,2 µm de largura, 26–29 estrias em 10 µm.

A espécie foi a mais comum na área de estudo e é bastante citada no país (ex. Faria et al. 2010, Marra et al. 2016). No Estado de São Paulo, *A. minutissimum* foi o primeiro táxon do gênero a ser citado com ilustrações e informações descritivas a partir de amostras de um trecho do Rio Paranapanema (como *Achnanthes minutissima* Kützing, Bicudo et al. 1993).

Apesar disso, além das medidas diferentes, as ilustrações mostram duas morfologias distintas para o mesmo táxon, sendo apenas o segundo espécime correspondente a *A. minutissimum*.

Mais de uma década depois, Moutinho et al. (2007) identificaram *A. minutissimum* no reservatório Cabuçu e Bicudo et al. (2009) na Reserva Biológica do Alto da Serra de Paranapiacaba, ambos em amostras perifíticas.

Bere (2010) analisou amostras de diversos substratos da comunidade bentônica no Rio Monjolinho em São Carlos e também citou *A. minutissimum*, contudo, as figuras 2–4 correspondem provavelmente a *A. tropicocatenatum* pelo formato valvar com ápices capitados e a porção mediana inflada. Além disso, a vista lateral forma uma colônia com duas frústulas de ápices levemente curvados em direção à valva arrafídea, características observadas no material tipo em Marquardt et al. (2017).

Por fim, o táxon foi ainda encontrado em outros ambientes dulcícolas e habitats no Estado (Faustino et al. 2016, Costa et al. 2017, Bartožek et al. 2018, Costa et al. Cap. 2).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, Lago das Ninféias, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255743); hidrofitotório, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255745); Lago dos Bugios, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255747); Lago das Garças, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255751); Lago das Ninféias, 7–XI–1996, D.C. Bicudo & L.L. Morandi (SP294899, SP294900); hidrofitotório, 7–XI–1996, D.C. Bicudo & L.L. Morandi (SP294903); Lago das Garças, 14–I–1997, D.C. Bicudo & L.L. Morandi (SP294904, SP294905); Lago do Monjolo, 1–III–1997, D.C. Bicudo & L.L. Morandi (SP294909); hidrofitotório, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427341, SP427342, SP427343); material sobre musgos e tronco de árvore, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427344).

*Achnanthidium peetersianum* C.E.Wetzel, Juttner & Ector in Wetzel & al. 2019, p. 345, figs 1N–AK, 3A–J

#### Figuras 16–19

Características métricas: 6,3–21,52 µm de comprimento; 1,8–2,9 µm de largura, 29–34 estrias em 10 µm.

*Achnanthidium peetersianum* foi o segundo táxon mais amplamente distribuído na área de estudo.

Foi recentemente publicado como um novo táxon para a Ciência (Wetzel et al. 2019a) e, após sua descrição, está sendo citado pela primeira vez no Brasil em estudo a respeito da diversidade de *Achnanthidium* em diversos ambientes aquáticos e diferentes habitats no Estado de São Paulo (Costa et al. Cap. 2). Entretanto, o mesmo já havia sido citado como *Achnanthidium cf. macrocephalum* (Hustedt) Round & Bukhtiyarova em estudo que englobou amostras de 10 represas localizadas em três bacias do Estado de São Paulo (Bartozek et al. 2018). No Paraná, espécimes identificados a nível genérico (Silva-Lehmkuhl et al. 2019) ou

como *A. macrocephalum* (Marra et al. 2016) também parecem corresponder a *A. peetersianum*.

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, Lago das Ninféias, 18-VII-1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255743); Lago das Ninféias, 7-XI-1996, D.C. Bicudo & L.L. Morandi (SP294899, SP294900); Lago dos Bugios, 7-XI-1996, D.C. Bicudo & L.L. Morandi (SP294902); Centro de Ciências e Tecnologia, lago, 15-I-1997, D.C. Bicudo & L.L. Morandi (SP294906, SP294907, SP294908); Jardim Botânico, Lago do Monjolo, 1-III-1997, D.C. Bicudo & L.L. Morandi (SP294909); hidrofitotério, 7-VI-2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427343); material sobre musgos e tronco de árvore, 7-VI-2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427344).

***Achnanthidium tropicocatenatum*** Marquardt, C.E.Wetzel & Ector in Marquardt et al. 2017, p. 318, figs 3–5

Figuras 20–22

Características métricas: 10,5–17,4 µm de comprimento; 2,6–3,3 µm de largura, 36–42 estrias em 10 µm.

Poucas valvas foram encontradas em dois ambientes da área de estudo, porém a identificação foi possível pois o táxon apresenta valvas características com região mediana inflada e ápices subcapitados a capitados.

*Achnanthidium tropicocatenatum* foi descrito recentemente a partir da análise de amostras das represas de Cachoeira do França e Jurupará no Estado de São Paulo (Marquardt et al. 2017).

Apesar disso, o táxon já havia sido observado anteriormente no Estado, citado como *A. catenatum* (Bicudo et al. 2009, Fontana & Bicudo 2012, Faustino et al. 2016, Costa et al. 2017) e *A. minutissimum* (Bere 2010). Finalmente, outros estudos taxonômicos incluíram a ocorrência do táxon em São Paulo (Bartozek et al. 2018, Marquardt et al. 2018, Costa et al. Cap. 2).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, Lago das Ninféias, 7–XI–1996, D.C. Bicudo & L.L. Morandi (SP294899); hidrofitotérrio, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427341).

***Achnanthidium* sp. 1**

Figuras 26–31

Características métricas: 5,9–20,9 µm de comprimento; 2,0–3,1 µm de largura, 28–30 estrias em 10 µm.

*Achnanthidium* sp. 1 aproxima-se morfologicamente de *A. minutissimum*, mas as espécies podem ser separadas pelos ápices estreitamente arredondados na primeira e rostrados na última.

A espécie vem sendo descrita como nova em estudo específico sobre *Achnanthidium* no Estado de São Paulo (Costa et al. Cap. 2).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, hidrofitotérrio, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255745); Lago dos Bugios, 7–XI–1996, D.C. Bicudo & L.L. Morandi (SP294902); hidrofitotérrio, 7–XI–1996, D.C. Bicudo & L.L. Morandi (SP294903); Lago do Monjolo, 1–III–1997, D.C. Bicudo & L.L. Morandi (SP294909); hidrofitotérrio, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427341, SP427342, SP427343); material sobres musgos e tronco de árvore, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427344, SP427346).

***Achnanthidium* sp. 2**

Figuras 23–25

Características métricas: 7,0–18,9 µm de comprimento; 2,2–3,3 µm de largura, 30–34 estrias em 10 µm.

*Achnanthidium* sp. 2 não apresenta fascia na área central diferindo de táxon próximo, *A. minutissimum*, que pode apresentar fascia uni ou bilateral. Além disso, *Achnanthidium* sp. 2 possui um formato valvar muitas vezes rômbico-lanceolado com ápices menos diferenciados e *A. minutissimum* apresenta ápices mais protraídos.

O táxon está sendo descrito em trabalho taxonômico a respeito de *Achnanthidium* no Estado de São Paulo a partir de amostras perifíticas da represa Hedberg (Costa et al. Cap. 2).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, Lago das Ninféias, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255743); Lago das Garças, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255751); Lago das Ninféias, 7–XI–1996, D.C. Bicudo & L.L. Morandi (SP294900); hidrofitotório, 7–XI–1996, D.C. Bicudo & L.L. Morandi (SP294903); Lago das Garças, 14–I–1997, D.C. Bicudo & L.L. Morandi (SP294904).

### ***Achnanthidium* sp. 3**

Figuras 32–33

Características métricas: 7,0–11,8 µm de comprimento; 2,0–2,8 µm de largura, 26–32 estrias em 10 µm.

*Achnanthidium* sp. 3 assemelha-se a *A. minutissimum* e *Achnanthidium* sp. 2, diferindo principalmente pelo menor comprimento valvar e menor densidade de estrias.

O táxon está sendo descrito a partir de amostras do Complexo Billings (Represa Rio Grande) na Região Metropolitana de São Paulo (Costa et al. Cap. 2).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, hidrofitotório, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427342, SP427343).

### ***Achnanthidium* sp. 6**

Figuras 36–42

Características métricas: 6,3–18,4 µm de comprimento; 2,6–3,8 µm de largura, 30–36 estrias em 10 µm.

*Achnanthidium* sp. 6 foi encontrado apenas em amostras do Lago das Garças, em coletas realizadas em diferentes anos. O táxon está atualmente sendo descrito como uma espécie nova para a Ciência a partir de amostras do mesmo Lago coletadas mais recentemente (Costa et al. Cap. 2).

*Achnanthidium* sp. 6 é bastante similar a *A. tropicocatenatum*, mas pode ser diferenciado pela região mediana mais inflada, ápices mais capitados, área central arredondada maior, menor densidade de estrias e, principalmente, pela vista lateral mais arqueada formando um “C” na primeira espécie (Marquardt et al. 2017, Costa et al. Cap. 2).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, Lago das Garças, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255751); 14–I–1997, D.C. Bicudo & L.L. Morandi (SP294904, SP294905).

#### *Achnanthidium* sp. 7

Figuras 34–35

Características métricas: 6,2–12,6 µm de comprimento; 2,0–3,2 µm de largura, 26–30 estrias em 10 µm.

*Achnanthidium* sp. 7 difere dos demais táxons inventariados pelas valvas elípticas com ápices amplamente arredondados. O táxon está sendo descrito a partir de amostras do Complexo Billings (braço Taquacetuba) na Região Metropolitana de São Paulo (Costa et al. Cap. 2).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, Lago das Ninféias, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255743); hidrofitotório, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255745); Lago do Monjolo, 1–III–1997, D.C. Bicudo & L.L. Morandi (SP294909); hidrofitotório, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427343).

***Gogorevia*** Kulikovskiy, Glushchenko, Maltsev & Kociolek 2020

*Gogorevia* foi descrito recentemente para abrigar um complexo de espécies de valvas lanceoladas ou elípticas com ápices protraídos, curtos e rostrados a amplamente arredondados; valva rafídea com área central estreita, retangular, às vezes com forma de cunha; valva arrafídea com área central assimétrica, com presença de cavum (Kulikovskiy et al. 2020).

***Gogorevia constricta*** (Torka) Kulikovskiy & Kociolek 2020

Basiônimo: *Cocconeis exigua* var. *constricta* Torka 1909, p. 131, fig. 3a.

Figuras 43–44

Características métricas: 9,1–13,1 µm de comprimento; 4,1–5,6 µm de largura, 24–26 estrias em 10 µm.

Recentemente transferido para *Gogorevia*, *G. constricta* distingue-se de *G. exilis*, anteriormente *Achnantes exigua* Grunow, exclusivamente pela constrição mediana em ambas as valvas.

A espécie já foi encontrada com diferentes nomenclaturas no Norte, Sul e Sudeste (Metzeltin & Lange-Bertalot 1998, Bicudo et al. 1993, Oliveira et al. 2001, Fontana & Bicudo 2012, Bartozek et al. 2013, Nardelli et al. 2016, Silva et al. 2017).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, Lago das Garças, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255751); 14–I–1997, D.C. Bicudo & L.L. Morandi (SP294904); hidrofitotério, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427341, SP427342, SP427343); material sobre musgos e tronco de árvore, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427344).

***Gogorevia exilis*** (Kützing) Kulikovskiy & Kociolek 2020

Basiônimo: *Stauroneis exilis* Kützing 1844, p. 105, pl. 30, fig. 21.

Figuras 45–46

Características métricas: 6,3–11,6 µm de comprimento; 3,6–4,5 µm de largura, 24–28 estrias em 10 µm.

Táxon bastante comum no país, foi citado diversas vezes como *Achnanthes exigua* (Bicudo et al. 1993, Moro & Fürstenberger 1993, Lobo et al. 2004) ou ainda como *Achnanthidium exiguum* em trabalhos mais recentes (Ferrari & Ludwig 2007, Bicudo et al. 2009, Bertolli et al. 2010, Santos et al. 2011, Marra et al. 2016, Bartozek et al. 2018). Foi transferido para *Lemnicola* há algum tempo (Plinski & Witkowski 2011), mas a nova combinação não foi aceita pela comunidade científica devido à necessidade de maiores estudos acerca do táxon. Contudo, estudo molecular recente aliado à morfologia descreveu o novo gênero para o complexo de espécies de *Achnanthes exigua* (Kulikovskiy et al. 2020).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, Lago das Garças, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255751); 14–I–1997, D.C. Bicudo & L.L. Morandi (SP294904, SP294905); Lago do Monjolo, 1–III–1997, D.C. Bicudo & L.L. Morandi (SP294909); hidrofitotório, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427341); material sobre musgos e tronco de árvore, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427344).

#### ***Lemnicola* Round & Basson 1997**

*Lemnicola* é um gênero monoespecífico, caracterizado pelas estrias bisseriadas, estauro largo e assimétrico na valva rafídea e bem reduzido ou ausente na valva arrafídea (Round & Basson 1997).

#### ***Lemnicola hungarica* (Grunow) Round & Basson 1997, p. 77, figs 4–7, 26–31**

Basiônimo: *Achnanthidium hungaricum* Grunow 1863, p. 146; pl. 13 (IV), fig. 8

Figuras 47–50

Características métricas: 14,8–31,4 µm de comprimento; 5,7–7,3 µm de largura, 21–24 estrias em 10 µm.

O táxon é bastante característico apesar da grande variação morfológica de sua frústula, podendo apresentar valvas lanceoladas a linear-lanceoladas, ápices cuneados, sub-rostrados ou rostrados (Carneiro & Bicudo 2007). *Lemnicola hungarica* já teve sua ocorrência documentada em amostras do PEFI e outras localidades do Estado de São Paulo (Carneiro & Bicudo 2007). Ainda, Costa et al. (2017) observaram a presença do táxon na represa Billings. Na região Sul, a espécie é bastante comum (Ferrari & Ludwig 2007, Schneck et al. 2008, Bertolli et al. 2010, Faria et al. 2010, Santos et al. 2011, Bes et al. 2012, Marra et al. 2016), e também foi observada na região Norte como *Achnanthes hungarica* (Metzeltin & Lange-Bertalot 1998).

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, hidrofitotério, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255745); Lago das Garças, 18–VII–1991, D.C. Bicudo, T.A.V. Ludwig & D.M. Figueiredo (SP255751); Lago das Ninféias, 7–XI–1996, D.C. Bicudo & L.L. Morandi (SP294899); Lago das Garças, 14–I–1997, D.C. Bicudo & L.L. Morandi (SP294904).

***Planothidium* Round & Bukhtiyarova 1996**

*Planothidium* possui estrias biseriadas a multisseriadas, interrompidas ou não na região central da valva arrafídea por uma depressão característica ('cavum' ou 'sinus'); na valva rafídea, a rafe é central com suas terminações sempre curvadas para o mesmo lado (Round & Bukhtiyarova 1996).

***Planothidium rostratoholarcticum* Lange-Bertalot & Bak in Bak & Lange-Bertalot 2015, p. 354, figs 8 a–s, 9 a–g**

Figuras 51–54

Características métricas: 10,7–12,7 µm de comprimento; 4,6–5,8 µm de largura, 14–17 estrias em 10 µm.

As populações do táxon foram encontradas apenas no Lago das Garças. A espécie assemelha-se a *P. rostratum* (Østrup) Lange-Bertalot, mas os espécimes observados possuem valvas

menores e ápices sub-rostrados, além de maior densidade de estrias do que o lectótipo de *P. rostratum* (C: 9–14 µm, L: 5–6 µm, E: 11–12 em 10 µm, Bak & Lange-Bertalot 2014). No Estado de São Paulo, dois trabalhos que permitem revisão taxonômica identificaram *P. rostratum*. Em Bicudo et al. (2009) a ilustração da valva apresentada e as características métricas são bastante semelhantes às encontradas no PEFI, dessa forma é possível que o táxon seja também correspondente a *P. rostratoholarcticum*. Já em Fontana & Bicudo (2012) não é possível identificar a partir da única valva ilustrada, mas a densidade de estrias é alta (18 em 10 µm), não concordando com *P. rostratum*.

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, Lago das Garças, 14–I–1997, D.C. Bicudo & L.L. Morandi (SP294904, SP294905).

***Planothidium victori*** Novis, Braidwood & Kilroy 2012, p. 22, figs 26–41, 161

Figuras 55–62

Características métricas: 7,0–17,0 µm de comprimento; 3,5–4,8 µm de largura, 14–18 estrias em 10 µm.

O táxon é semelhante a *P. frequentissimum* (Lange-Bertalot) Lange-Bertalot, que apresenta valvas rômbico-lanceoladas e área axial mais larga que o da espécie encontrada (Wetzel et al. 2019b).

*Planothidium victori* é citado para o Brasil e Estado de São Paulo pela primeira vez no presente estudo.

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, hidrofitotério, 7–XI–1996, D.C. Bicudo & L.L. Morandi (SP294903); 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427341, SP427342).

***Planothidium* sp.**

Figuras 63–64

Características métricas: 11,9–14,0 µm de comprimento; 4,7–5,3 µm de largura, 15–16 estrias em 10 µm.

Apenas duas valvas arrafídeas foram encontradas, assim não foi possível identificar o táxon.

Apesar de se aproximar morfologicamente a *P. victori*, a espécie se diferenciou por manter o formato valvar elíptico-lanceolado mesmo em espécime maior. A identificação só será possível mediante análise de mais espécimes.

Material examinado: BRASIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, Jardim Botânico, hidrofitotério, 7–VI–2011, P.D.A. Silva, G.C. Marquardt, S. Wengrat & B. Pellegrini (SP427343).

### **Considerações finais**

Um terço das espécies encontradas, pertencentes a *Achnanthidium*, estão sendo descritas em outro estudo como novidades para a Ciência a partir de amostras do Estado de São Paulo, além de outras duas já recentemente descritas para a região (*A. tropicocatenatum* e *Cocconeis amerieuglypta*). Assim, fica clara a importância de estudos taxonômicos aprofundados sobre grupos específicos de diatomáceas para o real conhecimento da biodiversidade de diatomáceas tanto no Estado quanto no país. Por fim, esse estudo soma uma importante contribuição a respeito do grupo de diatomáceas monorrafídeas no PEFI, incluindo também novas citações para o Estado de São Paulo e Brasil (*Platessa hustedtii* e *Planothidium victori*, respectivamente).

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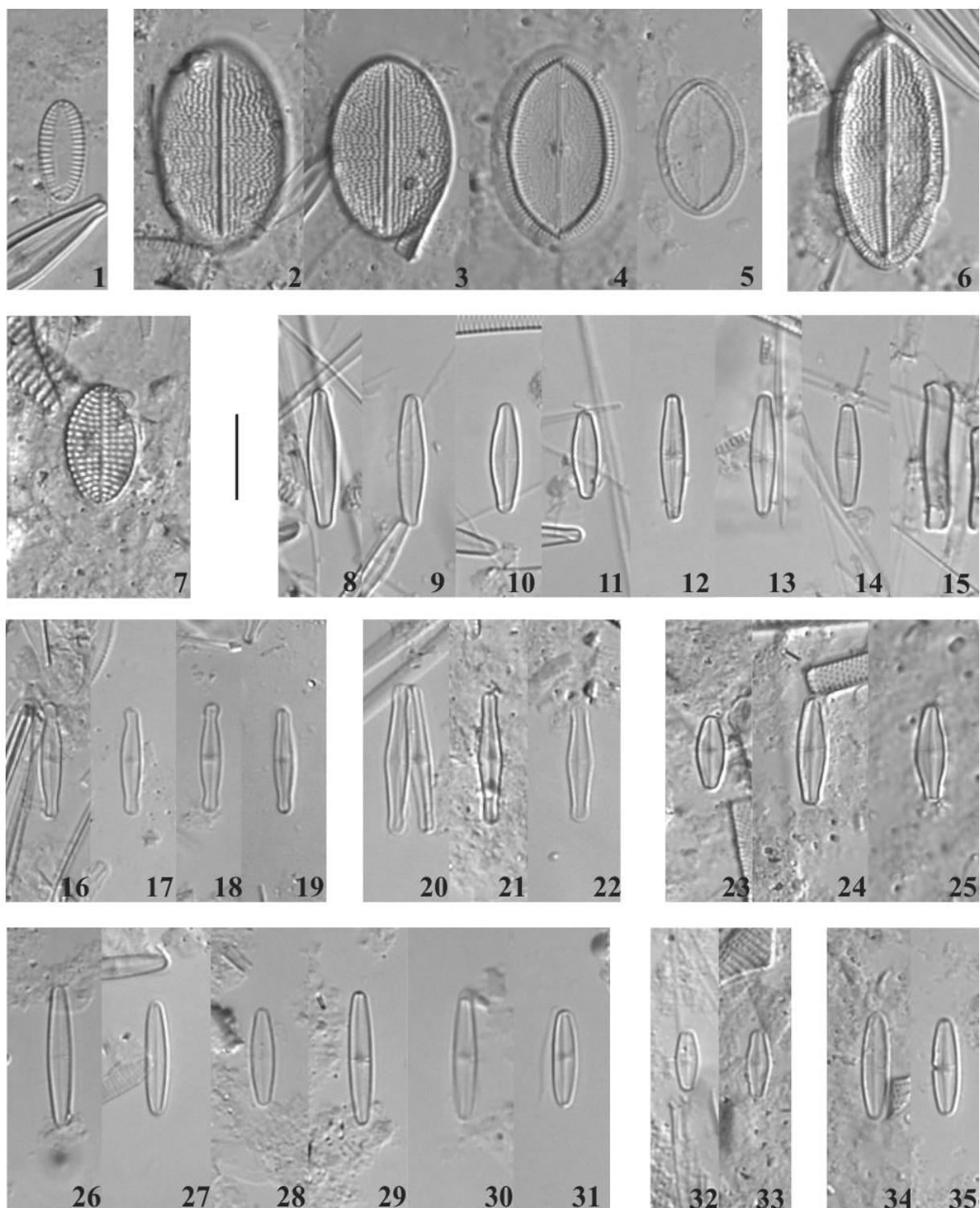
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**Tabela 1.** Informações gerais sobre as lâminas analisadas: números de herbário, data e local de coleta, coletores e habitat amostrado. \*Não foram encontrados táxons monorrafídeos.

| Número de Herbário                 | Data da coleta | Coletores   | Local  | Habitat   |
|------------------------------------|----------------|---|--|---|
| SP255743,<br>SP255744*             | 18/07/1991     | Bicudo, D.C., Ludwig,<br>T.A.V. & Figueiredo,<br>D.M.                 | Lago das Ninféias  | Perifítón,<br>fitoplâncton                        |
| SP255745,<br>SP255746*             | 18/07/1991     | Bicudo, D.C., Ludwig,<br>T.A.V. & Figueiredo,<br>D.M.                 | Hidrofitotério   | Fitoplâncton,<br>perifítón                        |
| SP255747,<br>SP255748*             | 18/07/1991     | Bicudo, D.C., Ludwig,<br>T.A.V. & Figueiredo,<br>D.M.                 | Lago dos Bugios  | Perifítón,<br>fitoplâncton                        |
| SP255749*,<br>SP255750*            | 18/07/1991     | Bicudo, D.C., Ludwig,<br>T.A.V. & Figueiredo,<br>D.M.                 | Córrego<br>Pirarungáua                                     | Perifítón,<br>fitoplâncton                        |
| SP255751,<br>SP255752*             | 18/07/1991     | Bicudo, D.C., Ludwig,<br>T.A.V. & Figueiredo,<br>D.M.                 | Lago das Garças  | Perifítón,<br>fitoplâncton                        |
| SP294899,<br>SP294900              | 07/11/1996     | Bicudo, D.C. &<br>Morandi, L.L.                                       | Lago das Ninféias  | Perifítón,<br>fitoplâncton                        |
| SP294902                           | 07/11/1996     | Bicudo, D.C. &<br>Morandi, L.L.                                       | Lago dos Bugios  | Fitoplâncton                                      |
| SP294903                           | 07/11/1996     | Bicudo, D.C. &<br>Morandi, L.L.                                       | Hidrofitotério   | Perifítón   |
| SP294904,<br>SP294905              | 14/01/1997     | Bicudo, D.C. &<br>Morandi, L.L.                                       | Lago das Garças  | Perifítón,<br>fitoplâncton                        |
| SP294906,<br>SP294907,<br>SP294908 | 15/01/1997     | Bicudo, D.C. &<br>Morandi, L.L.                                       | Lago do Centro de<br>Ciências e<br>Tecnologia<br>(Cientec) | Perifítón,<br>fitoplâncton,<br>metafítón          |
| SP294909                           | 01/03/1997     | Bicudo, D.C. &<br>Morandi, L.L.                                       | Lago do Monjolo  | Perifítón   |
| SP427341,<br>SP427342,<br>SP427343 | 07/06/2011     | Silva, P.D.A.,<br>Marquardt, G.C.,<br>Wengrat, S. &<br>Pellegrini, B. | Hidrofitotério   | Perifítón,<br>material sobre<br>musgos            |
| SP427344,<br>SP427346              | 07/06/2011     | Silva, P.D.A.,<br>Marquardt, G.C.,<br>Wengrat, S. &<br>Pellegrini, B. | Jardim Botânico  | Material sobre<br>musgos e<br>tronco de<br>árvore |

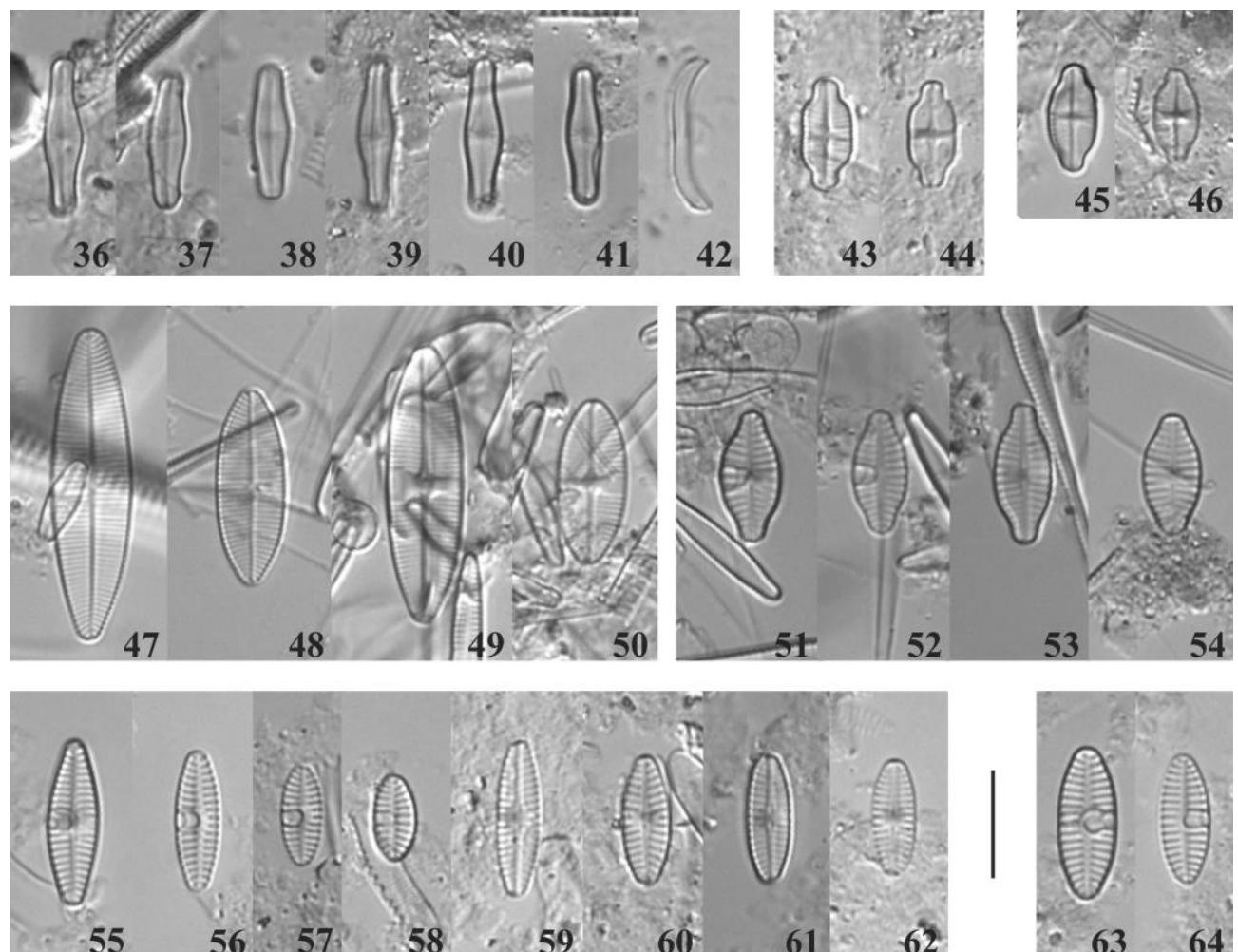
**Tabela 2.** Ocorrência de cada táxon por amostra/número de herbário.



Figuras 1–35. Representantes de diatomáceas monorrafídeas. 1. *Platessa hustedtii* (Krasske) Lange-Bertalot. 2–5. *Cocconeis amerieuglypta* L.F.Costa, C.E.Wetzel & Ector. 6. *Cocconeis euglypta* Ehrenberg. 7. *Cocconeis neodiminuta* Krammer. 8–15. *Achnanthidium minutissimum* (Kützing) Czarnecki. 16–19. *Achnanthidium peetersianum* C.E.Wetzel, Juttner & Ector. 20–22. *Achnanthidium tropicocatenatum* Marquardt, C.E.Wetzel & Ector. 23–24. *Achnanthidium*

sp. 2. 26–31. *Achnanthidium* sp. 1. 32–33. *Achnanthidium* sp. 3. 34–35. *Achnanthidium* sp. 7.

Escala = 10 µm.



Figuras 36–64. Representantes de diatomáceas monorrafídeas. 36–42. *Achnanthidium* sp. 6.

43–44. *Gogorevia constricta* (Torka) Kulikovskiy & Kociolek. 45–46. *Gogorevia exilis*

(Kützing) Kulikovskiy & Kociolek. 47–50. *Lemnicola hungarica* (Grunow) Round & Basson.

51–54. *Planothidium rostratoholarcticum* Lange-Bertalot & Bak. 55–62. *Planothidium victori*

Novis, Braidwood & Kilroy. 63–64. *Planothidium* sp. Escala = 10 µm.

## CAPÍTULO 4

### Monoraphid diatoms diversity in freshwater environments from São Paulo State, Brazil

#### Abstract

Monoraphid diatoms are not a monophyletic group despite their shared characteristic: just one of the valves possesses raphe. Considering the previous studies on two monoraphid genera (*Achnanthidium* and *Cocconeis*) in the State of São Paulo, this study aims to complete and enhance the knowledge of all other monoraphid diatoms diversity in the study area. Samples collected from freshwater environments throughout the State of São Paulo, covering all the hydrographic basins and emphasizing the Alto Tietê basin, were analyzed in LM and SEM. Thirty-four monoraphid taxa belonging to seven distinct genera were found in analyzed samples: *Achnanthes* (4), *Platessa* (3), *Gogorevia* (5) *Planothidium* (17), *Psammothidium* (2), *Lemnicola* (1), *Skabitschewskia* (1), and *Karayevia* (1). Great part of the monoraphid species were rare but still there was a great diversity, including eight new records for Brazil and seven more for São Paulo state.

#### Introduction

The history of the monoraphid diatoms began with all the taxa being described in *Achnanthes* Bory sensu lato. In the 90's a split process started with the establishment of a new family, Achnanthidiaceae D.G.Mann, including *Achnanthidium* Kützing and *Eucocconeis* Cleve ex Meister (Round et al. 1990), and then the description of some new genera belonging to Achnanthidiaceae: *Karayevia* Round & Bukhtiyarova ex Round, *Planothidium* Round & Bukhtiyarova, *Psammothidium* Bukhtiyarova & Round, *Rossithidium* Bukhtiyarova & Round and *Lemnicola* Round & Basson (Bukhtiyarova & Round 1996, Round & Bukhtiyarova 1996, Round & Basson 1997).

At least 17 monoraphid genera occur in freshwater, some of them are exclusively from this habitat and others can also occur in marine environments (Kociolek et al. 2020). Key characteristics to distinguish monoraphid genera include the shape of valve, areolae, distal raphe ends, and number of areolae rows per stria.

*Planothidium* was described to accommodate some *Achnanthes* species with a 'hoof-mark' structure on rapheless valve, bi-multiseriate striae, and distal raphe ends uniterally bent (Round & Bukhtiyarova 1996). In the same protologue, the authors described *Rossithidium*

having linear valves with parallel striae, usually continuous in the central area of the rapheless valve, with presence or absence of a stauros on the raphe valve, and distal raphe ends also uniterally bent. The last genus established by Round & Bukhtiyarova (1996) was *Karayevia*. Besides it was known as an Achnanthidiaceae taxa, currently it is grouped with *Madinithidium* C.Desrosiers, A.Witkowski & C.Riaux-Gobin (marine monoraphid taxon) in an independent clade of Stauroeidaceae D.G.Mann family (Kulikovskiy et al. 2019a).

In the same year, Bukhtiyarova & Round (1996) settled the name *Psammothidium* to designate taxa presenting raphe valve convex and rapheless valve concave, central area distinct and rectangular on raphe valve, distal raphe endings absent or bent to opposite sides, and areolae with different shapes. Usually, SEM analysis is needed to distinguish *Psammothidium* species (Bukhtiyarova & Round 1996). One year later, *Lemnicola* was characterized by the bisseriate striae, large and asymmetric stauros on raphe valve and very reduced on rapheless valve (Round & Basson 1997). Some other freshwater genera belonging to Achnanthidiaceae were described later (*Kolbesia* Round & Bukhtiyarova ex Round, *Crenotia* Wojtal, *Gliwiczia* Kulikovskiy, Lange-Bertalot & Witkowski, and *Skabitschewskia* Kulikovskiy & Lange-Bertalot). *Gogorevia* Kulikovskiy, Glushchenko, Maltsev & Kociolek is the most recently described, including species from *Achnanthes exigua* complex (Kulikovskiy et al. 2020).

Nowadays *Achnanthes* is almost restrict to marine and brackish taxa, and can be recognized by the presence of conspicuous areolae occluded by cribra (Toyoda et al. 2005). More recently, two freshwater genera were described in Achnanthaceae. *Platessa* Lange-Bertalot is characterized by small-elliptic valves, striae predominantly bisseriate and areolae occluded by hymen (Romero 2016). *Platebaikalia* Kulikovskiy, Glushchenko, Genkal & Kociolek is monospecific and can be easily distinguish from other in the group by multiserrate striae (Kulikovskiy et al. 2019b). The last family, Coccineidaceae, is composed of two genera occurring in freshwater despite of their preference for marine habitats (*Coccneis* Ehrenberg and *Anorthoneis* Grunow).

Studied Brazilian freshwater ecosystems present great occurrence and rich diversity of monoraphid taxa, including unidentified (generic level only) and some new taxa described mainly in Southeastern region (Marquardt et al. 2017, Costa et al. 2019, Costa et al. Cap. 2), but also in South (Wetzel & Ector 2014a, b), and North of the country (Metzeltin & Lange-Bertalot 1998, Tudesque et al. 2016, Morais et al. 2020). Despite of this, only other few studies were focused in this group of diatoms (Rodrigues & Moreira-Filho 1990, Carneiro & Bicudo 2007, Ferrari & Ludwig 2007, Costa et al. Cap. 2, 3). In the State of São Paulo, thirty-

two taxa were cited and illustrated until the present moment (Bicudo et al. 1993, Bicudo et al. 2009, Costa-Böddeker et al. 2012, Fontana & Bicudo 2012, Faustino et al. 2016, Bartozek et al. 2018, Costa et al. 2019, Marquardt et al. 2017, 2018).

Considering the lack of knowledge about monoraphid diversity, this group has been intensively studied in the State of São Paulo over the last years and the results for *Achnanthidium* and *Cocconeis* taxa were published or are on the way (Costa et al. 2019, Costa et al. Cap. 2). In addition, one study about monoraphid diversity carried out with samples from Parque Estadual das Fontes do Ipiranga (PEFI) is also in final phase to be submitted (Costa et al. Cap. 3). In order to encompass the other monoraphid taxa, this study aims to complete and enhance the knowledge of all monoraphid diatoms diversity in the State of São Paulo, excluding the two mentioned genera already studied.

## **Material and methods**

*Study area* – The state of São Paulo has a population of approximately 46 million inhabitants living in a territory of 248,219 km<sup>2</sup> (IBGE 2019). This area covers part of three Brazilian hydrographic regions: Paraná, Atlântico Sul and Atlântico Sudeste (ANA 2020); and it is divided in 22 hydrographic basins. Among them, the Alto Tietê basin has the greatest economic importance centralizing the most important industrial, commercial and financial complexes in the country (CBH-TJ 2010). In this way, the population distribution is directly linked to the economic bias, and the region houses almost half of the São Paulo state population, approximately 20 million habitants, in just over 2% of the state area (PERH 2006).

*Material* – Two sampling campaigns were carried out in freshwater environments throughout the state of São Paulo, covering all the hydrographic basins but emphasizing the Alto Tietê basin. Part of the collected material (344 samples) was analyzed here focusing on monoraphid diatom diversity, except for *Achnanthidium* and *Cocconeis*. The material included samples from different habitats (phytoplanktonic, periphytic, metaphytic, surface sediments). General information about sampling sites and collected samples were provided in previous study (Costa et al. Cap. 2). Kützing's sample 397 (Kützing Collection), type of *Stauroneis exilis* was also analyzed.

*Preparation* – Following ECS (2003), concentrated 35% H<sub>2</sub>O<sub>2</sub> and 37% HCl were added to the raw samples to digest the organic matter. Posteriorly, samples were cleaned with

deionized water. Two different procedures were performed subsequently: 1) preparation of permanent slides for light microscopy (LM), and 2) preparation of ‘stubs’ for scanning electron microscopy (SEM). Permanent slides were mounted with a small fraction of digested and cleaned materials deposited on slides and closed using Naphrax® mounting medium. For SEM analysis, small fractions of the processed materials were filtered through a polycarbonate membrane filter (3 µm), washed with deionized water, and posteriorly mounted on aluminum stubs. Before SEM analysis, stubs were coated with platinum in a BAL-TEC MED 020 (Wetzel & Ector 2014a).

*Microscopy* – For LM, a Leica light microscope equipped with phase-contrast was used, and the scanning electron microscopy analysis was performed with a Hitachi SU–70. CorelDraw X8 was used to organize the micrographs and to create the plates exposed here. The striae were counted above the stauros/fascia when it present.

*Distribution* – Only studies with taxonomic information were considered for Brazilian distribution and new records.

## Results and discussion

Thirty-four monoraphid taxa belonging to eight distinct genera were found in samples analyzed from São Paulo state: *Achnanthes* (4), *Plateissa* (3), *Gogorevia* (5), *Planothidium* (17), *Psammothidium* (2), *Lemnicola* (1), *Skabitschewskia* (1), and *Karayevia* (1). The current study presents 34 taxa classified and discussed below. The raphe valve was abbreviated as RV and the rapheless valve as SV (sternum valve).

Division Bacillariophyta Haeckel 1878

Subdivision Bacillariophytina Medlin & Kaczmarska 2004 emend. Adl et al. 2019

Class Bacillariophyceae Haeckel 1878 emend. Adl et al. 2019

Subclass Bacillariophycidae D.G.Mann in Round et al. 1990 emend. Adl et al. 2019

Order Mastogloiales D.G.Mann emend. E.J.Cox 2006

Family Achnanthaceae Kützing 1844

*Achnanthes* Bory 1822

***Achnanthes coarctata* (Brébisson ex W. Smith) Grunow**

Figure 1A–E

Basionym: *Achnanthidium coarctatum* Brébisson ex W. Smith 1855, p. 8; pl. 1, fig. 10

Morphometry: 21.6–33.8 µm long, 5.7–7.4 µm wide, RV: 15–17 striae in 10 µm, SV: 14–15 striae in 10 µm, 15–16 areolae in 10 µm.

The most significant difference between *A. coarctata* and *A. pseudocoarctata* Levkov & Tofilovska is the shape of the ciliate occlusions (Tofilovska et al. 2014), feature not observed in our population due to the rarity of the taxa, which could not be found in SEM analysis. However, the taxa also can be differentiated by the striae and areolae density on the rapheless valve: *A. coarctata* has greater striae density (12–14 striae/10 µm, 18–20 areolae/10 µm) than *A. pseudocoarctata* (8–10 striae/10 µm, 10–13 areolae/10 µm, Tofilovska et al. 2014). In this way, our specimens presented striae density more in accordance to *A. coarctata*.

Distribution: In Brazil, *A. coarctata* was already observed in the South region in Santa Catarina and Rio Grande do Sul states (Rodrigues 1984, Torgan 1985, Oliveira et al. 2002). For São Paulo state, this study provides the first mention to the species with taxonomic data.

***Achnanthes inflata* (Kützing) Grunow**

Figure 1F

Basionym: *Stauroneis inflata* Kützing 1844, p. 105; pl. 30, fig. 22

Morphometry: 30.1 µm long, 10.6 µm wide, SV: 13 striae in 10 µm.

Taxon very rare in the studied samples, but the species presents distinct morphological features allowing the identification: valves with the median portion widely inflate, with apices broadly rounded and capitate; a distinct transverse fascia in the central area of the raphe valve; on the rapheless valve the axial area is eccentric, positioned on the valve margin.

Distribution: The Brazilian occurrence of this taxon includes the states of São Paulo (Bicudo et al. 1993, Moutinho et al. 2007, Bicudo et al. 2009), Paraná (Moro & Fürstenberger 1993, Ferrari & Ludwig 2007, Bertolli et al. 2010), Santa Catarina (Souza-Mosimann 1982, Rodrigues & Moreira-Filho 1990, Fernandes 1996), and Rio Grande do Sul (Rodrigues 1984, Oliveira et al. 2001, Silva et al. 2017).

***Achnanthes subhudsonis* Hustedt**

Figure 1G–J

Morphometry: 13.9–15.2  $\mu\text{m}$  long, 3.6–3.9  $\mu\text{m}$  wide, SV: 22 striae in 10  $\mu\text{m}$ , SV: 21–23 striae in 10  $\mu\text{m}$ .

The species was recently transferred to *Achnanthidium* as *Achnanthidium subhudsonis* (Hustedt) H.Kobayasi (Kobayashi et al. 2006). However, according to other authors (Ohtsuka et al. 2007, Spaulding & Potapova 2014), *A. subhudsonis* probably does not belongs to *Achnanthes*, to *Achnanthidium*, or even to any already described genera. In this way, more study is necessary to allocate this taxon in a new genus.

The variety *Achnanthes subhudsonis* var. *kraeuselli* (Cholnoky) Cholnoky has lower striae density than the type species (16-18 in 10  $\mu\text{m}$ , Cholnoky 1954), and it is also radiate, but more information around this taxon is also needed.

Distribution: *Achnanthes subhudsonis* is cited here for the first time in Brazil.

#### *Achnanthes ziegleri* Lange-Bertalot

Figure 1K–M

Morphometry: 11.7–14.5  $\mu\text{m}$  long, 5.9–6.6  $\mu\text{m}$  wide, RV: inconspicuous striae, SV: 24 striae in 10  $\mu\text{m}$ .

Already transferred to *Platessa* as *Platessa ziegleri* (Lange-Bertalot) Lange-Bertalot (Krammer & Lange-Bertalot 2004), the taxon probably belongs to the new genus proposed to accommodate *Gogorevia exilis* (Kützing) Kulikovskiy & Kociolek and allied taxa. It can be compared to *Gogorevia* sp. 2, but differs mainly by its lanceolate axial area, widening towards central area on rapheless valve.

Distribution: The taxon was already recorded for Rio Grande do Sul state (Silva et al. 2017), however it can be another taxon due to the slight constriction on the median part of valve and linear axial area. In the State of São Paulo, this is the first record for *A. ziegleri*.

*Platessa* Lange-Bertalot 2004

#### *Platessa guianensis* R.Le Cohu, L.Tudesque & C.E.Wetzel

Figure 3A

Morphometry: 10.6  $\mu\text{m}$  long, 6.2  $\mu\text{m}$  wide, SV: 15 striae in 10  $\mu\text{m}$ .

Taxon was very rare, and just one valve was found in samples, however the morphology of it is very characteristic allowing the identification. Valves have elliptic-rhomboidal outline with

broadly rounded apices; and large axial area covered with numerous depressions on the rapheless valve surface (Tudesque et al. 2016).

**Distribution:** *Platessa guianensis* holotype was described from French Guyana and the paratype is from Amazon, Brazil (Tudesque et al. 2006). For São Paulo state, this study provides the first record.

***Platessa hustedtii* (Krasske) Lange-Bertalot**

Figure 3B–G

**Basionym:** *Cocconeis hustedtii* Krasske 1923, p. 193; fig. 10a, b

**Morphometry:** 11.8–14.2 µm long, 5.6–6.5 µm wide, RV: 19 striae in 10 µm, SV: 18–19 striae in 10 µm.

This taxon was found in greater abundance than the other *Platessa* species and all its valve views were illustrated in SEM. *Platessa hustedtii* obviously differs from *P. oblongella* (Østrup) C.E.Wetzel, Lange-Bertalot & Ector by presenting striae density more similar in both valves, besides the wider lanceolate axial area on rapheless valve. In SEM, *P. hustedtii* shows biserrate striae on both valves while *P. oblongella* has uniseriate striae on raphe valve.

**Distribution:** Cited for São Paulo (Costa et al. Cap. 3), Rio Grande do Sul and Paraná states, sometimes as *Achnanthes rupestoides* Hohn (Oliveira et al. 2002, Ferrari & Ludwig 2007, Moresco et al. 2011, Bes et al. 2012, Lobo et al. 2016, Silva et al. 2017), the taxa is already known occurring in Brazilian waters.

***Platessa oblongella* (Østrup) C.E.Wetzel, Lange-Bertalot & Ector**

Figure 3H–K

**Basionym:** *Achnanthes oblongella* Østrup 1902, p. 252 (34); pl. 1, fig. 9

**Morphometry:** 11.2–15.4 µm long, 5.0–7.0 µm wide, RV: 20–28 striae in 10 µm, SV: 11–17 striae in 10 µm.

*Platessa oblongella* has elliptic valves with broadly rounded apices; central area transapically expanded and bordered by shorter striae with different lengths on raphe valve, and large irregular axial area on rapheless valve (Wetzel et al. 2018). The different striae density between raphe and rapheless valve are clearly visible. Rapheless valve has lower density and conspicuous striae arranged by areolae visible in LM.

Distribution: Rosa & Garcia (2014) cited *P. oblongella* as *Karayevia oblongella* (Østrup) M.Aboal occurring in Rio Grande do Sul state. The taxon is cited here for the first time occurring in São Paulo state.

Família Achnanthidiaceae D.G. Mann 1990

*Gogorevia* Kulikovskiy, Glushchenko, Maltsev & Kociolek 2020

***Gogorevia constricta*** (Torka) Kulikovskiy & Kociolek

Figure 1N–T

Basionym: *Coccneis exigua* var. *constricta* Torka 1909, p. 131; fig. 3a

Morphometry: 13.0–15.3 µm long, 4.8–5.8 µm wide, RV: 28–30 striae in 10 µm, SV: 21–23 striae in 10 µm.

Hustedt (1921) described *A. exigua* var. *constricta* Hustedt as a new taxon, but later he realizes it was the same species as described by Torka (1909), *Coccneis exigua* var. *constricta*. He ignored his own taxon and transferred the *Coccneis* species to *Achnanthes* (Hustedt 1930). Recently, molecular and morphological investigations led to the transference of the taxon to the new genus *Gogorevia* (Kulikovskiy et al. 2020).

The taxon differs from *Gogorevia exilis* (Kützing) Kulikovskiy & Kociolek by the constriction in the median part of the valve. It usually can be slightly constricted on one side or on both making more difficult the distinction between both taxa, but in addition, *G. constricta* can also have shoulders more developed and wider valves than *G. exilis*.

Distribution: In the Northern region of Brazil, it was already mentioned as *Achnanthes exigua* var. *constricta* (Metzeltin & Lange-Bertalot 1998), whereas in Southern Brazil it was mainly identified as *Achnanthidium exiguum* var. *constrictum* (Grunow) N.A.Andresen, Stoermer & R.G.Kreis (Bartozek et al. 2013, Lobo et al. 2016, Nardelli et al. 2016, Silva et al. 2017), but also as *Achnanthes exigua* var. *constricta* (Oliveira et al. 2001), *A. exiguum* (Ferrari & Ludwig 2007) and *Achnanthes exigua* (Rodrigues & Moreira-Filho 1990, Lobo et al. 2004). In São Paulo State, it was cited as *Achnanthes exigua* var. *exigua* (Bicudo et al. 1993: fig. 35), *Achnanthidium exiguum* (Fontana & Bicudo 2012), and recently as *Gogorevia* (Costa et al. Cap. 3).

***Gogorevia exilis*** (Kützing) Kulikovskiy & Kociolek

Figure 1AF–AJ

Basionym: *Stauroneis exilis* Kützing 1844, p. 105, pl. 30, fig. 21.

Morphometry: 9.2–12.2 µm long, 4.5–4.8 µm wide, RV: 25–26 striae in 10 µm, SV: 24–25 striae in 10 µm.

The taxonomy of this taxon was confused for long time. Since its description, it was transferred to *Achnanthes* (Cleve & Grunow 1880), *Achnanthidium* (Czarnecki 1994), and *Lemnicola* (Plinski & Witkowski 2011). The last name, *Lemnicola exigua* (Grunow) Kulikovskiy, Witkowski & Plinski had not a great acceptance by the scientific community since some authors agreed that morphological approach was not enough and molecular data were necessary to decide the appropriate genus for this taxon (Potapova 2010b). Recently, a molecular and morphological study resulted in the description of a new genus to accommodate this species and allied taxa, transferring them to *Gogorevia* (Kulikovskiy et al. 2020).

Together with molecular, morphological analysis also shows that the previous genus was not the best place for *G. exilis*. On Table 1 features of some other genera are compared to *Gogorevia*. *Achnanthidium* species have usually slender valves, and they are at least three times longer than wide. In comparison, *G. exilis* and similar species of the complex have shorter and wider valves forming visible shoulders even when it is poorly developed. Other morphological characteristic differing the mentioned taxa from *Achnanthidium* is the central area on the rapheless valve, which in the latter genus it is often symmetrical, composed of 1–3 shortened striae slightly more spaced. *Gogorevia exilis* has an asymmetric central area, also with more distant striae but on one side forming a greater hyaline area up to the valve margin, and on the other side forming a weakly more distant striae. The central area on the raphe valve is also formed always by an asymmetrical fascia as in *Lemnicola*, but less common in *Achnanthidium* taxa. Despite that, *Lemnicola* has biseriate striae, linear to linear-elliptical valves with rounded to subrostrate apices (Round & Basson 1997, Taylor et al. 2014). *Gogorevia exilis* presents uniseriate striae but very occasionally biseriate near the apices and protracted rostrate apices (Taylor et al. 2014 as *Achnanthidium exiguum*).

Comparing *G. exilis* to other monoraphid genera as *Psammothidium* (Table 1) it is possible to notice the incongruity. *Psammothidium* has convex raphe valve and concave rapheless valve differing from other ‘achnanthoid’ genera. Besides that, the areolae are occluded internally by cibrium and not by hymenes as in *Lemnicola* and *Achnanthidium*.

Distribution: In Southern Brazil, it occurred in all the states (Ferrari & Ludwig 2007, Ribeiro et al. 2008, Bertolli et al. 2010, Faria et al. 2010, Santos et al. 2011, Lobo et al. 2016, Marra et

al. 2016 as *Achnanthidium exiguum*, and Rodrigues 1984, Rodrigues & Moreira-Filho 1990, Moro & Fürstenberger 1993, Lobo et al. 2004, as *Achnanthes exigua*). Also, it was cited for São Paulo state as *Achnanthes exigua* (Bicudo et al. 1993, Magrin & Senna 1997), and as *Achnanthidium exiguum* (Bicudo et al. 2009, Bartozek et al. 2018).

***Gogorevia parexigua* (Metzeltin & Lange-Bertalot) Kulikovskiy & Kocolek**

Figure 1AK

Basionym: *Achnanthes parexigua* Metzeltin & Lange-Bertalot 1998, p. 19; pl. 67, fig. 1-11, pl. 68, fig. 1-3

Morphometry: 18.6 µm long, 7.4 µm wide, SV: 19 striae in 10 µm.

*Gogorevia parexigua* bears many similarities with *G. constricta* considering valve outline and central area format. Despite this, the dimensions of the valve can separate both, the first having bigger valves. *Gogorevia parexigua* has broader apices, central area coarser in LM, and rounded to apically elongated areolae (Metzeltin & Lange-Bertalot 1998). In addition, the only one valve found showed the areolae visible in LM, characteristic never seen in *G. constricta*.

Distribution: Species described from Amazonas state in Brazilian Northern (Metzeltin & Lange-Bertalot 1998). It was also mentioned as *Achnanthidium exiguum* for Paraná state (Ferrari & Ludwig 2007: fig. 31), and recently mentioned occurring in Rio Grande do Sul as *Achnanthes parexigua* (Silva et al. 2017). For the state of São Paulo, it is firstly recorded in this study.

***Gogorevia* sp. 1**

Figure 1AL–AN

Morphometry: 17.5–23.0 µm long, 6.7–8.1 µm wide, RV: 34 striae in 10 µm, SV: 21–23 striae in 10 µm.

Taxon very rare in samples, not found in SEM analysis, and probably new. The species found is part of *G. exilis* complex, but more study is necessary, including SEM images.

It has bigger valves than *G. constricta* despite their resemblance by the presence of median constriction on one or both sides of the valve, similar valve outline, and apices format. The central area is also formed by a rectangular (or bow-tie shaped) fascia on raphe valve and asymmetrical spaced striae on rapheless valve as the usually found in this complex of species.

***Gogorevia* sp. 2**

Figure 1AO–AR

Morphometry: 13.0–14.4  $\mu\text{m}$  long, 5.7–6.1  $\mu\text{m}$  wide, RV: 28 striae in 10  $\mu\text{m}$ , SV: 20–21 striae in 10  $\mu\text{m}$ .

As *Gogorevia* sp. 1, valves were not found in SEM analysis and more studies are necessary. Resembling *G. exilis*, the taxon differs by its bigger apices, more subcapitate than rostrate, lower striae density in the rapheless valve, making the striae more visible and marked, and by the wider valves. *Achnanthes ziegleri* can be also confounded with *Gogorevia* sp. 2 by similar morphology, but the lanceolate axial area on the rapheless valve of the first can be one of the distinct features between them.

*Planothidium* Round & Bukhtiyarova 1996

***Planothidium alekseevae* Gogorev & Lange-Bertalot**

Figure 7J–L

Morphometry: 11.9–14.5  $\mu\text{m}$  long, 4.4–5.1  $\mu\text{m}$  wide, SV: 16–17 striae in 10  $\mu\text{m}$ .

Rare taxon, only rapheless valves were found. It bears some similarities with *P. rostratoholarticum* Lange-Bertalot & Bąk in valve outline and metrics features.

*Planothidium alekseevae* differs by having valves lanceolate to elliptic-lanceolate with rostrate apices instead of the elliptical valves with weakly elongated apices, usually broadly protracted and subrostrate in *P. rostratoholarticum* (Wetzel et al. 2019). In our specimens, we can notice the slender valve outline in *P. alekseevae* with more lanceolate valves narrowing to the rostrate apices while in our *P. rostratoholarticum* population the valves are elliptic with a weak shoulder. In addition, *P. biporomum* (M.H.Hohn & Hellerman) Lange-Bertalot has greater valves despite the similar morphology (13.0–26.0  $\mu\text{m}$  long, 6.0–7.0  $\mu\text{m}$  wide, Wetzel et al. 2013).

Distribution: First time cited for Brazil.

***Planothidium bagualense* C.E.Wetzel & Ector**

Figure 7Z

Morphometry: 15.3  $\mu\text{m}$  long, 6.5  $\mu\text{m}$  wide, SV: 13 striae in 10  $\mu\text{m}$ .

Rare taxon, only a rapheless valve was found. Presenting elliptic to elliptic-lanceolate valves with blunt rounded apices (Wetzel & Ector 2014a), this taxon has similar valve outline and

metric features to *P. hinzianum* C.E.Wetzel, Van de Vijver & Ector. Despite that, their rapheless valves can be distinguished by the slightly lower striae density in *P. bagualense* (*P. hinzianum*: 14–16 striae in 10 µm, Wetzel et al. 2019), and by possessing axial area very narrow, linear and straight, while *P. hinzianum* has axial area narrow, but widening asymmetrically towards the central area.

**Distribution:** Described from Rio Grande do Sul state, its distribution was assessed in the protologue, including several studies from South and Southeast regions (Wetzel & Ector 2014a). Later, the species was cited again in Southern Brazil (Ruwer & Rodrigues 2018, Silva et al. 2017).

***Planothidium brasiliense* C.E.Wetzel & S.Blanco**

Figure 7A–B, AM

**Morphometry:** 21.0–22.7 µm long, 7.4–7.6 µm wide, RV: 16 striae in 10 µm, SV: 15 striae in 10 µm.

Rare taxon observed from type sample identified in Wetzel et al. (2019). It has longer and wider valves than other ‘rostrate’ species but also differs from *P. incuriatum* C.E.Wetzel, Van de Vijver & Ector by presenting gently shoulders and apices more subcapitate than rostrate.

**Distribution:** It was described from sample collected in São Paulo state (Wetzel et al. 2019), and also analyzed in this study.

***Planothidium hinzianum* C.E.Wetzel, Van de Vijver & Ector**

Figure 7AA–AB

**Morphometry:** 10.5–15.6 µm long, 5.4–6.5 µm wide, SV: 14–15 striae in 10 µm.

With similar morphology, two other species are close to *P. hinzianum*: *P. bagualense* (see *P. bagualense*) and *P. victori* P.M.Novis, J.Braidwood & C.Kilroy. However, it differs from the latter by the wider valves (*P. victori*: 4.0–5.5, Wetzel et al. 2019) and by the axial area narrow straight and linear in *P. hinzianum*.

**Distribution:** First time cited for Brazil.

***Planothidium incuriatum* C.E.Wetzel, Van de Vijver & Ector**

Figure 4A–O

Morphometry: 16.7–22.9  $\mu\text{m}$  long, 5.7–6.8  $\mu\text{m}$  wide, RV: 13–14 striae in 10  $\mu\text{m}$ , SV: 13–14 striae in 10  $\mu\text{m}$ .

Great populations were observed allowing the complete LM and SEM analysis. Valves are lanceolate to elliptic-lanceolate with rostrate protracted apices (Wetzel et al. 2013). The cavum on rapheless valve has a large opening forming a second semicircular line greater than the horseshoe-shaped hyaline area and clearly visible on LM images.

Distribution: Described from France, it was already cited from Brazil for Rio Grande do Sul (Wetzel et al. 2013, Silva et al. 2017), and Paraná states (Silva-Lehmkuhl et al. 2019). It was also cited as *P. biporumum* (Bertolli et al. 2010, Faria et al. 2010), *P. lanceolatum* (Oliveira et al. 2001) and *P. frequentissimum* (Torgan et al. 2009). In São Paulo state, it is the first mention.

#### ***Planothidium lagerheimii* (Cleve) C.E.Wetzel & Ector**

Figure 7I

Basionym: *Cocconeis lagerheimii* Cleve, P.T., 1894. Le Diatomiste 2(19), p. 143, plate 3, figs 21–22

Morphometry: 25.6  $\mu\text{m}$  long, 13.2  $\mu\text{m}$  wide, SV: 8 striae in 10  $\mu\text{m}$ .

Rare taxon, only one rapheless valve was found. Valves of *P. lagerheimii* have an unusual shape compared with the most *Planothidium* species, they are wide lanceolate with cuneate apices. In addition, the rapheless valve presents wide axial area with irregular depressions in a linear apical direction (Wetzel & Ector 2014b), another distinct feature.

Distribution: Described from Ecuador, it was already cited in Southern, Paraná (Ferrari & Ludwig 2007 as *P. salvadorianum* (Hustedt) Lange-Bertalot), and Rio Grande do Sul states (Wetzel & Ector 2014b), and Southeastern Brazil, São Paulo state (Bicudo et al. 1993, Magrin & Senna 1997 as *Achnanthes salvadoriana* Hustedt).

#### ***Planothidium minutissimum* (Krasske) Lange-Bertalot**

Figure 7O–U, AL

Basionym: *Achnanthes lanceolata* var. *minutissima* Krasske 1938, Arch. Hydrobiol. 33, p. 513, pl. 6, figs 18–22

Morphometry: 8.1–8.9  $\mu\text{m}$  long, 3.7–4.0  $\mu\text{m}$  wide, RV: 17–20 striae in 10  $\mu\text{m}$ , SV: 17–19 striae in 10  $\mu\text{m}$ .

*Planothidium minutissimum* is part of the group of small *Planothidium* taxa without the horseshoe-shaped structure on rapheless valve. It has rhomboidal valves with rostrate apices, axial area narrowly lanceolate, and central area on rapheless valve with striae more spaced, forming a clear area on one side of the valve (Morales 2006).

It can be distinguished from *P. pumilum* Bąk & Lange-Bertalot by the slightly smaller valves, different valve outline and central area format on the rapheless valve.

Distribution: First time cited for Brazil.

***Planothidium potapovae* C.E.Wetzel & Ector**

Figure 7M–N

Morphometry: 15.5 µm long, 5.4–5.6 µm wide, SV: 13–14 striae in 10 µm.

Two rapheless valves were found with rostrate to subcapitate apices. Despite the apices more strongly rostrate in the type (Wetzel et al. 2019), another population from the USA is similar to our specimens (Potapova 2010a).

Distribution: First time cited for Brazil.

***Planothidium pumilum* Bąk & Lange-Bertalot**

Figure 7V–Y

Morphometry: 8.7–10.3 µm long, 4.3–4.8 µm wide, RV: 18–20 striae in 10 µm, SV: 14–16 striae in 10 µm.

Valves with a distinct set of features including the absence of the horseshoe-shaped structure (cavum or sinus). Small valves with elliptical to slightly linear-elliptical shape and obtusely rounded apices; axial area very narrow, slightly widening towards the small central area transapically extended on raphe valve; and axial area very narrow, straight, and central area absent on rapheless valve (Bąk & Lange-Bertalot 2014).

*Planothidium granum* (Hohn & Hellerman) Lange-Bertalot seems to be similar but has rhomboidal outline and lower striae density (12–16 in 10 µm, Morales 2006).

Distribution: First time cited for Brazil.

***Planothidium rostratoholarticum* Lange-Bertalot & Bąk**

Figure 5A–N

Morphometry: 7.9–13.3 µm long, 4.5–5.5 µm wide, RV: 14–17 striae in 10 µm, SV: 14–16 striae in 10 µm.

Great populations were observed allowing the complete LM and SEM analysis. When compared with *P. rostratum* (Østrup) Lange-Bertalot, our populations are in accordance with the smaller specimens with greater striae density find in Europe for *P. rostratoholarcticum* (Bak & Lange-Bertalot 2014). The latter authors presented the measurements of the lectotype of *P. rostratum* illustrated in Krammer & Lange-Bertalot (1991) for comparison: 9–14 µm long, 5–6 µm wide, striae density 11–12 in 10 µm. In addition, the apices of *P. rostratum* are rostrate and long while in *P. rostratoholarcticum* it is rostrate but truncate.

Bak & Lange-Bertalot (2014) also discussed the difference between paleotropical (South East Asia, Africa) and Holarctic populations concerning the number of areolae rows in each stria, however our population showed the entire range mentioned for both cited populations (2–4 rows).

Distribution: *Planothidium rostratoholarcticum* is common in South and Southeast Brazil, however it was usually confounded and cited as other taxa, mainly as *P. rostratum* (Bicudo et al. 2009, Bertolli et al. 2010, Fontana & Bicudo 2012, Bartozek et al. 2013, Marra et al. 2016), but also as *P. frequentissimum* (Schneck et al. 2007, Costa et al. 2017), and *P. biporomum* (Silva et al. 2010).

#### ***Planothidium* aff. *rostrolanceolatum* Van de Vijver, Kopalová & Zidarova**

#### Figure 7C–D

Morphometry: 24.9–25.3 µm long, 7.1–7.6 µm wide, RV: 13 striae in 10 µm, SV: 12 striae in 10 µm.

The rare rapheless valve found presents a sinus structure and not a cavum, making it part of a minor group of *Planothidium* species. Similar to *P. rostrolanceolatum*, species described from Antarctic region (Van de Vijver et al. 2013), our taxon probably correspond to another one being described. SEM analysis of other population from São Paulo shows distinct number of areolae rows per stria, and different depressions on the valve face when compared with the Antarctic taxon (C.E.Wetzel, pers. com.).

#### ***Planothidium straubianum* C.E.Wetzel, Van de Vijver & Ector**

#### Figure 6A–Q

Morphometry: 6.1–11.9  $\mu\text{m}$  long, 3.3–4.0  $\mu\text{m}$  wide, RV: 16–18 striae in 10  $\mu\text{m}$ , SV: 16–18 striae in 10  $\mu\text{m}$ .

A great population was found allowing the complete LM and SEM analysis. The elliptic-lanceolate valves of this taxon can be confounded with small specimens of *P. hinzianum* and *P. victori*. The apices are obtusely rounded in *P. straubianum* while they are rostrate in *P. victori* and acutely rounded in *P. hinzianum*, besides the slightly wider valves in the latter (5.0–6.5  $\mu\text{m}$ , Wetzel et al. 2019).

Distribution: First time cited for Brazil.

***Planothidium victori* P.M.Novis, J.Braidwood & C.Kilroy**

Figure 7AC–AD

Morphometry: 10.0–13.4  $\mu\text{m}$  long, 4.3–4.4  $\mu\text{m}$  wide, SV: 18–19 striae in 10  $\mu\text{m}$ .

Rare valves were observed. Our specimens present apices more subrostrate than really rostrate as described in Wetzel et al. (2019), but general morphometric features are in accordance.

Distribution: Already cited in the State of São Paulo (Costa et al. Cap. 3).

***Planothidium* sp. 1**

Figure 7E–F

Morphometry: 20.7–23.7  $\mu\text{m}$  long, 7.7–8.7  $\mu\text{m}$  wide, RV: 11 striae in 10  $\mu\text{m}$ , SV: 11 striae in 10  $\mu\text{m}$ .

Rare, two valves elliptic-lanceolate with subrostrate apices were observed. The rapheless valve has a wide lanceolate axial area and a cavum with a large unilateral hyaline area horseshoe-shaped on central area. The raphe valve has a linear straight axial area and a rectangular central area.

*Planothidium robustius* Lange-Bertalot is morphologically similar but it presents axial area wider and central area rounded on raphe valve. On rapheless valves it presents typical marks in axial area (Hustedt 1937), feature not observed in our specimens.

***Planothidium* sp. 2**

Figure 7G–H

Morphometry: 30.8  $\mu\text{m}$  long, 13.9  $\mu\text{m}$  wide, SV: 12 striae in 10  $\mu\text{m}$ .

Two valves were found but from different samples. Valves are lanceolate with rostrate apices. The rapheless valve has a linear straight axial area widening towards the central area, which has a large unilateral hyaline area horseshoe-shaped (cavum). The raphe valve presents an axial area straight and linear, and central area rounded but extended uniterally. Striae are punctuate with visible areolae.

*Planothidium lanceolatoides* (Sovereign) Lange-Bertalot is morphologically close to our taxon by the metrics features, however its apices are apiculate and the central area on raphe valve is clearly differentiate. The central area of *P. lanceolatoides* is variable, ranging from rectangular to bow tie-shaped (Potapova 2015). *Planothidium heteromorphum* (Grunow) Lange-Bertalot also differs by its striae strongly radiate, sometimes curved, and it has also apices more pointed as seen in population illustrated in Metzeltin & Lange-Bertalot (1998).

Distribution: This taxon was already cited as *Achnanthes heteromorpha* and *P. heteromorphum* for Santa Catarina and Paraná states (Rodrigues & Moreira-Filho 1990, Santos et al. 2011, Marra et al. 2016).

### ***Planothidium* sp. 3**

#### Figure 7AE–AI

Morphometry: 15.3–17.0 µm long, 8.4–9.2 µm wide, RV: 13–14 striae in 10 µm, SV: 14 striae in 10 µm.

The valves are elliptic to elliptic-lanceolate with rostrate apices bearing similar valve outline with the most *Planothidium* species. The rapheless valve has a wide lanceolate axial area and central area with a unilateral hyaline area horseshoe-shaped, the cavum. The raphe valve has an axial area straight and linear, and central area rounded to elliptic, sometimes extended uniterally.

The taxon is probably a new species. It differs from others by a set of features including its shorter and wider valves, lower striae density on both valves, central and axial area shape.

Distribution: Bartožek et al. (2013) and Ruwer & Rodrigues (2018) cited for Paraná a very similar taxon as *P. rostratum* morphotype I, and *Planothidium* aff. *rostratum*, respectively.

### ***Planothidium* sp. 4**

#### Figure 7AJ–AK

Morphometry: 11.9–14.0  $\mu\text{m}$  long, 4.7–5.3  $\mu\text{m}$  wide, RV: 15–16 striae in 10  $\mu\text{m}$ , SV: 14 striae in 10  $\mu\text{m}$ .

The same sample was already analyzed in Costa et al. Cap. 3, and the species from PEFI was already mentioned and illustrated as *Planothidium* sp. The species is very rare and resembles *P. victori* but they present different hood shapes.

*Psammothidium* Bukhtiyarova & Round 1996

***Psammothidium subatomoides* (Hustedt) Bukhtiyarova & Round**

Figure 8C–D, H–I

Basionym: *Navicula subatomoides* Hustedt in Schmidt et al. 1936, pl. 404, fig. 33–35

Morphometry: 8.4–8.9  $\mu\text{m}$  long, 4.1–4.7  $\mu\text{m}$  wide, RV: 46 striae in 10  $\mu\text{m}$ , SV: 42 striae in 10  $\mu\text{m}$ .

Only raphe valves were found in samples. *Psammothidium subatomoides* has elliptic valves, with bow-tied central area bordered by 4–5 striae on raphe valve (Bukhtiyarova & Round 1996). The observed species is morphologically very similar to *P. daonense* (Lange-Bertalot) Lange-Bertalot, however it has greater valves, usually longer and linear, and it has lower striae density than *P. subatomoides* (10–26 long, 5–8 wide, 27–32 striae in 10  $\mu\text{m}$ , A. *daonense* in Lange-Bertalot 1999).

Distribution: The taxon occurred in Rio Grande do Sul (Schneck et al. 2007, 2008) and it was also cited for Paraná state accompanying only measurements (Santos et al. 2011). The first record in the state of São Paulo is the present study.

***Psammothidium ventralis* (Krasske) Bukhtiyarova & Round**

Figure 8A–B

Basionym: *Navicula ventralis* Krasske 1923: 197, fig. 13

Morphometry: 10.4  $\mu\text{m}$  long, 4.9  $\mu\text{m}$  wide, SV: 28 striae in 10  $\mu\text{m}$ .

Rare species, with raphe and rapheless valves observed in different samples. *Psammothidium ventralis* has elliptic valves with broad capitulated apices; axial area linear, central area wide and bordered by 6–7 striae on raphe valve; axial area broadly lanceolate on rapheless valve (Bukhtiyarova & Round 1996). This taxon easily differs from the other *Psammothidium* found here by the valve outline with apices not differentiate and broadly rounded in *P. subatomoides*.

Distribution: Already cited in Brazil from Paraná state as *Navicula ventralis* (Contin 1990).

*Lemnicola* Round & Basson 1997

***Lemnicola hungarica*** (Grunow) Round & Basson

Figures 1AP–AW, 2A–E

Basionym: *Achnanthidium hungaricum* Grunow 1863, p. 146; pl. 13 (IV), fig. 8

Morphometry: 14.8–33.2 µm long, 6.9–8.2 µm wide, RV: 20–23 striae in 10 µm, SV: 18–20 striae in 10 µm.

*Lemnicola hungarica* is the type species of the genus and it presents typical morphological features: linear to linear-elliptic valves narrowing to rounded or subrostrate apices; central area forming an asymmetric stauros on raphe valve and asymmetric but reduced or even absent on rapheless valve (Round & Basson 1997, Potapova 2010c).

Distribution: Widely spread in Brazil, it occurred in North (as *Achnanthes hungarica* (Grunow) Grunow, Metzeltin & Lange-Bertalot 1998), South (Ferrari & Ludwig 2007, Schneck et al. 2008, Bertolli et al. 2010, Faria et al. 2010, Santos et al. 2011, Bes et al. 2012, Marra et al. 2016) and Southeast regions (Carneiro & Bicudo 2007, Costa et al. 2017).

*Skabitschewskia* Kulikovskiy & Lange-Bertalot 2015

***Skabitschewskia peragalloi*** (Brun & Héribaud) Kulikovskiy & Lange-Bertalot

Figure 8E–F

Basionym: *Achnanthes peragalloi* Brun & Héribaud in Héribaud 1893, p. 50; pl. 1, fig. 4

Morphometry: 17.1 µm long, 8.1 µm wide, SV: 22 striae in 10 µm.

The species was rare and just the rapheless valve of *S. peragalloi* was observed in few samples. It is morphologically very distinct from other taxa by possessing elliptic-lanceolate valves with rostrate to subcapitate apices, wide lanceolate axial area and cavum on rapheless valve. Its raphe valve has a narrow linear axial area and a bow tie-shaped central area, the striae are uniserial (bi- on rapheless valve), and strongly radiate (Potapova 2019).

Distribution: Already mentioned occurring in Paraná state as *Achnanthes peragalli* (Contin 1990).

Order Naviculales Bessey 1907

Family Stauroneidaceae D.G. Mann 1990

*Karayevia* Round & Bukhtiyarova ex Round 1998

***Karayevia clevei* (Grunow) Bukhtiyarova**

Figure 8G

Basionym: *Achnanthes clevei* Grunow in Cleve & Grunow 1880, p. 21

Morphometry: 14.8 µm long, 5.4 µm wide, SV: 16 striae in 10 µm.

As some other species, only the rapheless valve of one specimen was observed. This taxon has lanceolate valves with subrostrate apices, and coarsely punctate striae on rapheless valve. The valve outline is distinct from other *Karayevia* species as *K. laterostrata* (Hustedt) Bukhtiyarova by the presence of shoulders in this one. The latter also has narrow capitate to subcapitate apices (Potapova 2010d).

Distribution: First time cited for Brazil.

### Final considerations

Fifteen species are new records: eight are the first cited occurrence in Brazil (*Achnanthes subhudsonis*, *Planothidium alekseevae*, *P. hinzianum*, *P. minutissimum*, *P. potapovae*, *P. pumilum*, *P. straubianum*, *Karayevia clevei*), and other seven taxa are also cited here for the first time in São Paulo state (*Achnanthes coarctata*, *A. ziegleri*, *Platessa guianensis*, *P. oblongella*, *Gogorevia parexigua*, *Planothidium incuriatum*, *Psammothidium subatomoides*). Most species were rare in the samples analyzed, but they still account for the high richness of monoraphid diatoms in the region. Among the rare taxa, the rapheless valve was the most found and illustrated probably due to its distinct and easily recognizable characteristics. This study highlights the need of floristic surveys, including the rare species, to improve the knowledge of biodiversity of freshwater tropical diatoms.

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Table 1. Morphological comparison among the type species of some monoraphid genera from literature. RV: raphe valve, SV: sternum valve/rapheless valve, Nd: no data.

|                          | <i>Gogorevia exilis</i>   | <i>Gogorevia rinatii</i>   | <i>Lemnicola hungarica</i>  | <i>Psammothidium marginulatum</i> | <i>Achnanthidium microcephalum</i>  |
|--------------------------|---|--|---|-----------------------------------|---|
| <b>Study</b>             | Taylor et al. (2014) as <i>Achnanthidium exiguum</i>  | Kulikovskiy et al. (2020a)   | Potapova (2010c)  | Bukhtiyarova & Round (1996)       | Novais et al. (2015)  |
| <b>Girdle view</b>       | Narrow rectangular weakly curved  | Narrow rectangular weakly curved   | Nd  | RV convex, SV concave             | Slightly arched, RV concave, SV convex  |
| <b>Valve outline</b>     | Narrowly lanceolate with shoulders poorly developed   | Lanceolate to elliptical   | Linear to linear-elliptical   | Lanceolate/elliptical             | Linear-elliptic to linear-lanceolate  |
| <b>Apices</b>            | Protracted, short rostrate, broadly rounded   | Protracted, short rostrate, broadly rounded  | Narrowed, rounded, or subrostrate   | Always rounded                    | Protracted, rostrate to subcapitate   |
| <b>Length</b>            | 5.1–12.3  | 11.5–15.0  | 8–40  | 11–15                             | 6.2–14.2  |
| <b>Width</b>             | 3.3–5.1   | 5–6  | 4.5–7.5   | 5–6                               | 2.4–3.6   |
| <b>Striae</b>            | Uniseriate (very occasionally biseriate near the apices)  | Uniseriate (sometimes biseriate near the axial area, at valve ends)  | Biseriate   | Uniseriate                        | Uniseriate  |
| <b>Striae on mantle</b>  | Nd  | Nd   | One row of elongated areolae  | Striae uninterrupted along mantle | One row of narrow, elongated, frequently slit-like areolae  |
| <b>Striation pattern</b> | Almost parallel becoming more radiate towards the apices (SV); Radiate throughout, more strongly radiate near the apices (RV) | Almost parallel becoming more radiate towards the apices (SV); Radiate throughout, more strongly radiate near the apices | Slightly radiate in the valve center, becoming strongly radiate at the apices | Radiate                           | Radiate throughout the entire valve, becoming more strongly radiate and slightly denser near the apices |
| <b>Rapheless valve</b>   |   |  |   |                                   |   |
| <b>Axial area</b>        | Very narrow, linear, opening rather abruptly to the central area  | Narrow   | Narrow linear   | Wide, elliptical                  | Narrow linear, often slightly widening towards the valve margin   |

Continues

**Table 1. Continuation.**

|                        | <i>Gogorevia exilis</i>   | <i>Gogorevia rinatii</i>  | <i>Lemnicola hungarica</i>  | <i>Psammothidium marginulatum</i>  | <i>Achnanthidium microcephalum</i>   |
|------------------------|---|---|---|--|--|
| <b>Central area</b>    | Asymmetrical, on one side forming a hyaline area up to the valve margin, on other side formed by weakly more distant striae | Asymmetrical wedge-shaped   | Very small, often asymmetrical, irregularly shaped, transverse                                  | Nd   | Weakly elliptical or almost absent, usually bordered by one more widely spaced stria on both sides                 |
| <b>Striae in 10 um</b> | 24–30   | 25–30   | 18–24   | 27–30  | 30   |
| <b>Areolae</b>         | Apically elongated to rounded, covered by individual hymenes  | Elongated to rounded, occluded by individual hymenes  | Rounded to apically elongated at the valve margin, occluded by hymenes                          | Small, closed internally by cribrum  | Rounded to transapically elongated areolae, near the valve margin usually slit-like                                |
| <b>Raphe valve</b>     |   |   |   |  |  |
| <b>Axial area</b>      | Very narrow, linear, opening rather abruptly to the central area  | Nd  | Linear  | Wide, narrowing to apices (triangular)   | Narrow linear, slightly expanded towards the centre  |
| <b>Central area</b>    | More or less symmetrical<br>Narrow, rectangular to wedge-shaped fascia reaching the valve margins                           | More or less symmetrical, narrow, rectangular to wedge-shaped fascia reaching the valve margins                             | Asymmetrical  | Rectangular occupying $\frac{3}{4}$ of the breadth and bounded by 8–11 striae                          | Almost absent, slightly elliptical to, more rarely, forming a rectangular fascia                                   |
| <b>Raphe</b>           | Straight; proximal ends straight, clearly expanded; distal ends deflected to opposite sides, terminating in drop-like pores | Straight; proximal ends straight, clearly expanded; distal ends deflected to opposite sides, terminating in drop-like pores | Proximal ends drop-like; distal ends short, slightly expanded, and turned toward opposite sides | Straight; proximal ends simple, widely spaced; distal ends simple, short, curved to one side or absent | Straight, filiform; proximal ends straight slightly expanded; distal ends slightly deflected in the same direction |
| <b>Striae in 10 um</b> | 27–31   | 26–32.5   | 18–24   | 27–30  | 30–32  |
| <b>Areolae</b>         | Very small, usually rounded, covered by individual hymenes  | Very small, usually rounded, occluded by individual hymenes   | Rounded to apically elongated at the valve margin, occluded by hymenes                          | Small, closed internally by cribrum  | Rounded or transapically elongated, sometimes slit-like towards the valve margin                                   |

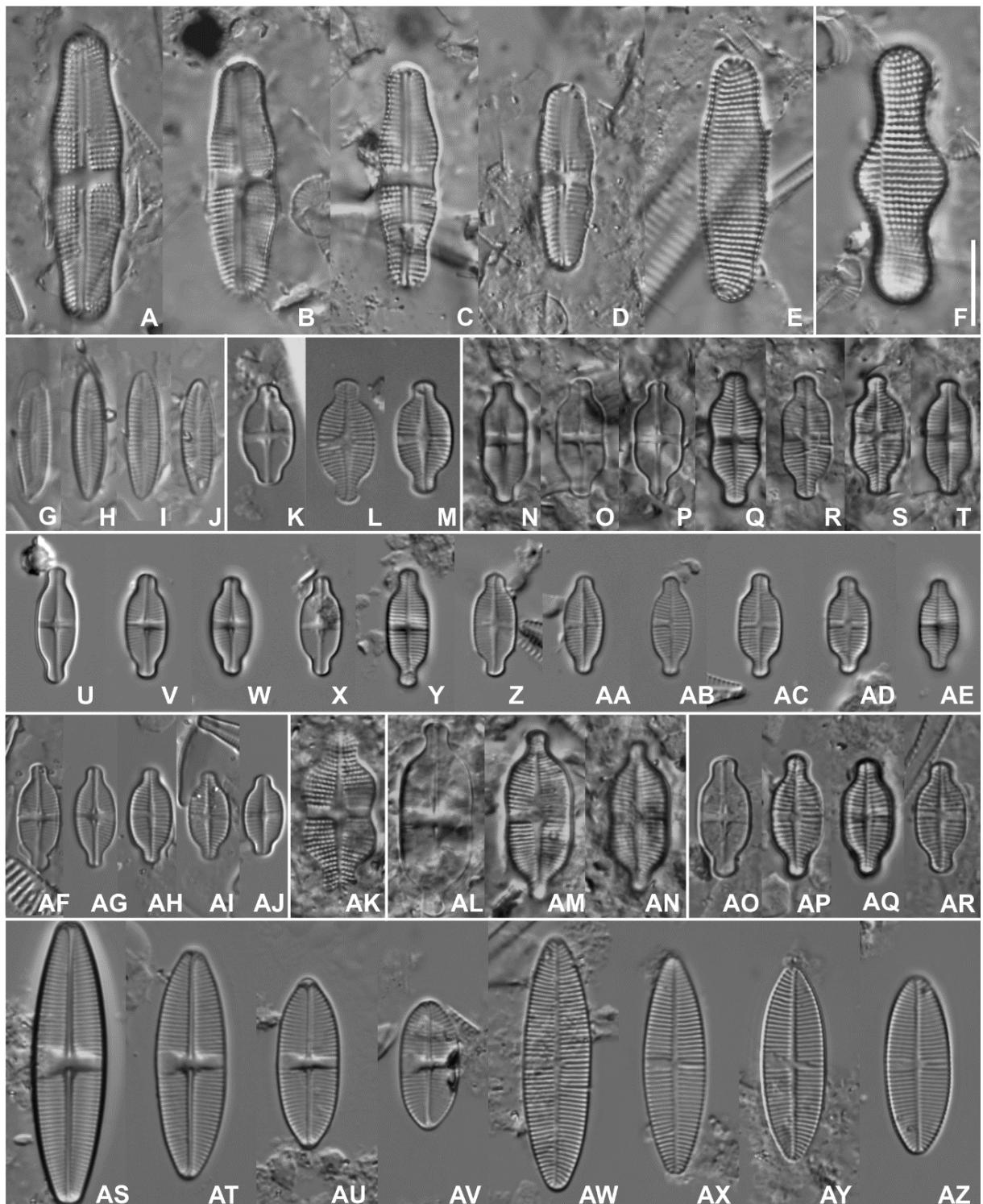


Figure 1. A–AZ. LM photomicrographies of some monoraphid species found. A–E. *Achnanthes coarctata* (SP428513). F. *Achnanthes inflata* (SP428513). G–J. *Achnanthes subhudsonis* (SP469262). K–M. *Achnanthes ziegleri* (SP469306). N–T. *Gogorevia constricta* (SP469207). U–AE. *Gogorevia exigua* type material (*Stauroneis exilis*, Kützing's sample 397, Kützing Collection). AF–AJ. *Gogorevia exigua* (SP255742). AK. *Gogorevia paraexigua* (SP469207). AL–AN. *Gogorevia* sp. 1 (SP469207). AO–AZ. *Gogorevia* sp. 1 (SP469207).

**AR.** *Gogorevia* sp. 2 (SP428921). **AS–AZ.** *Lemnicola hungarica* (SP427991). Scale = 10 µm.

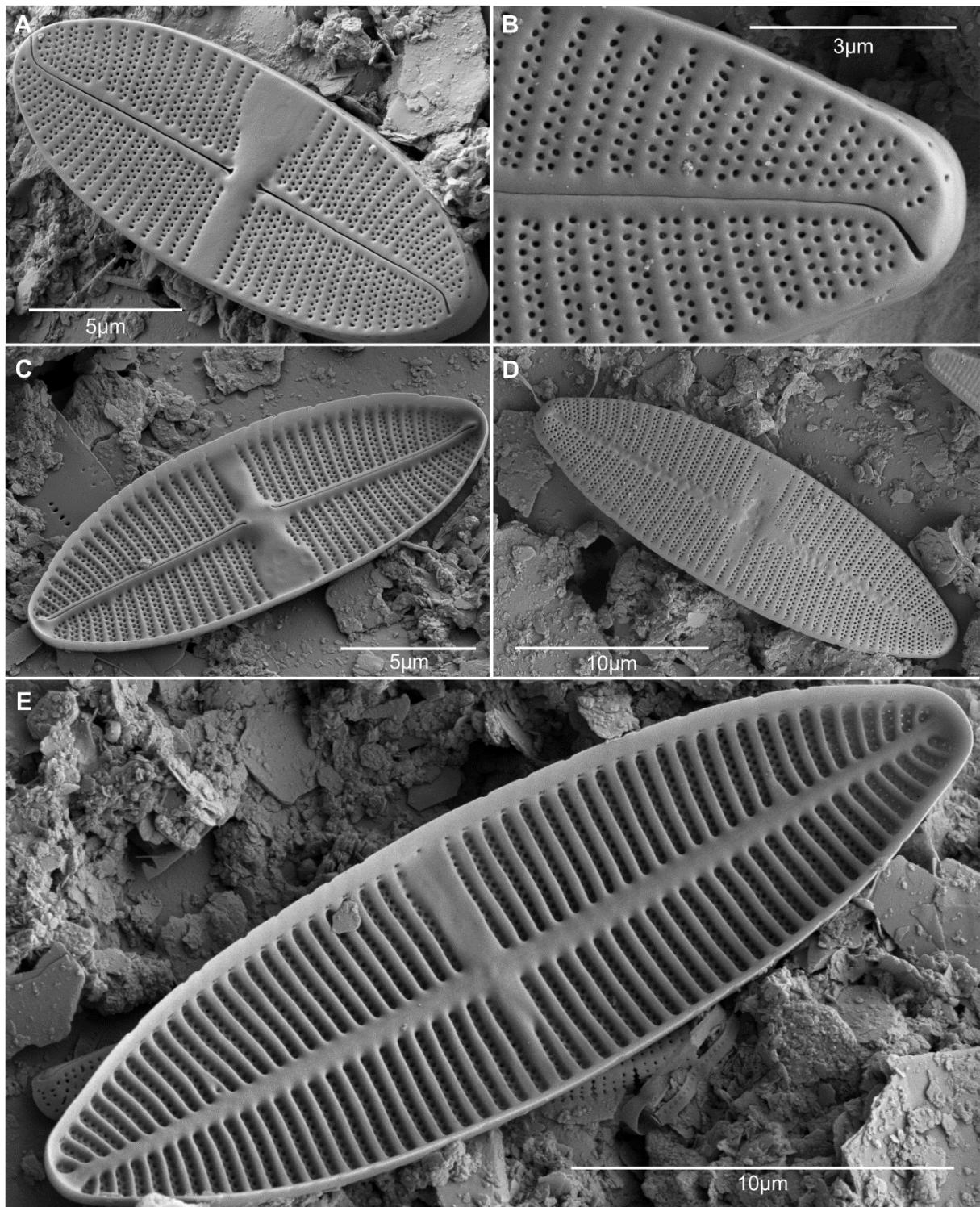
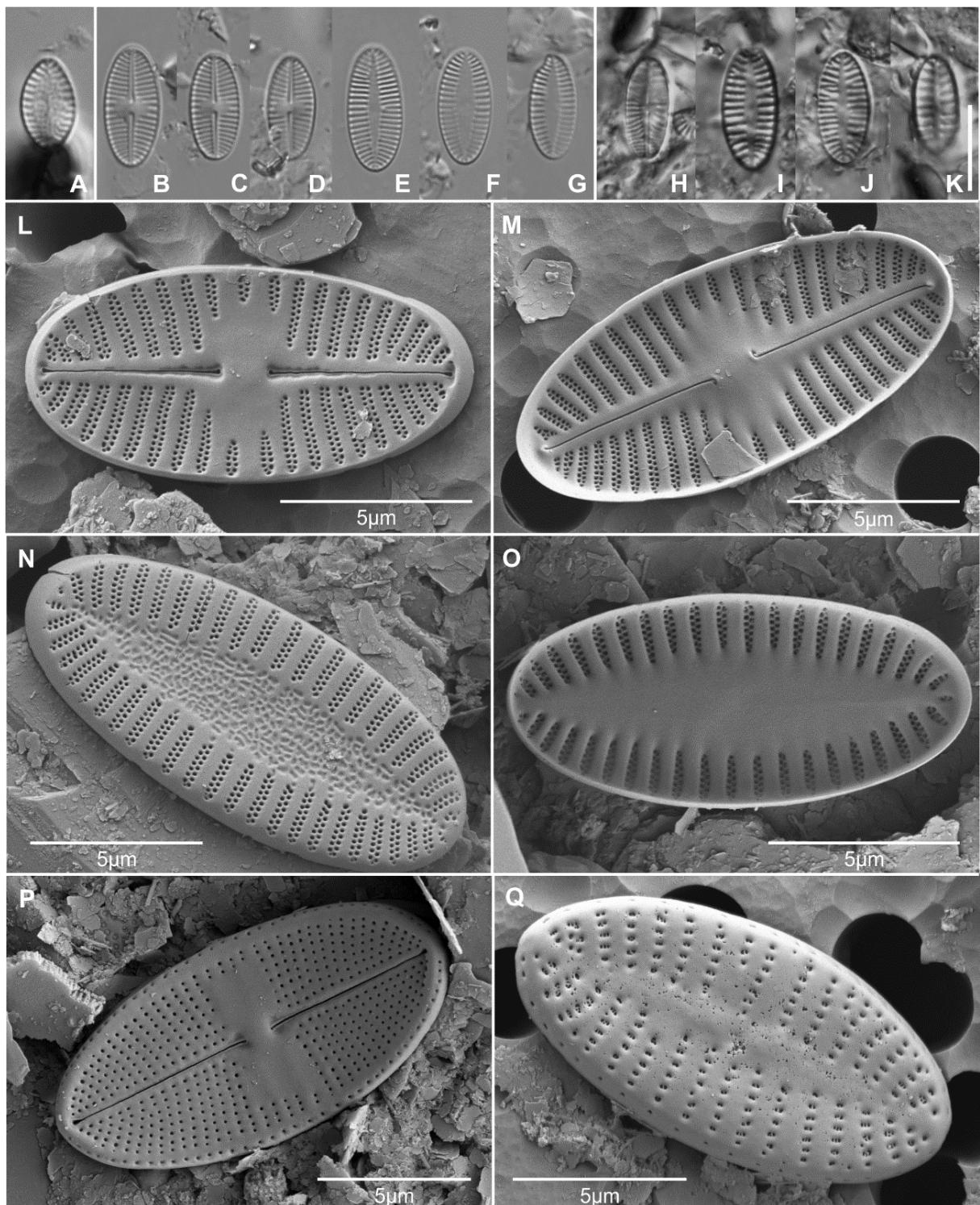


Figure 2. **A–E.** SEM photomicrographies of *Lemnicola hungarica* (SP427991). **A–C.** Raphe valves. **A.** External view. **B.** Detail of external distal raphe ending. **C.** Internal view. **D, E.** Rapheless valves. **D.** External view. **E.** Internal view.



**Figure 3. A–K.** LM photomicrographies of *Platessa* species found. **A.** *Platessa guianensis* (SP371176). **B–G.** *Platessa hustedtii* (SP255765). **H–K.** *Platessa oblongella* (SP469207). **L–Q.** SEM photomicrographies of two *Platessa* species. **L–O.** *Platessa hustedtii* (SP255765). **P, Q.** *Platessa oblongella* (SP469207). Scale = 10  $\mu\text{m}$ , except when indicated.

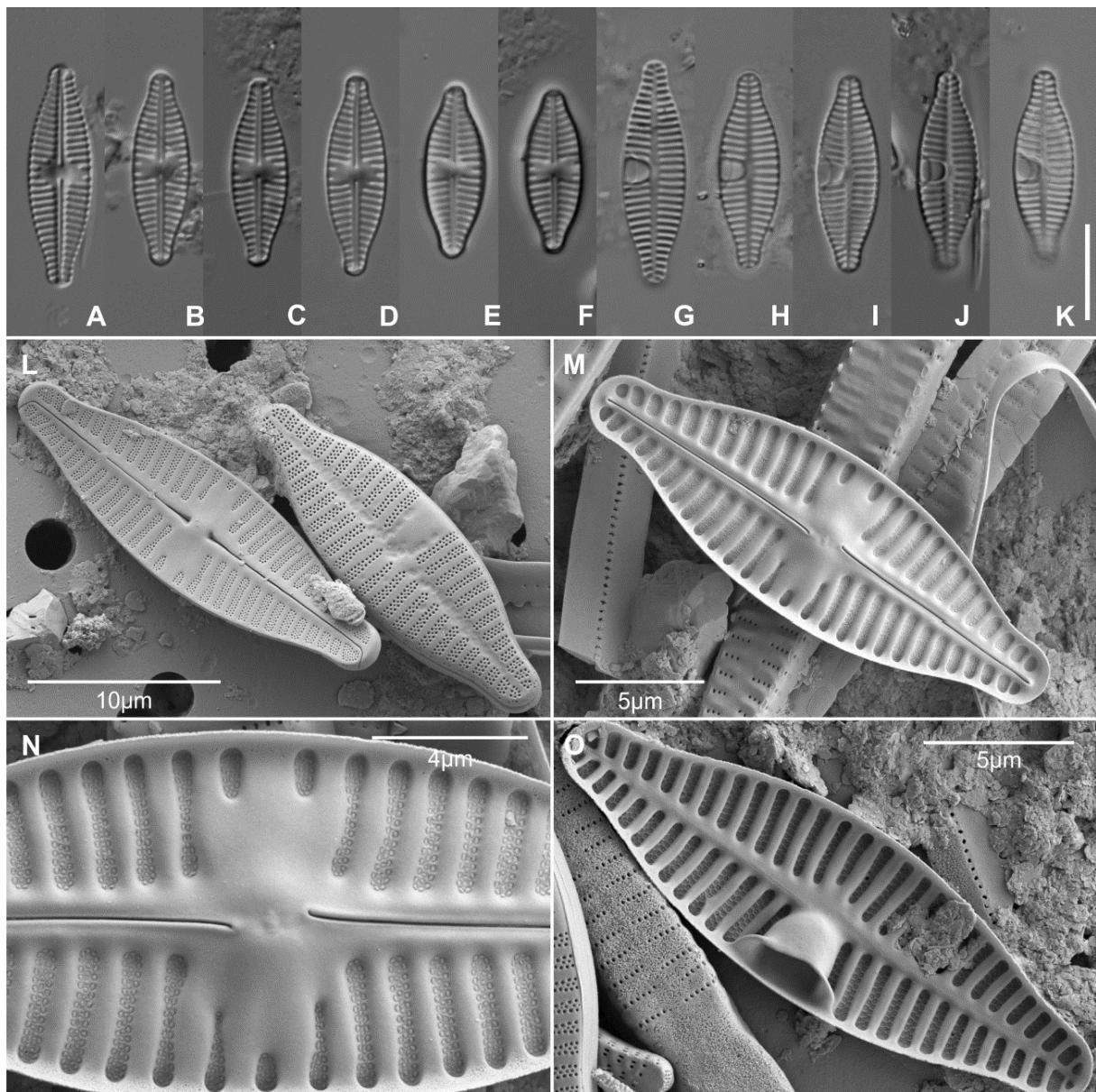


Figure 4. **A–K.** LM photomicrographies of *Planothidium incuriatum* (SP469539). **A–F.** Raphe valves. **G–K.** Rapheless valves. **L–O.** SEM photomicrographies of *Planothidium incuriatum* (SP469539). **L.** Raphe and rapheless valves. **M, N.** External view of the same raphe valve. **N.** Detail of proximal raphe endings and striae/areolae. **O.** Rapheless valve view. Scale = 10  $\mu\text{m}$ , except when indicated.

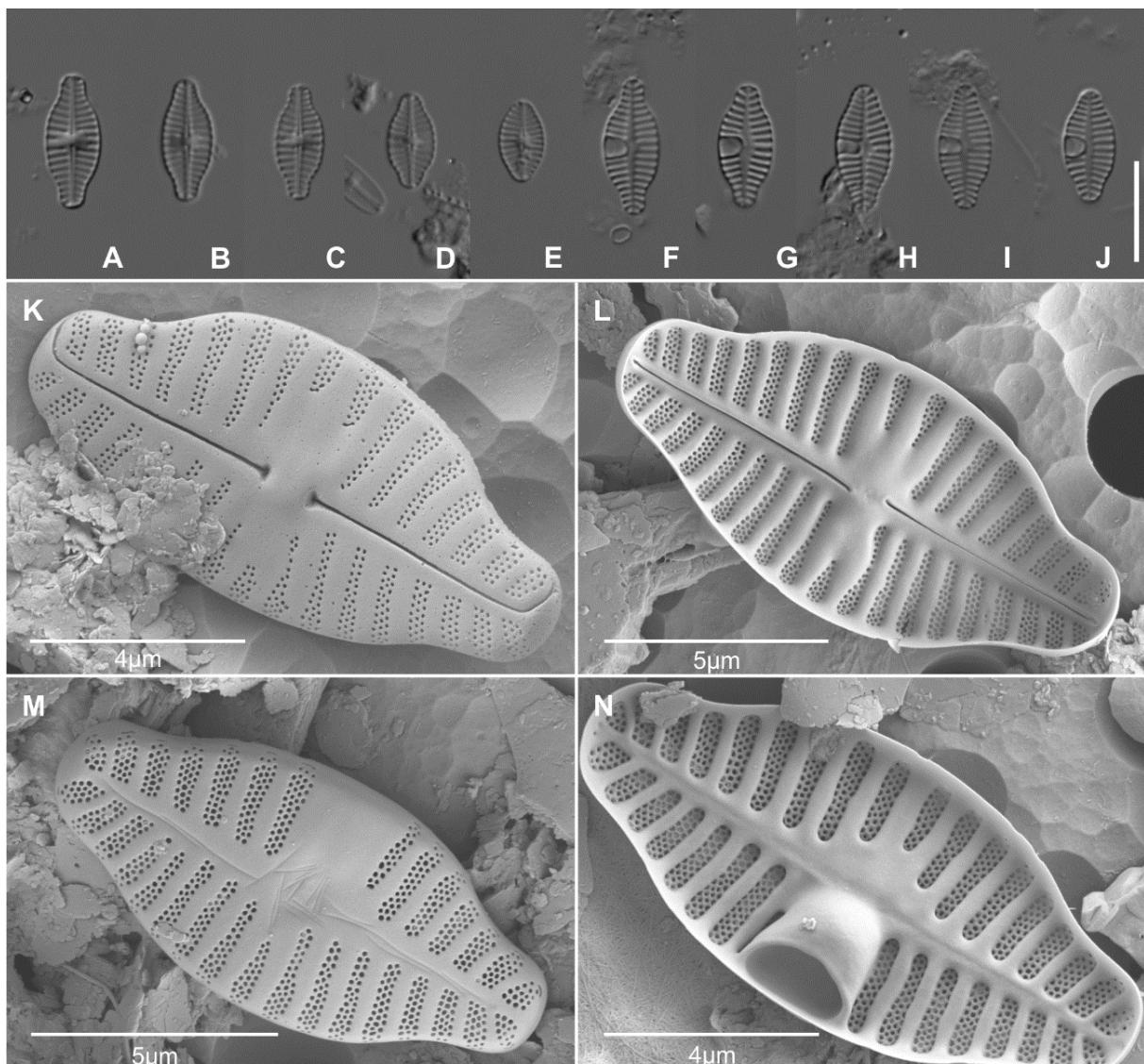


Figure 5. A–J. LM photomicrographies of *Planothidium rostratoholarcticum* (SP469530). A–E. Raphe valves. F–J. Rapheless valves. K–N. SEM photomicrographies of *Planothidium rostratoholarcticum* (SP469530). K, L. Raphe valves. K. External view. L. Internal view. M, N. Rapheless valves. M. External view. N. Internal view. Scale = 10  $\mu\text{m}$ , except when indicated.

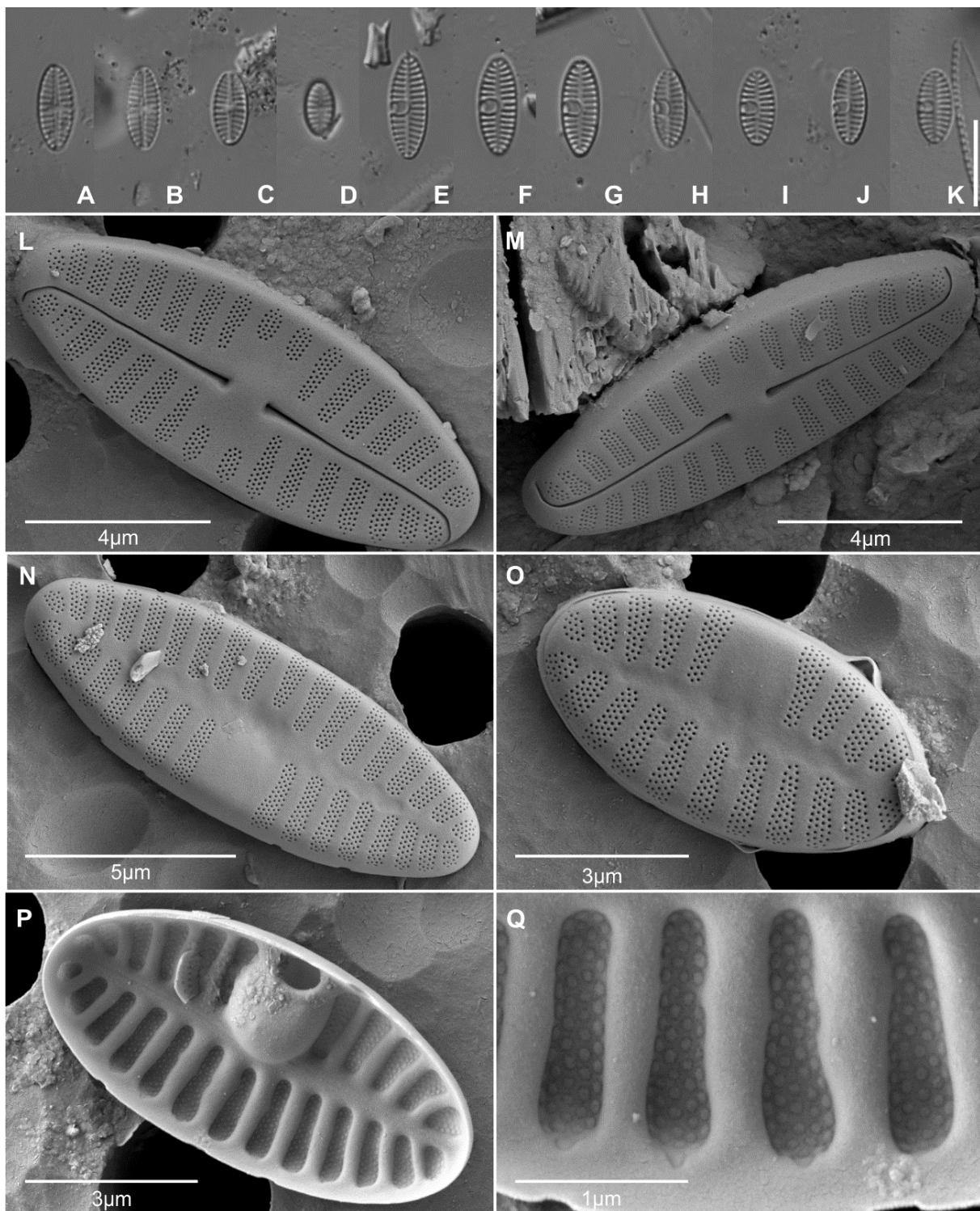


Figure 6. **A–K.** LM photomicrographies of *Planothidium straubianum* (SP255728). **A–D.** Raphe valves. **E–K.** Rapheless valves. **L–Q.** SEM photomicrographies of *Planothidium straubianum* (SP255728). **L, M.** External view of raphe valves. **N–Q.** Rapheless valves. **N, O.** External view. **P.** Internal view. **Q.** Detail of internal areolae. Scale = 10  $\mu\text{m}$ , except when indicated.

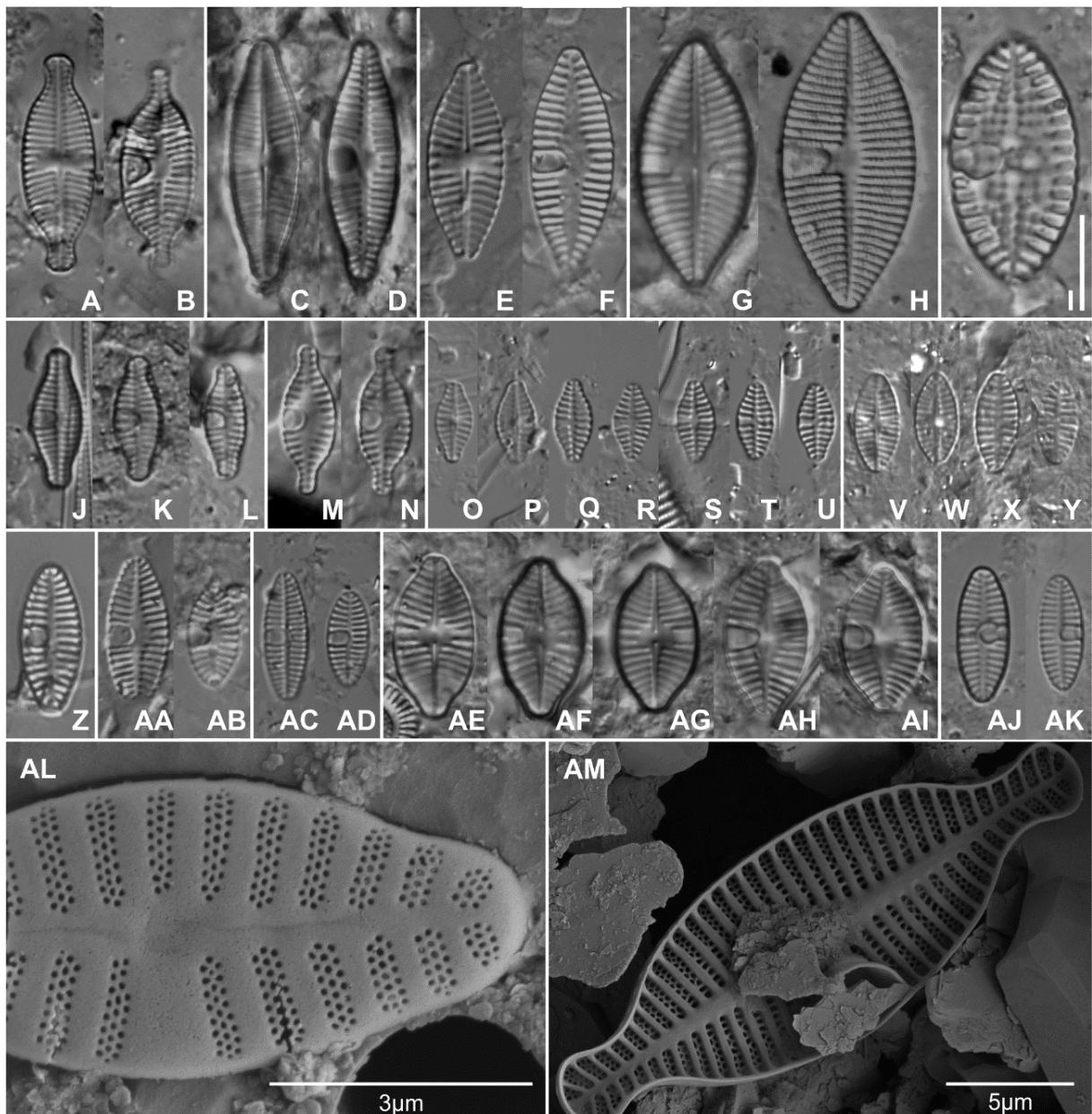


Figure 7. **A–AI.** LM photomicrographies of some monoraphid species found. **A, B.** *Planothidium brasiliense* (SP255765). **C, D.** *Planothidium* aff. *rostrolanceolatum* (SP469232). **E, F.** *Planothidium* sp. 1 (SP469281). **G, H.** *Planothidium* sp. 2 (SP469208, SP469232). **I.** *Planothidium lagerheimii* (SP469281). **J–L.** *Planothidium alekseevae* (SP371182). **M, N.** *Planothidium potapovae* (SP469281). **O–U.** *Planothidium minutissimum* (SP255742). **V–Y.** *Planothidium pumilum* (SP469204). **Z.** *Planothidium bagualense* (SP255763). **AA, AB.** *Planothidium hinziaum* (SP469281). **AC, AD.** *Planothidium victori* (SP255765). **AE–AI.** *Planothidium* sp. 3 (SP469207, SP469232). **AJ, AK.** *Planothidium* sp. 4 (SP427343). **AL, AM.** SEM photomicrographies of two *Planothidium* species. **AL.** External view of *Planothidium*

*minutissimum* rapheless valve (SP255742). **AM.** Internal view of *Planothidium brasiliense* rapheless valve (SP255765). Scale = 10  $\mu\text{m}$ , except when indicated.

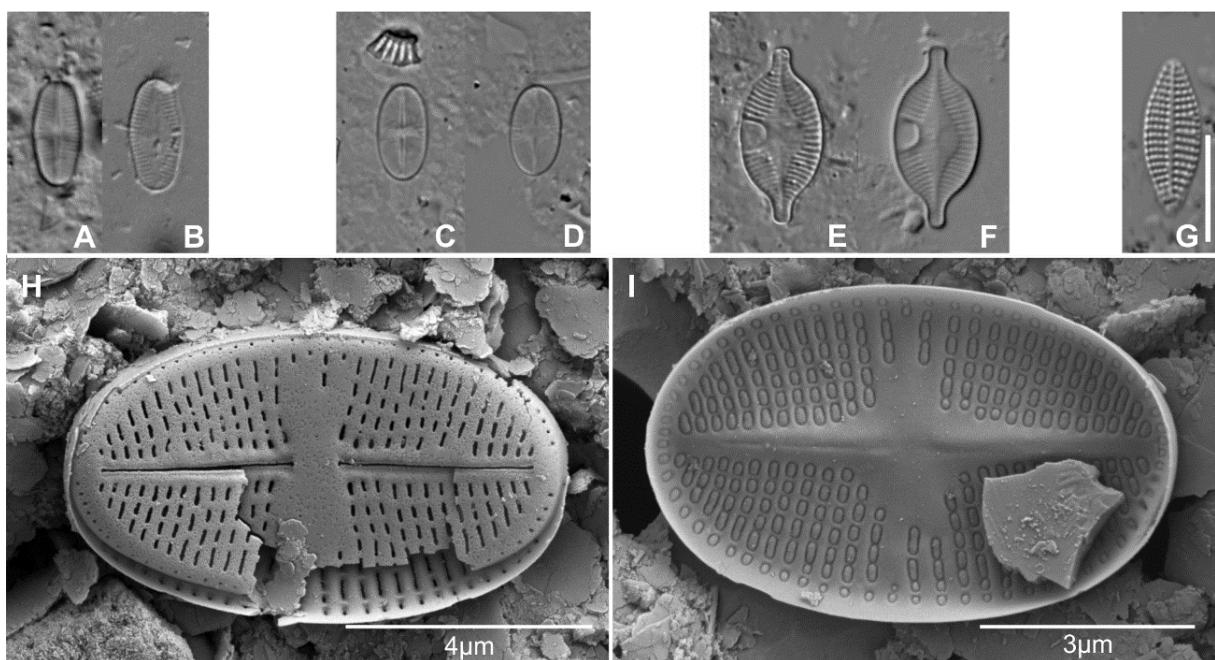


Figure 8. **E–F.** LM photomicrographies of some monoraphid species found. **A, B.** *Psammothidium ventralis* (SP469393, SP469417). **C–D.** *Psammothidium subatomoides* (SP469207). **E, F.** *Skabitschewskia peragalloi* (SP469232, SP469492). **G.** *Karayevia clevei* (SP255765). **H, I.** SEM photomicrographies of *Psammothidium subatomoides* (SP469207). **H.** External view of raphe valve. **I.** Internal view of rapheless valve. Scale = 10  $\mu\text{m}$ , except when indicated.

Supplementary table 1. Codes of monoraphid diatom taxa found in this study.

| Taxa   | Code |
|--|------|
| <i>Achnanthes coarctata</i> (Brébisson ex W.Smith) Grunow                            | ACOA |
| <i>Achnanthes inflata</i> (Kützing) Grunow   | AINF |
| <i>Achnanthes subhudsonis</i> Hustedt  | ASUB |
| <i>Achnanthes ziegleri</i> Lange-Bertalot  | AZIE |
| <i>Plateissa guianensis</i> R.Le Cohu, L.Tudesque & C.E.Wetzel                       | PLGU |
| <i>Plateissa hustedtii</i> (Krasske) Lange-Bertalot                                  | PLHU |
| <i>Plateissa oblongella</i> (Østrup) C.E.Wetzel, Lange-Bertalot & Ector              | PLOB |
| <i>Gogorevia exilis</i> (Kützing) Kulikovskiy & Kociolek                             | GOEX |
| <i>Gogorevia constricta</i> (Torka) Kulikovskiy & Kociolek                           | GOCO |
| <i>Gogorevia parexigua</i> (Metzeltin & Lange-Bertalot) Kulikovskiy & Kociolek       | GOPA |
| <i>Gogorevia</i> sp. 1   | GSP1 |
| <i>Gogorevia</i> sp. 2   | GSP2 |
| <i>Planothidium alekseevae</i> Gogorev & Lange-Bertalot                              | PALE |
| <i>Planothidium bagualense</i> C.E.Wetzel & Ector                                    | PBAG |
| <i>Planothidium brasiliense</i> C.E.Wetzel & S.Blanco                                | PBRA |
| <i>Planothidium hinzianum</i> C.E.Wetzel, Van de Vijver & Ector                      | PHIN |
| <i>Planothidium incuriatum</i> C.E.Wetzel, Van de Vijver & Ector                     | PINC |
| <i>Planothidium largerheimii</i> (Cleve) C.E.Wetzel & Ector                          | PLAG |
| <i>Planothidium minutissimum</i> (Krasske) Lange-Bertalot                            | PMIN |
| <i>Planothidium potapovae</i> C.E.Wetzel & Ector                                     | PPOT |
| <i>Planothidium pumilum</i> Bąk & Lange-Bertalot                                     | PPUM |
| <i>Planothidium rostratoholarticum</i> Lange-Bertalot & Bąk                          | PRHO |
| <i>Planothidium</i> aff. <i>rostrolanceolatum</i> Van de Vijver, Kopalová & Zidarova | PRLA |
| <i>Planothidium straubianum</i> C.E.Wetzel, Van de Vijver & Ector                    | PSTR |
| <i>Planothidium victori</i> P.M.Novis, J.Braidwood & C.Kilroy                        | PVIC |
| <i>Planothidium</i> sp. 1  | PSP1 |
| <i>Planothidium</i> sp. 2  | PSP2 |
| <i>Planothidium</i> sp. 3  | PSP3 |
| <i>Planothidium</i> sp. 4  | PSP4 |

*Continues*

**Supplementary table 1. Cont.**

| <b>Taxa</b>  | <b>Code</b> |
|--|-------------|
| <i>Psammothidium subatomoides</i> (Hust.) Bukhtiyarova & Round           | PSSU        |
| <i>Psammothidium ventralis</i> (Krasske) Bukhtiyarova & Round            | PSVE        |
| <i>Lemnicola hungarica</i> (Grunow) Round & Basson                       | LHUN        |
| <i>Skabitschewskia peragalloi</i> (Brun & Héribaud) Round & Bukhtiyarova | SKPE        |
| <i>Karayevia clevei</i> (Grunow) Bukhtiyarova                            | KACL        |

Supplementary Table 2. Presence/absence of monoraphid diatom taxa found in this study.

| Herbarium number | Site   | ACOA     | AINF | ASUB | AZIE     | PLGU | PLHU | PLOB | GOCO     | GOEX | GOPA | GSP1 | GSP2 | PALE     | PBAG | PBRA | PHIN | PINC     | PLAG | PMIN     | PPOT | PPUM     | PRHO     | PRLA | PSTR | PVIC | PSP1 | PSP2     | PSP3     | PSP4     | PSSU     | PSVE     | LHUN | SKPE | KACL |
|------------------|--------|----------|------|------|----------|------|------|------|----------|------|------|------|------|----------|------|------|------|----------|------|----------|------|----------|----------|------|------|------|------|----------|----------|----------|----------|----------|------|------|------|
| SP469253         | AT1-S  | 0        | 0    | 0    | <b>1</b> | 0    | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | <b>1</b> | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0        | 0    |      |      |
| SP469255         | AT3-S  | 0        | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0        | 0    |      |      |
| SP469275         | AT1V-F | 0        | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | <b>1</b> | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0        | 0    |      |      |
| SP469297         | AT1I-F | 0        | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0        | 0    |      |      |
| SP469244         | BB2-S  | 0        | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0        | 0    |      |      |
| SP469247         | BB5-S  | 0        | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | <b>1</b> | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0        | 0    | 0    |      |
| SP469515         | BB2V-F | 0        | 0    | 0    | 0        | 0    | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0        | 0    |      |      |
| SP469520         | BB2I-F | 0        | 0    | 0    | 0        | 0    | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0        | <b>1</b> | 0    | 0    |      |
| SP469523         | BB5I-F | 0        | 0    | 0    | 0        | 0    | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0        | 0    | 0    |      |
| SP469545         | BB2V-P | 0        | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0        | <b>1</b> | 0    | 0    |      |
| SP469554         | BB2I-P | 0        | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | <b>1</b> | 0        | 0    |      |      |
| SP469557         | BB5I-P | 0        | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | <b>1</b> | 0        | 0        |      |      |      |
| SP401560         | RG2I-F | 0        | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | <b>1</b> | 0        | 0    |      |      |
| SP401567         | CC9I-F | 0        | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | <b>1</b> | 0        | 0    |      |      |
| SP401579         | CC9V-F | 0        | 0    | 0    | 0        | 0    | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | <b>1</b> | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | <b>1</b> | 0        | 0        |          |      |      |      |
| SP401584         | RG2-S  | 0        | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | <b>1</b> | 0        | 0        |      |      |      |
| SP401586         | RG4-S  | 0        | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | <b>1</b> | 0        | 0        |      |      |      |
| SP401589         | RP7-S  | 0        | 0    | 0    | 0        | 0    | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0        | 0    |      |      |
| SP401592         | TQ10-S | 0        | 0    | 0    | 0        | 0    | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | <b>1</b> | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | <b>1</b> | 0        | 0        |          |      |      |      |
| SP427898         | RG2I-P | 0        | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | <b>1</b> | 0        | 0        |      |      |      |
| SP427900         | RG4I-P | <b>1</b> | 0    | 0    | 0        | 0    | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | <b>1</b> | 0        | 0        |          |      |      |      |
| SP427902         | RP7I-P | 0        | 0    | 0    | 0        | 0    | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0        | 0    |      |      |
| SP427903         | CC9I-P | 0        | 0    | 0    | 0        | 0    | 0    | 0    | <b>1</b> | 0    | 0    | 0    | 0    | 0        | 0    | 0    | 0    | 0        | 0    | 0        | 0    | 0        | 0        | 0    | 0    | 0    | 0    | <b>1</b> | 0        | 0        |          |          |      |      |      |

Continues

Supplementary table 2. Cont.

| Herbarium number | Site    | ACOA | AINF | ASUB | AZIE | PLGU | PLHU | PLOB | GOCO | GOEX | GOPA | GSP1 | GSP2 | PALE | PBAG | PBRA | PHIN | PINC | PLAG | PMIN | PPOT | PPUM | PRHO | PRLA | PSTR | PVIC | PSP1 | PSP2 | PSP3 | PSP4 | PSSU | PSVE | LHUN | SKPE | KACL |   |
|------------------|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|---|
| SP427907         | RG4V-P  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |   |
| SP427910         | CC9V-P  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |   |
| SP427911         | TQ10V-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP428921         | CB1-S   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |   |
| SP428923         | CB3-S   | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |   |
| SP428936         | CB1I-F  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP428938         | CB3I-F  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469433         | FU2I-F  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0 |
| SP469200         | FU2-S   | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0 |
| SP427591         | CG2I-F  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469558         | CG2I-P  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469249         | CA2-S   | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469271         | CA2V-F  | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469293         | CA2I-F  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469294         | CA3I-F  | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP428507         | GU1-S   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0 |
| SP428508         | GU2-S   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0 |
| SP428512         | GU6-S   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0 |
| SP428513         | GU7-S   | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0 |
| SP428514         | GU8-S   | 1    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0 |
| SP469456         | GU2V-F  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0 |

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Supplementary table 2. Cont.

| Herbarium number | Site    | ACOA     | AINF     | ASUB | AZIE | PLGU | PLHU     | PLOB     | GOCO     | GOEX     | GOPA | GSP1 | GSP2 | PALE | PBAG     | PBRA     | PHIN     | PINC | PLAG     | PMIN     | PPOT     | PPUM     | PRHO | PRLA | PSTR | PVIC     | PSP1     | PSP2 | PSP3 | PSP4     | PSSU     | PSVE     | LHUN     | SKPE | KACL |   |
|------------------|---------|----------|----------|------|------|------|----------|----------|----------|----------|------|------|------|------|----------|----------|----------|------|----------|----------|----------|----------|------|------|------|----------|----------|------|------|----------|----------|----------|----------|------|------|---|
| SP469461         | GU7V-F  | <b>1</b> | 0        | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    |   |
| SP469466         | GU12V-F | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    |   |
| SP469469         | GU1I-F  | 0        | 0        | 0    | 0    | 0    | <b>1</b> | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    |   |
| SP469474         | GU6I-F  | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | <b>1</b> | 0    | 0        | 0        | 0        | <b>1</b> | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | 0        | <b>1</b> | 0    | 0    |   |
| SP469475         | GU7I-F  | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | <b>1</b> | 0        | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | <b>1</b> | 0        | 0    |      |   |
| SP469476         | GU8I-F  | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | <b>1</b> | 0        | 0    |      |   |
| SP469240         | HB1-S   | 0        | 0        | 0    | 0    | 0    | 0        | 0        | <b>1</b> | <b>1</b> | 0    | 0    | 0    | 0    | 0        | <b>1</b> | 0        | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | <b>1</b> | 0        | 0        |      |      |   |
| SP469508         | HB1V-F  | 0        | 0        | 0    | 0    | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0    | <b>1</b> | 0        | 0        | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    |   |
| SP469511         | HB1I-F  | 0        | 0        | 0    | 0    | 0    | 0        | 0        | <b>1</b> | <b>1</b> | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | <b>1</b> | 0        | 0    |      |   |
| SP469534         | HB1V-P  | 0        | 0        | 0    | 0    | 0    | 0        | 0        | <b>1</b> | <b>1</b> | 0    | 0    | 0    | 0    | 0        | <b>1</b> | 0        | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | <b>1</b> | 0        | 0    |      |   |
| SP469539         | HB1I-P  | 0        | 0        | 0    | 0    | 0    | 0        | <b>1</b> | <b>1</b> | 0        | 0    | 0    | 0    | 0    | <b>1</b> | 0        | 0        | 0    | 0        | <b>1</b> | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | <b>1</b> | 0        | 0        |      |      |   |
| SP469239         | IP3-S   | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    |   |
| SP469504         | IP3V-F  | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    |   |
| SP469507         | IP3I-F  | 0        | <b>0</b> | 0    | 0    | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    |   |
| SP469530         | IP3V-P  | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0 |
| SP469232         | IT1-S   | 0        | 0        | 0    | 0    | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | <b>1</b> | <b>1</b> | 0    | 0    | 0    | <b>1</b> | <b>1</b> | 0    | 0    | 0        | <b>1</b> | <b>1</b> | 0        | 0    |      |   |
| SP469236         | IT5-S   | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    |   |
| SP469492         | IT1V-F  | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | 0        | 0        | <b>1</b> | 0    | 0    |   |
| SP469526         | IT1V-P  | 0        | 0        | 0    | 0    | 0    | 0        | 0        | <b>1</b> | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | <b>1</b> | 0        | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | <b>1</b> | 0        | 0        |      |      |   |
| SP428846         | JC6V-F  | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0    | 0    | 0        | <b>1</b> | 0        | 0        | 0    |      |   |
| SP428847         | JC7V-F  | 0        | 0        | 0    | 0    | 0    | 0        | 0        | 0        | 0        | 0    | 0    | 0    | 0    | 0        | 0        | 0        | 0    | 0        | 0        | 0        | <b>1</b> | 0    | 0    | 0    | 0        | 0        | 0    | 0    | <b>1</b> | 0        | 0        | 0        |      |      |   |

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Supplementary table 2. Cont.

| Herbarium number | Site   | ACOA | AINF | ASUB | AZIE | PLGU | PLHU | PLOB | GOCO | GOEX | GOPA | GSP1 | GSP2 | PALE | PBAG | PBRA | PHIN | PINC | PLAG | PMIN | PPOT | PPUM | PRHO | PRLA | PSTR | PVIC | PSP1 | PSP2 | PSP3 | PSP4 | PSSU | PSVE | LHUN | SKPE | KACL |   |
|------------------|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|---|
| SP428865         | JC7-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP428839         | JA1V-F | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |      |   |
| SP428857         | JA1-S  | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |      |   |
| SP427988         | JU1V-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |   |
| SP427997         | JU2V-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP468830         | JU1I-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |   |
| SP468850         | JU2-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |   |
| SP469208         | JP1-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469421         | JP1V-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469483         | LG1V-F | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469484         | LG1I-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |   |
| SP469486         | LG1-S  | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469317         | NI1I-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469318         | NI1V-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469576         | NI1-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469212         | PI1-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 1    | 0    |   |
| SP469214         | PI3-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0 |
| SP469425         | PI1V-F | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469445         | PI1I-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    |   |
| SP469492         | PI1V-P | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469503         | PI1I-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469259         | PC1-S  | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |
| SP469262         | PC4-S  | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |

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Supplementary table 2. Cont.

| Herbarium number | Site   | ACOA | AINF | ASUB | AZIE | PLGU | PLHU | PLOB | GOCO | GOEX | GOPA | GSP1 | GSP2 | PALE | PBAG | PBRA | PHIN | PINC | PLAG | PMIN | PPOT | PPUM | PRHO | PRLA | PSTR | PVIC | PSP1 | PSP2 | PSP3 | PSP4 | PSSU | PSVE | LHUN | SKPE | KACL |   |   |   |
|------------------|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|---|---|---|
| SP469369         | PC1V-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0 |   |   |
| SP469281         | PC1V-F | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |   |   |
| SP469284         | PC4V-F | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |   |   |
| SP469303         | PC1I-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |   |   |
| SP469306         | PC4I-F | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |   |   |
| SP469551         | PC4V-P | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |   |   |
| SP469552         | PC4I-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |   |   |   |
| SP427984         | PA1V-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0 |   |   |
| SP427985         | PA1I-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0 | 0 |   |
| SP427992         | PA1V-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0 | 0 |   |
| SP468847         | PA1-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0 | 0 |   |
| SP427923         | PN2V-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0 | 0 |   |
| SP468845         | PN2-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0 | 0 |   |
| SP427990         | RA2V-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0 | 0 |   |
| SP427991         | RA2I-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1 | 0 | 0 |
| SP468860         | RA2-S  | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0 | 0 |   |
| SP427916         | RC1V-F | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0 | 0 |   |
| SP427919         | RC1I-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0 | 0 |   |
| SP427921         | RC3I-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0 | 0 |   |
| SP469207         | SI3-S  | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 1    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0 | 0 |   |
| SP469400         | SI3V-F | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0 | 0 |   |
| SP469420         | SI3I-F | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0 | 0 |   |
| SP469263         | SG1-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0 | 0 |   |

Continues

Supplementary table 2. Cont.

| Herbarium number | Site   | ACOA | AINF | ASUB | AZIE | PLGU | PLHU | PLOB | GOCO | GOEX | GOPA | GSP1 | GSP2 | PALE | PBAG | PBRA | PHIN | PINC | PLAG | PMIN | PPOT | PPUM | PRHO | PRLA | PSTR | PVIC | PSP1 | PSP2 | PSP3 | PSP4 | PSSU | PSVE | LHUN | SKPE | KACL |
|------------------|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| SP469266         | SG4-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |      |
| SP469285         | SG1V-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |      |      |
| SP469288         | SG4V-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |      |
| SP469307         | SG1I-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |
| SP469310         | SG4I-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| SP469372         | SG1V-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |
| SP469375         | SG4V-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |
| SP469381         | SG1I-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |
| SP469384         | SG4I-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |
| SP469230         | SH2-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| SP469454         | SH2V-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| SP469490         | SH2I-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| SP469524         | SH2V-P | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| SP469204         | SE3-S  | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |
| SP469397         | SE3V-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |
| SP469417         | SE3I-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| SP427986         | TA1V-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |
| SP427987         | TA1I-P | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |
| SP468857         | TA1-S  | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| SP468858         | TA2-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| SP428920         | TG3-S  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| SP428926         | TG3I-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    |
| SP428929         | TG3V-F | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |

Continues

**Supplementary table 2. Cont.**

## CONSIDERAÇÕES FINAIS

- O gênero *Planothidium* foi o mais rico taxonomicamente na área de estudo, representado por 17 táxons, seguido por *Achnanthidium* (13), *Cocconeis* (9), *Gogorevia* (5), *Achnanthes* (4), *Platessa* (3), *Psammothidium* (2), *Karayevia* (1), *Lemnicola* (1), *Skabitschewskia* (1). Dos 56 táxons identificados pertencendo aos 10 gêneros mencionados, 39 foram identificados em nível específico, nove foram considerados novas espécies, sete foram identificados em nível genérico e uma sofreu mudança de status (*Cocconeis tropicoacuta*).
- Duas espécies atualmente classificadas em *Achnanthes*, *A. subhudsonis* e *A. ziegleri*, provavelmente correspondem a outros gêneros e merecem mais atenção e estudo. Da mesma forma, duas espécies raras de *Gogorevia* encontradas na área de estudo são provavelmente novas espécies (*Gogorevia* sp. 1 e *Gogorevia* sp. 2).
- Vinte táxons foram reportados pioneiramente para o Estado de São Paulo, sendo nove deles também novas citações para o Brasil, quais sejam: *Achnanthes subhudsonis*, *Planothidium alekseevae*, *P. hinzianum*, *P. minutissimum*, *P. potapovae*, *P. pumilum*, *P. straubianum*, *P. victori* e *Karayevia clevei*.
- Das 346 amostras analisadas da área de estudo, 52 não apresentaram representantes de diatomáceas monorrafídeas.
- Considerando os três habitats avaliados nas amostras do Projeto AcquaSed, as diatomáceas monorrafídeas foram mais diversas na comunidade do fitoplâncton (31 táxons) e dos sedimentos superficiais (30 táxons) e menos diversas no perifítton (22 táxons). Contudo, considerando a proporção de espécies por amostras analisadas de cada habitat (57 amostras de sedimentos superficiais, 108 de fitoplâncton e 59 de perifítton), o sedimento apresentou a maior diversidade de táxons e o fitoplâncton a menor.
- Nas amostras do projeto AcquaSed, as espécies com a maior frequência de ocorrência foram todas pertencentes a *Achnanthidium*: *A. minutissimum* (63%), seguida de *A. tropicocatenatum* (61%) e *A. peetersianum* (40%). Em relação à abundância máxima, *Achnanthidium* sp. nov. 6 se destacou, atingindo abundâncias de 89,5% e 86,7% em amostras do fitoplâncton do Lago das Garças e 62,1% em amostra de sedimento da Represa Rio Grande (RG4).
- A preferência ecológica de 10 espécies foi avaliada conforme o critério de corte estabelecido de frequência de ocorrência  $\geq 10\%$  nos diferentes habitats. Em relação aos nutrientes, mais da metade dos táxons (*A. peruvianum*, *A. peetersianum*, *A. tropicocatenatum*, *A. minutissimum*, *Achnanthidium* sp. nov. 4 e *Achnanthidium* sp. nov. 7) apresentou seus ótimos ecológicos distribuídos no intervalo de concentração de fósforo total referente a

ambientes oligo- a mesotróficos ( $14,1\text{--}47,3 \mu\text{g L}^{-1}$ ). Da mesma forma, o ótimo para condutividade ocorreu principalmente em valores baixos a moderados ( $< 100 \mu\text{S cm}^{-1}$ ) e em águas ligeiramente ácidas a ligeiramente alcalinas (ótimo de pH entre 6,6–7,4), com exceção de *Achnanthidium* sp. nov. 7 que preferiu ambientes alcalinos (pH 7,9). *Achnanthidium* sp. nov. 1 apresentou preferências intermediárias entre ambientes eutróficos e mesotróficos (PT 39,0–77,8  $\mu\text{g L}^{-1}$ ). Já, *Achnanthidium* sp. nov. 2 e *Achnanthidium* sp. nov. 6 tiveram preferência ecológica por ambientes eutróficos com alta condutividade. Por fim, apenas *Achnanthidium* sp. 9 teve seu ótimo ecológico em águas hipereutróficas.

- Destaca-se o uso da microscopia eletrônica de varredura para o aprofundamento do conhecimento das espécies de *Achnanthidium*, além de sua grande importância na identificação das espécies. Devido à proximidade das características morfológicas entre espécies deste gênero, a utilização do MEV foi essencial para melhor observação de suas estruturas, principalmente da rafe e estrias.
- A partir da integração de informações taxonômicas e ecológicas das espécies do gênero *Achnanthidium*, este estudo contribui, com bases mais consistentes, para o melhor conhecimento das populações tropicais e de seu uso na bioindicação.
- Destaca-se a importância de estudos florísticos incluindo as espécies raras para o conhecimento mais amplo e aprofundado da biodiversidade das diatomáceas monorrafídeas.

## **ANEXO 1**

LISTAGEM DOS TÁXONS COM SUAS RESPECTIVAS AUTORIAS E CÓDIGOS

**Tabela 1.** Códigos dos táxons monorrafídeos encontrados e suas respectivas autorias.

| Código      | Táxon  |
|-------------|--|
| <b>APER</b> | <i>Aachnanthidium peruvianum</i> E.Morales & Ector                             |
| <b>APEE</b> | <i>Achnanthidium peetersianum</i> C.E.Wetzel, Jüttner & Ector                  |
| <b>ADTR</b> | <i>Achnanthidium tropicocatenatum</i> Marquardt, C.E.Wetzel & Ector            |
| <b>ADMI</b> | <i>Achnanthidium minutissimum</i> (Kützing) Czarnecki                          |
| <b>ASP1</b> | <i>Achnanthidium</i> sp. nov. 1  |
| <b>ASP2</b> | <i>Achnanthidium</i> sp. nov. 2  |
| <b>ASP3</b> | <i>Achnanthidium</i> sp. nov. 3  |
| <b>ASP4</b> | <i>Achnanthidium</i> sp. nov. 4  |
| <b>ASP5</b> | <i>Achnanthidium</i> sp. nov. 5  |
| <b>ASP6</b> | <i>Achnanthidium</i> sp. nov. 6  |
| <b>ASP7</b> | <i>Achnanthidium</i> sp. nov. 7  |
| <b>ASP8</b> | <i>Achnanthidium</i> sp. nov. 8  |
| <b>ASP9</b> | <i>Achnanthidium</i> sp. 9   |
| <b>CCAM</b> | <i>Coccneis amerieuglypta</i> L.F.Costa, C.E.Wetzel & Ector                    |
| <b>CEUG</b> | <i>Coccneis euglypta</i> Ehrenberg   |
| <b>CFEU</b> | <i>Coccneis feuerbornii</i> Hustedt  |
| <b>CFLU</b> | <i>Coccneis fluvialis</i> J.H.Wallace  |
| <b>CNTH</b> | <i>Coccneis neothumensis</i> Krammer   |
| <b>CPAC</b> | <i>Coccneis tropicoacuta</i> L.F.Costa & C.E.Wetzel                            |
| <b>ACOA</b> | <i>Achnanthes coarctata</i> (Brébisson ex W.Smith) Grunow                      |
| <b>AINF</b> | <i>Achnanthes inflata</i> (Kützing) Grunow                                     |
| <b>ASUB</b> | <i>Achnanthes subhudsonis</i> Hustedt  |
| <b>AZIE</b> | <i>Achnanthes ziegleri</i> Lange-Bertalot                                      |
| <b>PLGU</b> | <i>Platessa guianensis</i> R.Le Cohu, L.Tudesque & C.E.Wetzel                  |
| <b>PLHU</b> | <i>Platessa hustedtii</i> (Krasske) Lange-Bertalot                             |
| <b>PLOB</b> | <i>Platessa oblongella</i> (Østrup) C.E.Wetzel, Lange-Bertalot & Ector         |
| <b>GOEX</b> | <i>Gogorevia exilis</i> (Kützing) Kulikovskiy & Kocielek                       |
| <b>GOCO</b> | <i>Gogorevia constricta</i> (Torka) Kulikovskiy & Kocielek                     |
| <b>GOPA</b> | <i>Gogorevia parexigua</i> (Metzeltin & Lange-Bertalot) Kulikovskiy & Kocielek |
| <b>GSP1</b> | <i>Gogorevia</i> sp. 1   |
| <b>GSP2</b> | <i>Gogorevia</i> sp. 2   |
| <b>PALE</b> | <i>Planothidium alekseevae</i> Gogorev & Lange-Bertalot                        |
| <b>PBAG</b> | <i>Planothidium bagualense</i> C.E.Wetzel & Ector                              |
| <b>PBRA</b> | <i>Planothidium brasiliense</i> C.E.Wetzel & S.Blanco                          |
| <b>PHIN</b> | <i>Planothidium hinzianum</i> C.E.Wetzel, Van de Vijver & Ector                |
| <b>PINC</b> | <i>Planothidium incuriatum</i> C.E.Wetzel, Van de Vijver & Ector               |
| <b>PLAG</b> | <i>Planothidium largerheimii</i> (Cleve) C.E.Wetzel & Ector                    |
| <b>PMIN</b> | <i>Planothidium minutissimum</i> (Krasske) Lange-Bertalot                      |
| <b>PPOT</b> | <i>Planothidium potapovae</i> C.E.Wetzel & Ector                               |

*Continua*

**Tabela 1.** Cont.

| Código      | Táxon  |
|-------------|--|
| <b>PPUM</b> | <i>Planothidium pumilum</i> Bąk & Lange-Bertalot                                     |
| <b>PRHO</b> | <i>Planothidium rostratoholarticum</i> Lange-Bertalot & Bąk                          |
| <b>PRLA</b> | <i>Planothidium</i> aff. <i>rostrolanceolatum</i> Van de Vijver, Kopalová & Zidarova |
| <b>PSTR</b> | <i>Planothidium straubianum</i> C.E.Wetzel, Van de Vijver & Ector                    |
| <b>PVIC</b> | <i>Planothidium victori</i> P.M.Novis, J.Braidwood & C.Kilroy                        |
| <b>PSP1</b> | <i>Planothidium</i> sp. 1  |
| <b>PSP2</b> | <i>Planothidium</i> sp. 2  |
| <b>PSP3</b> | <i>Planothidium</i> sp. 3  |
| <b>PSP4</b> | <i>Planothidium</i> sp. 4  |
| <b>PSSU</b> | <i>Psammothidium subatomoides</i> (Hust.) Bukhtiyarova & Round                       |
| <b>PSVE</b> | <i>Psammothidium ventralis</i> (Krasske) Bukhtiyarova & Round                        |
| <b>LHUN</b> | <i>Lemnicola hungarica</i> (Grunow) Round & Basson                                   |
| <b>SKPE</b> | <i>Skabitschewskia peragalloi</i> (Brun & Héribaud) Round & Bukhtiyarova             |
| <b>KACL</b> | <i>Karayevia clevei</i> (Grunow) Bukhtiyarova  |

## **ANEXO 2**

ABUNDÂNCIA RELATIVA (%) DOS TÁXONS ENCONTRADOS EM AMOSTRAS DE  
SEDIMENTOS SUPERFICIAIS

**Tabela 2.** Abundância relativa (%) dos táxons encontrados nas amostras de sedimento superficial do Projeto Acquased. Código das espécies conforme Tabela 1. Em negrito: valores acima de zero.

| Local de coleta | APER       | APEE       | ADTR        | ADM1       | ASP1       | ASP2       | ASP3       | ASP4       | ASP6        | ASP7       | ASP9       | CCAM | CEUG | CFEU       | CFLU | CNTH | CPAC | ACOA       | AZIE | GOCO       | GOEX       | GSP2 | PHIN       | PINC | PRHO       | PSTR       | PVIC | PSP3 | LHUN | SKPE |
|-----------------|------------|------------|-------------|------------|------------|------------|------------|------------|-------------|------------|------------|------|------|------------|------|------|------|------------|------|------------|------------|------|------------|------|------------|------------|------|------|------|------|
| AT1             | <b>0,4</b> | <b>0,4</b> | <b>2,7</b>  | <b>0,4</b> | 0          | <b>0,4</b> | 0          | <b>0,2</b> | 0           | 0          | 0          | 0    | 0    | <b>0,2</b> | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | <b>0,2</b> | 0    | 0          | 0          | 0    | 0    |      |      |
| AT3             | 0          | 0          | <b>2,4</b>  | 0          | 0          | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| BB2             | 0          | 0          | 0           | <b>0,2</b> | 0          | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| BB5             | 0          | 0          | 0           | 0          | 0          | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| RG2             | 0          | 0          | 0           | 0          | 0          | 0          | <b>25</b>  | 0          | <b>62,1</b> | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| RG4             | 0          | 0          | <b>1,2</b>  | 0          | 0          | 0          | <b>1,2</b> | 0          | <b>6,8</b>  | <b>1,4</b> | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    |      |      |      |
| RP6             | 0          | <b>0,4</b> | <b>2,2</b>  | <b>1,4</b> | 0          | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| RP7             | 0          | <b>1,2</b> | 0           | 0          | 0          | 0          | 0          | 0          | <b>0,8</b>  | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | <b>0,4</b> | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| CC9             | 0          | 0          | 0           | 0          | 0          | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| TQ10            | 0          | 0          | <b>6,5</b>  | <b>0,6</b> | <b>0,2</b> | 0          | 0          | 0          | 0           | 0          | <b>0,2</b> | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    |      |      |      |
| CB1             | 0          | <b>0,6</b> | <b>1,4</b>  | <b>1,9</b> | <b>0,2</b> | 0          | <b>0,6</b> | <b>0,8</b> | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    |      |      |      |
| CB3             | 0          | <b>0,2</b> | <b>0,7</b>  | <b>0,2</b> | 0          | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| FU2             | 0          | 0          | <b>0,8</b>  | 0          | 0          | 0          | 0          | 0          | 0           | 0          | <b>0,2</b> | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| CG2             | 0          | <b>0,8</b> | 0           | 0          | 0          | 0          | 0          | <b>0,4</b> | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0,0,       | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| FR3             | 0          | <b>0,4</b> | 0           | 0          | 0          | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| CA2             | 0          | 1          | <b>6,6</b>  | <b>3,2</b> | 0          | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | <b>0,4</b> | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| CA3             | 0          | <b>0,2</b> | <b>2,7</b>  | 0          | 0          | 0          | 0          | <b>0,2</b> | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| GU1             | 0          | 4          | <b>13,1</b> | 1          | <b>0,2</b> | 0          | 0          | <b>1,2</b> | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| GU2             | 0          | <b>2,4</b> | <b>7,2</b>  | <b>7,4</b> | 1          | 0          | 0          | <b>0,4</b> | 0           | 0          | 0          | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | 0          | 0          | 0    | 0    |      |      |
| GU6             | 0          | 0          | 0           | <b>0,2</b> | <b>0,4</b> | 0          | 0          | 0          | 0           | 0          | <b>1,4</b> | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | <b>1,4</b> | 0    | 0          | 0    | 0          | <b>0,8</b> | 0    |      |      |      |
| GU7             | 0          | 0          | 0           | 0          | 0          | <b>3,5</b> | 0          | 0          | 0           | 0          | <b>0,8</b> | 0    | 0    | 0          | 0    | 0    | 0    | 0          | 0    | 0          | 0          | 0    | 0          | 0    | <b>0,2</b> | 0          | 0    |      |      |      |

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Tabela 2. Cont.

| Local de coleta | APER       | APEE       | ADTR        | ADM1       | ASP1       | ASP2       | ASP3 | ASP4       | ASP6        | ASP7       | ASP9       | CCAM       | CEUG       | CFEU | CFLU       | CNTH       | CPAC | ACOA | AZIE       | GOCO       | GOEX       | GSP2 | PHIN       | PINC       | PRHO       | PSTR       | PVIC       | PSP3 | LHUN | SKPE |
|-----------------|------------|------------|-------------|------------|------------|------------|------|------------|-------------|------------|------------|------------|------------|------|------------|------------|------|------|------------|------------|------------|------|------------|------------|------------|------------|------------|------|------|------|
| <b>GU8</b>      | 0          | 0          | 0           | 0          | <b>0,2</b> | <b>0,6</b> | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | <b>0,6</b> | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    |      |      |
| <b>GU12</b>     | 0          | 0          | 0           | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    |      |      |
| <b>HB1</b>      | 0          | 0          | <b>0,6</b>  | <b>2,8</b> | 0          | <b>3,6</b> | 0    | 0          | 0           | 0          | 0          | <b>2,2</b> | 0          | 0    | 0          | 0          | 0    | 0    | <b>0,6</b> | <b>0,2</b> | 0          | 0    | <b>0,8</b> | 0          | 0          | 0          | <b>1</b>   | 0    |      |      |
| <b>IP3</b>      | 0          | 0          | 0           | <b>1</b>   | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    |      |      |
| <b>IT1</b>      | 0          | 0          | <b>1,4</b>  | <b>3,6</b> | 0          | 0          | 0    | <b>0,6</b> | <b>6,1</b>  | 0          | 0          | 0          | 0          | 0    | <b>0,2</b> | 0          | 0    | 0    | 0          | <b>0,2</b> | 0          | 0    | 0          | 0          | 0          | <b>0,4</b> | 0          |      |      |      |
| <b>IT5</b>      | 0          | <b>0,4</b> | <b>0,2</b>  | <b>2,4</b> | 0          | 0          | 0    | <b>0,6</b> | <b>1,4</b>  | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    |      |      |
| <b>JC6</b>      | 0          | 0          | <b>2,8</b>  | <b>0,8</b> | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    | 0    |      |
| <b>JC7</b>      | 0          | <b>0,4</b> | <b>5,8</b>  | <b>4,6</b> | 0          | 0          | 0    | <b>1,2</b> | 0           | 0          | <b>0,2</b> | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | <b>0,2</b> | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    |      |      |
| <b>JA1</b>      | <b>2,6</b> | <b>0,3</b> | <b>0,3</b>  | <b>2,3</b> | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0          | <b>0,8</b> | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    | 0    |      |
| <b>JU1</b>      | 0          | <b>0,2</b> | <b>0,6</b>  | <b>0,6</b> | 0          | <b>0,8</b> | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    | 0    |      |
| <b>JU2</b>      | 0          | <b>0,2</b> | <b>0,2</b>  | <b>0,4</b> | 0          | <b>0,2</b> | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    | 0    |      |
| <b>JP1</b>      | <b>0,6</b> | <b>0,4</b> | <b>1,6</b>  | 0          | 0          | 0          | 0    | 0          | 0           | <b>0,2</b> | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | <b>0,2</b> | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    | 0    |      |
| <b>JP4</b>      | 0          | <b>0,8</b> | <b>0,4</b>  | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    | 0    | 0    |
| <b>LG1</b>      | 0          | 0          | 0           | <b>1</b>   | <b>0,2</b> | 0          | 0    | 0          | <b>45,9</b> | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | <b>0,6</b> | <b>1,5</b> | 0          | 0    | 0          | 0          | <b>0,2</b> | 0          | 0          | 0    | 0    |      |
| <b>NI1</b>      | 0          | <b>0,8</b> | <b>7,4</b>  | 0          | <b>0,2</b> | 0          | 0    | 0          | 0           | 0          | 0          | <b>0,6</b> | 0          | 0    | 0          | 0          | 0    | 0    | <b>0,4</b> | 0          | <b>0,2</b> | 0    | 0          | 0          | 0          | 0          | 0          | 0    | 0    |      |
| <b>PI1</b>      | 0          | <b>0,2</b> | <b>2,8</b>  | <b>1,6</b> | <b>0,2</b> | 0          | 0    | 0          | 0           | 0          | <b>1,6</b> | 0          | 0          | 0    | <b>0,6</b> | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | <b>1,2</b> | 0          | <b>0,2</b> |      |      |      |
| <b>PI3</b>      | 0          | 0          | <b>1</b>    | <b>0,4</b> | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    | 0    |      |
| <b>PC1</b>      | 0          | 0          | <b>3,6</b>  | <b>7,8</b> | <b>1,2</b> | <b>0,6</b> | 0    | <b>0,2</b> | 0           | <b>0,6</b> | 0          | 0          | <b>0,2</b> | 0    | 0          | 0          | 0    | 0    | <b>0,4</b> | 0          | 0          | 0    | 0          | <b>0,2</b> | 0          | 0          | 0          | 0    |      |      |
| <b>PC4</b>      | 0          | 0          | <b>16,1</b> | <b>4,6</b> | <b>0,4</b> | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | <b>0,2</b> | 0          | 0    | 0    | <b>0,4</b> | 0          | 0          | 0    | <b>0,4</b> | 0          | 0          | 0          | 0          | 0    |      |      |
| <b>PA1</b>      | 0          | <b>0,8</b> | <b>3,3</b>  | <b>3,9</b> | 0          | 0          | 0    | <b>0,2</b> | <b>0,8</b>  | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | <b>1,6</b> | 0          | 0    | 0          | 0          | 0          | <b>0,2</b> | 0          | 0    |      |      |
| <b>PB3</b>      | 0          | <b>1</b>   | 0           | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    | 0    |      |
| <b>PB4</b>      | 0          | <b>1,8</b> | 0           | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    | 0    |      |
| <b>PB5</b>      | 0          | 0          | 0           | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0    | 0    |      |

Continua

**Tabela 2. Cont.**

## **ANEXO 3**

ABUNDÂNCIA RELATIVA (%) DOS TÁXONS ENCONTRADOS EM AMOSTRAS DE  
FITOPLÂNCTON

**Tabela 3.** Abundância relativa (%) dos táxons encontrados nas amostras de fitoplâncton do Projeto Acquased. Código das espécies conforme Anexo 1 e dos locais de amostragem segundo Anexo 2. Em negrito: valores acima de zero.

| Loca de coleta | APER | APEE       | ADTR       | ADM1       | ASP1       | ASP2     | ASP3       | ASP4       | ASP6        | ASP7       | ASP8 | ASP9 | CCAM | CFEU | CFLU | CPAC       | AZIE       | PLHU | PLOB       | GOCO       | GOEX | PBRA | PHIN | PINC       | PLAG       | PPOT | PRHO | PSTR       | PVIC | PSP3 | LHUN |
|----------------|------|------------|------------|------------|------------|----------|------------|------------|-------------|------------|------|------|------|------|------|------------|------------|------|------------|------------|------|------|------|------------|------------|------|------|------------|------|------|------|
| AT1V           | 0    | <b>0,6</b> | <b>8,4</b> | <b>1,6</b> | 0          | <b>1</b> | 0          | 0          | 0           | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | <b>0,2</b> | 0    | 0    | 0          | 0    |      |      |
| AT1I           | 0    | <b>0,8</b> | <b>2,6</b> | <b>0,4</b> | 0          | 0        | 0          | <b>0,2</b> | 0           | 0          | 0    | 0    | 0    | 0    | 0    | <b>0,2</b> | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| AT3V           | 0    | <b>0,2</b> | <b>1,5</b> | <b>0,2</b> | 0          | 0        | 0          | 0          | <b>0,7</b>  | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| AT3I           | 0    | 0          | <b>0,2</b> | 0          | 0          | 0        | 0          | 0          | 0           | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| BB2V           | 0    | 0          | <b>0,2</b> | 0          | 0          | 0        | 0          | 0          | 0           | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | <b>0,2</b> | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| BB2I           | 0    | 0          | <b>0,6</b> | 0          | 0          | 0        | 0          | 0          | 0           | 0          | 0    | 0    | 0    | 0    | 0    | 0          | <b>0,2</b> | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          |      |      |      |
| BB5V           | 0    | 0          | 0          | 0          | 0          | 0        | 0          | 0          | 0           | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| BB5I           | 0    | 0          | 0          | 0          | 0          | 0        | 0          | 0          | 0           | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| RG2I           | 0    | 0          | <b>5,4</b> | 0          | 0          | 0        | <b>2,9</b> | 0          | <b>1,2</b>  | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | <b>0,8</b> |      |      |      |
| RG4V           | 0    | 0          | 0          | <b>0,6</b> | <b>0,2</b> | 0        | 0          | 0          | <b>35,2</b> | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| RG4I           | 0    | <b>0,2</b> | <b>0,4</b> | <b>0,2</b> | 0          | 0        | 0          | <b>0,4</b> | 0           | <b>0,2</b> | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| RP6V           | 0    | 0          | <b>0,8</b> | <b>0,8</b> | 0          | 0        | 0          | 0          | <b>0,2</b>  | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| RP6I           | 0    | <b>0,2</b> | 0          | <b>0,4</b> | 0          | 0        | 0          | <b>0,2</b> | 0           | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| RP7V           | 0    | <b>1,6</b> | 0          | <b>6,4</b> | 0          | 0        | <b>1,6</b> | 0          | 0           | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| RP7I           | 0    | <b>1,1</b> | <b>3,4</b> | <b>2,3</b> | 0          | 0        | 0          | 0          | 0           | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| CC9V           | 0    | 0          | 0          | <b>0,2</b> | 0          | 0        | 0          | 0          | <b>4,5</b>  | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | <b>0,2</b> | 0          | 0    | 0    | 0    | <b>0,2</b> | 0          | 0    | 0    | <b>0,4</b> |      |      |      |
| CC9I           | 0    | 0          | 0          | 0          | <b>0,3</b> | 0        | 0          | 0          | <b>3,7</b>  | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | <b>0,6</b> |      |      |      |
| TQ10V          | 0    | 0          | 0          | <b>0,6</b> | 0          | 0        | 0          | 0          | <b>11</b>   | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| TQ10I          | 0    | 0          | 0          | 0          | 0          | 0        | 0          | 0          | <b>0,6</b>  | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |
| CB1V           | 0    | 0          | <b>0,2</b> | <b>6</b>   | 0          | 0        | 0          | <b>0,4</b> | <b>0,4</b>  | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          |      |      |      |
| CB1I           | 0    | <b>0,2</b> | 0          | <b>0,2</b> | 0          | 0        | 0          | <b>1,4</b> | 0           | 0          | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0    |      |      |

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Tabela 3. Cont.

| Loca de coleta | APER | APEE       | ADTR       | ADM1        | ASP1       | ASP2       | ASP3 | ASP4       | ASP6       | ASP7       | ASP8 | ASP9       | CCAM        | CFEU       | CFLU       | CPAC       | AZIE | PLHU | PLOB       | GOCO | GOEX       | PBRA       | PHIN       | PINC | PLAG | PPOT       | PRHO       | PSTR | PVIC       | PSP3 | LHUN |
|----------------|------|------------|------------|-------------|------------|------------|------|------------|------------|------------|------|------------|-------------|------------|------------|------------|------|------|------------|------|------------|------------|------------|------|------|------------|------------|------|------------|------|------|
| CB3V           | 0    | 0          | <b>0,2</b> | <b>9</b>    | 0          | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          |      |      |
| CB3I           | 0    | 0          | <b>0,2</b> | <b>3,2</b>  | 0          | 0          | 0    | 0          | <b>2</b>   | <b>0,4</b> | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          |      |      |
| FU2V           | 0    | 0          | <b>1</b>   | 0           | 0          | 0          | 0    | <b>0,2</b> | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          |      |      |
| FU2I           | 0    | <b>0,2</b> | <b>3,6</b> | 0           | 0          | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| CG2V           | 0    | 0          | 0          | 0           | 0          | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| CG2I           | 0    | 0          | 0          | 0           | 0          | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | <b>0,2</b> | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| FR3V           | 0    | 0          | <b>0,6</b> | 0           | 0          | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| FR3I           | 0    | <b>0,2</b> | <b>3,6</b> | 0           | 0          | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| CA2V           | 0    | <b>0,2</b> | <b>4,3</b> | <b>3,3</b>  | 0          | <b>0,4</b> | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | <b>0,4</b> | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| CA2I           | 0    | 0          | <b>1,7</b> | <b>0,6</b>  | 0          | <b>0,2</b> | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | <b>0,2</b> | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          |      |      |
| CA3V           | 0    | <b>0,4</b> | <b>4,6</b> | 0           | 0          | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| CA3I           | 0    | 0          | <b>0,6</b> | <b>0,8</b>  | 0          | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| GU1V           | 0    | <b>0,8</b> | <b>2,2</b> | <b>3</b>    | 0          | 0          | 0    | <b>0,2</b> | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| GU1I           | 0    | <b>0,6</b> | <b>8,2</b> | <b>4,4</b>  | <b>0,2</b> | 0          | 0    | <b>2</b>   | 0          | 0          | 0    | 0          | 0           | 0          | 0          | <b>0,4</b> | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| GU2V           | 0    | <b>0,6</b> | <b>14</b>  | <b>3</b>    | <b>0,8</b> | 0          | 0    | <b>3</b>   | 0          | 0          | 0    | <b>0,4</b> | <b>0,2</b>  | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          |      |      |
| GU2I           | 0    | <b>0,6</b> | 0          | <b>63,8</b> | 0          | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0           | <b>0,2</b> | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| GU6V           | 0    | 0          | 0          | 0           | 0          | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| GU6I           | 0    | 0          | <b>0,2</b> | <b>1</b>    | 0          | 0          | 0    | 0          | 0          | 0          | 0    | 0          | <b>11,2</b> | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | <b>0,4</b> | 0    | 0    | <b>0,2</b> | 0          | 0    | 0          |      |      |
| GU7V           | 0    | 0          | 0          | <b>0,6</b>  | <b>0,2</b> | <b>0,8</b> | 0    | 0          | 0          | 0          | 0    | <b>1,4</b> | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          |      |      |
| GU7I           | 0    | 0          | 0          | <b>1,2</b>  | 0          | 0          | 0    | 0          | 0          | 0          | 0    | <b>4,8</b> | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | <b>0,2</b> | 0    | 0    | <b>0,2</b> | 0          | 0    | <b>0,6</b> |      |      |
| GU8V           | 0    | 0          | 0          | 0           | 0          | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| GU8I           | 0    | 0          | <b>0,4</b> | <b>2</b>    | 0          | <b>0,6</b> | 0    | 0          | <b>0,2</b> | 0          | 0    | 0          | <b>0,8</b>  | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0          | 0    |      |
| GU12V          | 0    | <b>0,8</b> | <b>0,2</b> | <b>1,6</b>  | <b>0,4</b> | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0           | 0          | 0          | 0          | 0    | 0    | 0          | 0    | 0          | <b>0,4</b> | 0          | 0    | 0    | 0          | <b>0,4</b> | 0    | 0          | 0    | 0    |

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Tabela 3. Cont.

| Loca de coleta | APER | APEE       | ADTR        | ADM1        | ASP1       | ASP2     | ASP3 | ASP4       | ASP6        | ASP7 | ASP8 | ASP9 | CCAM       | CFEU | CFLU | CPAC | AZIE | PLHU | PLOB | GOCO       | GOEX       | PBRA | PHIN | PINC       | PLAG       | PPOT | PRHO | PSTR       | PVIC       | PSP3 | LHUN |
|----------------|------|------------|-------------|-------------|------------|----------|------|------------|-------------|------|------|------|------------|------|------|------|------|------|------|------------|------------|------|------|------------|------------|------|------|------------|------------|------|------|
| GU12I          | 0    | <b>0,2</b> | <b>0,2</b>  | <b>0,4</b>  | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          |      |      |
| HB1V           | 0    | <b>0,4</b> | <b>0,4</b>  | <b>14,8</b> | <b>0,8</b> | <b>1</b> | 0    | 0          | <b>2</b>    | 0    | 0    | 0    | <b>0,2</b> | 0    | 0    | 0    | 0    | 0    | 0    | 0          | <b>0,2</b> | 0    | 0    | <b>0,2</b> | 0          | 0    | 0    | 0          | 0          |      |      |
| HB1I           | 0    | 0          | 0           | <b>1,4</b>  | 0          | <b>2</b> | 0    | 0          | <b>0,4</b>  | 0    | 0    | 0    | <b>0,2</b> | 0    | 0    | 0    | 0    | 0    | 0    | 0          | <b>0</b>   | 0    | 0    | 0          | <b>0,6</b> | 0    | 0    | <b>0,4</b> | 0          |      |      |
| IP3V           | 0    | 0          | <b>0,6</b>  | 0           | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | <b>0,6</b> | 0    | 0    | 0          | 0          |      |      |
| IP3I           | 0    | 0          | <b>0,8</b>  | <b>4</b>    | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | <b>0,4</b> | 0          | 0    | 0    | <b>3</b>   | 0          | 0    | 0    | 0          | 0          |      |      |
| IT1V           | 0    | 0          | <b>3,1</b>  | <b>0,4</b>  | 0          | 0        | 0    | 0          | <b>13,6</b> | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          |      |      |
| IT1I           | 0    | <b>0,2</b> | <b>0,2</b>  | 0           | 0          | 0        | 0    | 0          | <b>1,2</b>  | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          |      |      |
| IT5V           | 0    | <b>0,4</b> | 0           | 0           | 0          | 0        | 0    | 0          | <b>12,5</b> | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          |      |      |
| IT5I           | 0    | <b>1,2</b> | <b>32,3</b> | 0           | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          |      |      |
| JC6V           | 0    | <b>0,4</b> | <b>2,2</b>  | 0           | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          |      |      |
| JC6I           | 0    | 0          | 0           | <b>0,8</b>  | 0          | 0        | 0    | <b>0,2</b> | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          |      |      |
| JC7V           | 0    | 0          | <b>0,2</b>  | <b>0,6</b>  | 0          | 0        | 0    | <b>0,2</b> | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          |      |      |
| JC7I           | 0    | 0          | <b>0,6</b>  | <b>0,2</b>  | 0          | 0        | 0    | <b>0,2</b> | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          |      |      |
| JA1V           | 0    | 0          | 0           | 0           | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | <b>0,4</b> | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          |      |      |
| JA1I           | 0    | 0          | 0           | 0           | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          |      |      |
| JU1V           | 0    | 0          | 0           | <b>0,8</b>  | <b>0,2</b> | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          |      |      |
| JU1I           | 0    | 0          | 0           | <b>0,2</b>  | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | <b>0,2</b> | 0    |      |
| JU2V           | 0    | 0          | <b>0,4</b>  | <b>1,2</b>  | <b>0,2</b> | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | <b>0,4</b> | 0          | 0    | 0    | <b>0,2</b> | 0          | 0    | 0    | 0          | 0          |      |      |
| JU2I           | 0    | 0          | 0           | 0           | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    |      |
| JP1V           | 0    | 0          | <b>0,9</b>  | 0           | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    |      |
| JP1I           | 0    | 0          | 0           | 0           | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    |      |
| JP4V           | 0    | 0          | <b>0,6</b>  | 0           | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    |      |
| JP4I           | 0    | 0          | <b>0,2</b>  | 0           | 0          | 0        | 0    | 0          | 0           | 0    | 0    | 0    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    | 0    | 0          | 0          | 0    |      |

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Tabela 3. Cont.

| Loca de coleta | APER       | APEE       | ADTR       | ADM1       | ASP1       | ASP2 | ASP3 | ASP4       | ASP6        | ASP7       | ASP8       | ASP9       | CCAM       | CFEU | CFLU | CPAC | AZIE       | PLHU       | PLOB       | GOCO       | GOEX | PBRA | PHIN       | PINC       | PLAG       | PPOT | PRHO       | PSTR       | PVIC | PSP3       | LHUN |
|----------------|------------|------------|------------|------------|------------|------|------|------------|-------------|------------|------------|------------|------------|------|------|------|------------|------------|------------|------------|------|------|------------|------------|------------|------|------------|------------|------|------------|------|
| LG1V           | 0          | 0          | 0          | <b>1,9</b> | 0          | 0    | 0    | 0          | <b>89,5</b> | 0          | <b>1,5</b> | 0          | 0          | 0    | 0    | 0    | <b>0,2</b> | 0          | 0          | 0          | 0    | 0    | 0          | 0          | <b>0,4</b> | 0    | 0          | 0          | 0    | 0          |      |
| LG1I           | 0          | <b>0,2</b> | 0          | <b>1,8</b> | 0          | 0    | 0    | 0          | <b>86,7</b> | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | <b>0,4</b> | 0          | 0    | 0          | <b>0,2</b> | 0    |            |      |
| NI1V           | 0          | <b>0,6</b> | <b>8,4</b> | <b>0,4</b> | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | <b>0,8</b> | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    |            |      |
| NI1I           | 0          | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          |      |
| PI1V           | 0          | 2          | 1          | <b>0,4</b> | <b>2,2</b> | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | <b>0,2</b> | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          |      |
| PI1I           | 0          | <b>0,4</b> | 3          | <b>0,2</b> | 0          | 0    | 0    | 0          | 0           | 0          | 0          | <b>0,2</b> | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          |      |
| PI3V           | 0          | <b>0,4</b> | <b>2,4</b> | <b>0,2</b> | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          |      |
| PI3I           | 0          | <b>0,2</b> | 1          | 0          | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          | 0    |
| PC1V           | <b>0,2</b> | <b>0,4</b> | 4          | 6          | <b>0,4</b> | 0    | 0    | <b>0,2</b> | 0           | 0          | 0          | 0          | <b>0,4</b> | 0    | 0    | 0    | 0          | <b>0,2</b> | <b>0,6</b> | <b>0,2</b> | 0    | 0    | <b>0,2</b> | <b>0,4</b> | <b>0,2</b> | 0    | <b>0,2</b> | <b>0,6</b> | 0    |            |      |
| PC1I           | 0          | 0          | 2          | <b>2,8</b> | <b>0,6</b> | 0    | 0    | <b>0,2</b> | 0           | 0          | 0          | <b>0,2</b> | 0          | 0    | 0    | 0    | 0          | <b>0,4</b> | <b>0,4</b> | 0          | 0    | 0    | 0          | 0          | <b>0,2</b> | 0    | 0          | 0          | 0    | 0          |      |
| PC4V           | 0          | 0          | 7,2        | <b>1,8</b> | 0          | 0    | 0    | <b>0,6</b> | <b>0,4</b>  | <b>0,2</b> | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | <b>0,6</b> | 0    | 0          | 0          | 0    | 0          |      |
| PC4I           | 0          | 0          | 2,2        | <b>0,8</b> | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          | 0    |
| PA1V           | 0          | 0          | <b>0,2</b> | <b>1,2</b> | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | <b>0,2</b> |      |
| PA1I           | 0          | 0          | <b>0,8</b> | 0          | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          | 0    |
| PB3V           | 0          | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          | 0    |
| PB3I           | 0          | <b>0,8</b> | <b>2,3</b> | 0          | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          | 0    |
| PB4V           | 0          | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          | 0    |
| PB4I           | 0          | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          | 0    |
| PB5V           | 0          | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          | 0    |
| PB5I           | 0          | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          | 0    |
| PN2V           | 0          | <b>2,8</b> | <b>2,2</b> | <b>3</b>   | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | <b>0,4</b> |      |
| PN2I           | 0          | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          | 0    |
| RC1V           | 0          | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0           | 0          | 0          | 0          | 0          | 0    | 0    | 0    | 0          | 0          | 0          | 0          | 0    | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0    | 0          | 0    |

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Tabela 3. Cont.

| Loca de coleta | APER       | APEE       | ADTR        | ADM1        | ASP1       | ASP2       | ASP3       | ASP4        | ASP6       | ASP7       | ASP8 | ASP9       | CCAM       | CFEU | CFLU | CPAC       | AZIE | PLHU       | PLOB       | GOCO       | GOEX       | PBRA       | PHIN       | PINC | PLAG       | PPOT       | PRHO       | PSTR       | PVIC       | PSP3 | LHUN |
|----------------|------------|------------|-------------|-------------|------------|------------|------------|-------------|------------|------------|------|------------|------------|------|------|------------|------|------------|------------|------------|------------|------------|------------|------|------------|------------|------------|------------|------------|------|------|
| RC1I           | 0          | <b>0,6</b> | <b>2,3</b>  | <b>8,5</b>  | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0          | <b>0,6</b> | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          |      |      |
| RC3I           | <b>0,3</b> | 0          | 0           | <b>1,6</b>  | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0          | <b>0,3</b> | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | <b>0,6</b> |      |      |
| SI3V           | <b>1,4</b> | <b>0,2</b> | <b>0,4</b>  | 0           | 0          | 0          | 0          | 0           | 0          | <b>0,2</b> | 0    | <b>0,4</b> | 0          | 0    | 0    | <b>0,6</b> | 0    | 0          | <b>1</b>   | <b>0,4</b> | 0          | 0          | 0          | 0    | 0          | 0          | 0          | <b>0,2</b> | <b>0,2</b> |      |      |
| SI3I           | <b>2,6</b> | <b>0,3</b> | <b>1,9</b>  | <b>2,2</b>  | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0          | 0          | 0    | 0    | <b>3,2</b> | 0    | 0          | <b>0,3</b> | <b>0,3</b> | 0          | 0          | 0          | 0    | 0          | <b>0,3</b> | 0          | 0          | 0          |      |      |
| SG1V           | 0          | 0          | 0           | <b>0,4</b>  | 0          | 0          | 0          | <b>0,4</b>  | <b>0,2</b> | 0          | 0    | 0          | <b>1,2</b> | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | <b>0,2</b> |      |      |
| SG1I           | 0          | 0          | <b>16</b>   | <b>6,6</b>  | <b>0,2</b> | <b>2,2</b> | 0          | 0           | 0          | <b>0,6</b> | 0    | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | <b>0,2</b> | 0          | 0    | 0          | 0          | 0          | <b>0,2</b> |            |      |      |
| SG4V           | 0          | <b>0,2</b> | <b>0,8</b>  | <b>0,8</b>  | <b>0,2</b> | 0          | <b>0,2</b> | 0           | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | <b>0,2</b> | 0          | 0          | 0          | 0    | <b>0,2</b> | 0          | 0          | 0          | <b>0,2</b> |      |      |
| SG4I           | 0          | 0          | <b>1,6</b>  | <b>2,4</b>  | 0          | 0          | <b>0,4</b> | <b>0,4</b>  | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0          | 0    | <b>0,2</b> | 0          | 0          | 0          | 0          |      |      |
| SH2V           | 0          | 0          | 0           | <b>0,4</b>  | 0          | 0          | 0          | 0           | <b>4,3</b> | 0          | 0    | 0          | <b>0,4</b> | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0          | 0    | 0          | <b>0,2</b> | 0          | 0          | 0          | 0    |      |
| SH2I           | 0          | 0          | 0           | <b>5,4</b>  | 0          | 0          | 0          | 0           | <b>1,2</b> | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0          | 0    | <b>0,2</b> | 0          | 0          | 0          | 0          |      |      |
| SE3V           | 0          | 0          | 0           | <b>0,2</b>  | <b>0,2</b> | <b>0,2</b> | 0          | 0           | 0          | 0          | 0    | 0          | <b>0,2</b> | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          |      |      |
| SE3I           | 0          | 0          | 0           | 0           | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          |      |      |
| TA1V           | 0          | 0          | <b>0,4</b>  | <b>0,4</b>  | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          |      |      |
| TA1I           | 0          | <b>0,2</b> | <b>1,4</b>  | <b>0,4</b>  | 0          | 0          | 0          | 0           | 0          | 0          | 0    | 0          | <b>0,2</b> | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          | 0          |      |      |
| TG3V           | 0          | <b>0,2</b> | <b>0,9</b>  | <b>0,5</b>  | <b>0,2</b> | 0          | 0          | <b>23,2</b> | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | 0          | 0          | 0    | <b>0,7</b> | 0          | 0          | 0          | 0          |      |      |
| TG3I           | 0          | 0          | <b>0,6</b>  | <b>2,2</b>  | 0          | 0          | 0          | <b>2,2</b>  | 0          | 0          | 0    | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0          | 0          | <b>0,2</b> | 0    | 0          | 0          | <b>0,4</b> | 0          |            |      |      |
| TU1V           | 0          | 0          | <b>10,2</b> | <b>11,1</b> | <b>0,2</b> | <b>1,4</b> | 0          | <b>0,5</b>  | 0          | <b>0,2</b> | 0    | 0          | 0          | 0    | 0    | 0          | 0    | <b>0,4</b> | 0          | <b>0,2</b> | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0          |            |      |      |
| TU1I           | 0          | 0          | <b>10,4</b> | <b>9,4</b>  | 0          | 0          | 0          | <b>1,6</b>  | 0          | <b>0,2</b> | 0    | 0          | 0          | 0    | 0    | 0          | 0    | 0          | 0          | 0          | <b>0,2</b> | 0          | 0          | 0    | 0          | 0          | <b>0,4</b> | 0          |            |      |      |

## **ANEXO 4**

ABUNDÂNCIA RELATIVA (%) DOS TÁXONS ENCONTRADOS EM AMOSTRAS DE  
PERIFÍTON

**Tabela 4.** Abundância relativa (%) dos táxons encontrados nas amostras de perifítion do Projeto Acquased. Código das espécies conforme Anexo 1 e dos locais de amostragem segundo Anexo 2. Em negrito: valores acima de zero.

| Local de coleta | APEE       | ADTR        | ADM1        | ASP1        | ASP2        | ASP3       | ASP4       | ASP6        | ASP7        | ASP8 | ASP9       | CCAM       | CEUG       | CFEU | ACOA       | AZIE       | GOCO       | GOEX     | PINC | PMIN       | PRHO       | LHUN |
|-----------------|------------|-------------|-------------|-------------|-------------|------------|------------|-------------|-------------|------|------------|------------|------------|------|------------|------------|------------|----------|------|------------|------------|------|
| <b>BB2V</b>     | 0          | 0           | <b>0,2</b>  | 0           | 0           | 0          | 0          | 0           | 0           | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | 0          |      |
| <b>BB2I</b>     | 0          | <b>0,4</b>  | 0           | 0           | 0           | 0          | 0          | 0           | 0           | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | <b>1</b>   |      |
| <b>BB5V</b>     | 0          | 0           | 0           | 0           | 0           | 0          | 0          | 0           | 0           | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | 0          |      |
| <b>BB5I</b>     | 0          | 0           | 0           | 0           | 0           | 0          | 0          | 0           | 0           | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | <b>1,8</b> |      |
| <b>RG2I</b>     | 0          | <b>1,98</b> | <b>14</b>   | <b>11,9</b> | 0           | <b>9,5</b> | 0          | <b>6,5</b>  | <b>0,6</b>  | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | <b>1,8</b> |      |
| <b>RG4V</b>     | 0          | 0           | <b>1,8</b>  | <b>0,6</b>  | 0           | 0          | 0          | <b>13,2</b> | 0           | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | <b>1,4</b> |      |
| <b>RG4I</b>     | 0          | 0           | <b>3</b>    | <b>0,4</b>  | <b>0,8</b>  | 0          | 0          | 0           | 0           | 0    | 0          | 0          | 0          | 0    | <b>0,2</b> | 0          | <b>0,2</b> | 0        | 0    | 0          | <b>8</b>   |      |
| <b>RP7V</b>     | <b>2,2</b> | 0           | 0           | 0           | 0           | 0          | 0          | 0           | 0           | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | 0          |      |
| <b>RP7I</b>     | 0          | 0           | <b>0,2</b>  | 0           | 0           | 0          | 0          | 0           | 0           | 0    | 0          | 0          | 0          | 0    | <b>0,2</b> | 0          | 0          | 0        | 0    | 0          | 0          |      |
| <b>CC9V</b>     | 0          | <b>0,2</b>  | <b>11,8</b> | <b>0,4</b>  | 0           | 0          | 0          | 0           | 0           | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | <b>0,2</b> |      |
| <b>CC9I</b>     | <b>0,2</b> | 1           | <b>19,2</b> | <b>5,6</b>  | 0           | <b>4,6</b> | 0          | <b>0,4</b>  | 0           | 0    | 0          | <b>1,6</b> | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | <b>0,2</b> |      |
| <b>TQ10V</b>    | 0          | <b>17,7</b> | <b>11,5</b> | <b>2,2</b>  | 0           | 0          | 0          | <b>0,6</b>  | <b>4,8</b>  | 0    | 0          | 0          | <b>1,6</b> | 0    | 0          | 0          | 0          | 0        | 0    | 0,2        | 0          |      |
| <b>TQ10I</b>    | 0          | <b>22,4</b> | 2           | 0           | <b>0,6</b>  | 0          | 0          | <b>2,2</b>  | <b>14,4</b> | 0    | 0          | <b>0,2</b> | <b>0,4</b> | 0    | 0          | 0          | 0          | 0        | 0    | 0          | 0          |      |
| <b>CB1I</b>     | <b>1,6</b> | <b>0,2</b>  | <b>45</b>   | <b>1,6</b>  | 0           | 0          | <b>5,2</b> | 0           | 0           | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | 0          |      |
| <b>CG2I</b>     | <b>2,8</b> | <b>0,2</b>  | <b>0,2</b>  | 0           | 0           | 0          | <b>0,2</b> | 0           | 0           | 0    | <b>0,2</b> | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | 0          |      |
| <b>FR3V</b>     | <b>1,2</b> | <b>19,9</b> | <b>0,2</b>  | 0           | 0           | 0          | 0          | 0           | 0           | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | 0          |      |
| <b>FR3I</b>     | <b>4,2</b> | <b>22</b>   | 0           | 0           | 0           | 0          | 0          | 0           | 0           | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | 0          | 0          |      |
| <b>HB1V</b>     | 0          | 0           | 0           | 0           | <b>0,8</b>  | 0          | 0          | 0           | 0           | 0    | 0          | 0          | 0          | 0    | 0          | 0          | <b>0,2</b> | 0        | 0    | 0          | <b>0,2</b> |      |
| <b>HB1I</b>     | 0          | <b>0,4</b>  | 6           | 0           | <b>13,4</b> | 0          | 0          | <b>0,8</b>  | 0           | 0    | 0          | 0          | 0          | 0    | 0          | <b>0,2</b> | 0          | <b>2</b> | 0    | 0          | <b>1</b>   |      |
| <b>IP3V</b>     | 0          | 0           | <b>0,4</b>  | 0           | 0           | 0          | 0          | <b>1</b>    | 0           | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          | 0        | 0    | <b>4,2</b> | 0          |      |

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Tabela 4. Cont.

| Local de coleta | APEE        | ADTR        | ADM1        | ASP1       | ASP2 | ASP3       | ASP4       | ASP6       | ASP7       | ASP8        | ASP9 | CCAM | CEUG       | CFEU | ACOA       | AZIE       | GOCO       | GOEX | PINC       | PMIN       | PRHO       | LHUN |
|-----------------|-------------|-------------|-------------|------------|------|------------|------------|------------|------------|-------------|------|------|------------|------|------------|------------|------------|------|------------|------------|------------|------|
| IT1V            | 0           | 0           | <b>12,6</b> | <b>1,2</b> | 0    | 0          | 0          | <b>4</b>   | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | <b>0,4</b> | 0          | 0          |      |
| JU1V            | <b>0,2</b>  | <b>0,4</b>  | <b>2,5</b>  | <b>0,2</b> | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | <b>0,2</b> |      |
| JU1I            | 0           | <b>0,2</b>  | <b>0,2</b>  | 0          | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |
| JP1V            | <b>3,8</b>  | <b>27,5</b> | <b>0,2</b>  | 0          | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |
| JP1I            | <b>8,7</b>  | <b>23,9</b> | 0           | 0          | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |
| JP4V            | <b>8</b>    | <b>7,6</b>  | 0           | 0          | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |
| JP4I            | <b>18,3</b> | <b>11,8</b> | 0           | 0          | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |
| LG1I            | 0           | 0           | <b>72,3</b> | 0          | 0    | 0          | 0          | <b>4,5</b> | 0          | <b>14,8</b> | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |
| NI1V            | <b>0,4</b>  | <b>6,6</b>  | <b>0,6</b>  | 0          | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | <b>0,4</b> | 0    | 0          | 0          | <b>0,4</b> | 0    | 0          | 0          | 0          |      |
| NI1I            | <b>0,8</b>  | <b>4,4</b>  | 0           | 0          | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | <b>0,2</b> | 0    | 0          | 0          | 0          |      |
| PI1V            | <b>1,8</b>  | <b>14,9</b> | <b>2,6</b>  | <b>0,4</b> | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | <b>0,2</b> | 0          | 0          | 0    | 0          | 0          | 0          |      |
| PI1I            | <b>1</b>    | <b>9,8</b>  | <b>3,4</b>  | 0          | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | <b>0,4</b> | 0          |      |
| PI3V            | <b>3</b>    | <b>38,8</b> | <b>2,8</b>  | 0          | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |
| PI3I            | <b>3,2</b>  | <b>27,1</b> | <b>7,8</b>  | 0          | 0    | 0          | <b>0,4</b> | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |
| PC1V            | 0           | <b>4</b>    | <b>22,9</b> | 0          | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | <b>0,2</b> | 0    | 0          | 0          | 0          |      |
| PC1I            | 0           | <b>11,9</b> | <b>10,9</b> | <b>0,4</b> | 0    | 0          | <b>0,6</b> | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |
| PC4V            | 0           | <b>45,5</b> | <b>17,6</b> | <b>1,1</b> | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |
| PC4I            | 0           | <b>35</b>   | <b>13,8</b> | 0          | 0    | 0          | <b>0,8</b> | 0          | <b>1,4</b> | 0           | 0    | 0    | 0          | 0    | 0          | <b>0,2</b> | 0          | 0    | 0          | 0          | 0          |      |
| PA1V            | <b>0,4</b>  | <b>9</b>    | <b>5,5</b>  | <b>1,9</b> | 0    | 0          | 0          | <b>0,7</b> | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | <b>1,3</b> |      |
| PA1I            | <b>1,3</b>  | <b>3,8</b>  | <b>6,4</b>  | <b>0,2</b> | 0    | <b>0,4</b> | <b>0,2</b> | <b>1,5</b> | 0          | 0           | 0    | 0    | 0          | 0    | 0          | <b>0,6</b> | 0          | 0    | 0          | 1          | 0          |      |
| PB4I            | 0           | 0           | 0           | 0          | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |
| PB5I            | 0           | <b>0,2</b>  | 0           | 0          | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |
| PN2V            | <b>3,4</b>  | <b>0,2</b>  | 0           | <b>0,2</b> | 0    | 0          | 0          | 0          | 0          | 0           | 0    | 0    | 0          | 0    | 0          | 0          | 0          | 0    | 0          | 0          | 0          |      |

Continua

**Tabela 4. Cont.**

