GISELE CAROLINA MARQUARDT

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Tese apresentada ao Instituto de Botânica da Secretaria do Meio Ambiente do Estado de São Paulo como parte dos requisitos exigidos para 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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"O meu mundo não é como o dos outros, quero demais, exijo demais; há em mim uma sede de infinito, uma angústia constante que eu nem mesma compreendo, pois estou longe de ser uma pessimista; sou antes uma exaltada, com uma alma intensa, violenta, atormentada, uma alma que não se sente bem onde está, que tem saudade… sei lá de quê!"

Florbela Espanca, Cartas a Guido Battelli.

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Todos vocês são coautores deste trabalho.

SUMÁRIO

Resumo

As diatomáceas (Bacillariophyceae) são algas unicelulares, microscópicas, de distribuição cosmopolita, que ocorrem em diferentes ecossistemas. Sua principal característica é a parede celular bivalve composta quimicamente por sílica, cuja superfície é ricamente esculpida e o conjunto das duas valvas de um indivíduo constitui a frústula. Tais algas são sensíveis a uma variedade de fatores ambientais necessários para seu ótimo crescimento sendo, por isso, consideradas excelentes indicadores de mudanças que ocorrem nos ecossistemas aquáticos, em especial da eutrofização. Apesar disso, o conhecimento das diatomáceas das regiões tropicais ainda é considerado bastante insuficiente. A atual pesquisa é parte do projeto AcquaSed (2010-2014) e enfoca seis sistemas tropicais e suas comunidades de diatomáceas. Para tanto, foram amostrados seis reservatórios localizados nas bacias do Alto Paranapanema (três estações de amostragem) e do Ribeira do Iguape/Litoral Sul (17 estações de amostragem), situados no sul do Estado de São Paulo. Amostras de água coletadas durante o verão e inverno e de sedimentos superficiais amostrados no inverno foram utilizadas para analisar as características ambientais dos seis reservatórios, além de suas respectivas comunidades de diatomáceas. Apesar do paradigma estabelecido sobre a dispersão ubíqua dos microrganismos, estudos taxonômicos recentes confirmaram um componente endêmico distintivo na flórula de diatomáceas de água doce em diversas localidades ao redor do mundo e, no Brasil, um crescente número de trabalhos taxonômicos contendo descrição de novas espécies vem sendo publicado. Na presente tese, algumas espécies que ocorrem na área de estudo e relacionadas no banco de dados do projeto AcquaSed são apresentadas como novas com base em análises de microscopia de luz (ML) e eletrônica de varredura (MEV), além do suporte de dados ecológicos, da análise de materiais-tipo e da comparação com populações de diferentes hábitats e ecossistemas. As amostras coletadas nos reservatórios brasileiros revelaram uma flórula muito diversa, com espécies desconhecidas e únicas de diatomáceas, que clarificam a importância de avaliar a identidade e a distribuição dessas algas nas regiões tropicais. A composição das comunidades de diatomáceas reflete todo um complexo de variáveis ecológicas de um local particular. Consequentemente, estudos taxonômicos e levantamentos florísticos não só apoiam projetos de pesquisa aplicada como também permitem o monitoramento da qualidade da água, além de uma ampla gama de questões de pesquisa básica de revisão e monografias taxonômicas, reconstrução filogenética, estudos biogeográficos, ecológicos, fisiológicos, de restauração e conservação. Do ponto de vista ecológico, investigou-se os principais direcionadores da biodiversidade e da distribuição de diatomáceas nos seis reservatórios e avaliaram-se seus efeitos também em conjuntos de diatomáceas de diferentes categorias de raridade, evidenciando a importância desse grupo que compõe a maior parte da comunidade, mas que é, em geral, excluído das análises estatísticas. O presente estudo mostra que os processos na natureza são dependentes da escala e que os limites da dispersão podem ser mais importantes em pequenas escalas (< 100 km). Além disso, a área de estudo não impactada pode ser considerada um fator importante na determinação das contribuições relativas dos componentes geográficos na estrutura da comunidade de diatomáceas. Finalmente, expôs-se que altos níveis de conectividade hidrológica favorecem a troca de organismos através da dispersão passiva, aumentando a similaridade na composição das metacomunidades e diminuindo a diversidade beta, demonstrando que a conectividade hidrológica desempenha um papel de capital importância na explicação da dinâmica das metacomunidades. Consequentemente, esta variável não deve ser negligenciada em modelos que predigam o funcionamento do ecossistema. Para fins de conservação, os resultados sugerem uma proteção em grande escala, assegurando a diversidade beta entre sítios e conservando a diversidade total. Por fim, o desenvolvimento e o teste de diferentes abordagens de biomonitoramento tornam-se fundamentais no fornecimento de informações sobre o estado atual dos ecossistemas de água doce.

Palavras-chave: bioindicação, diatomácea, diversidade beta, fitoplâncton, represa de abastecimento, RMSP, sedimentos superficiais.

Abstract

Diatoms (Bacillariophyceae) are cosmopolitan, unicellular, microscopic algae that occur in different ecosystems. Their main morphological feature is the silica built cell wall possessing a rich sculpted surface that constitutes the frustule. Diatoms are sensitive to a variety of environmental features necessary for its optimal growth that makes them excellent indicators of aquatic ecosystems changes and especially the eutrophication. Despite this, knowledge about diatoms of tropical regions is still considered very insufficient. Present research is part of the AcquaSed project (2010-2014) and focuses, therefore, on tropical systems and their diatom communities. For this purpose, six reservoirs located in the Alto Paranapanema (3 sampling stations) and Ribeira do Iguape/Litoral Sul (17 sampling units) basins locates in the south of the State of São Paulo were sampled. Water (summer and winter) and surface sediments (winter) samples were collected for the analyses of the reservoirs environmental characteristics and their respective diatom communities. Despite the established paradigm that microorganisms are ubiquitous dispersed, recent taxonomic studies confirmed a distinctive endemic component in the freshwater diatom flora of several localities round the world, and in Brazil a growing number of taxonomic works containing new species description has been published. In the present thesis, some species that occur in the study area and were included in the AcquaSed database are presented as new based on light (LM) and scanning electron microscopy (SEM) analyses, and also as supported by ecological information, type material analysis, and comparison among populations of different habitats and ecosystems. Analyses of samples collected from Brazilian reservoirs revealed a very diverse diatom flora, with unknown and unique diatom species enlightening the importance of tropical diatoms identity and distribution evaluation. Diatom community composition reflects an entire complex of ecological characteristics of a particular location. In this sense, taxonomical studies and floristic surveys are of utmost importance for applied research projects, such as water quality monitoring as well as of a wide range of basic research such as taxonomic monographs and reviews, phylogenetic reconstruction, biogeography, ecology, physiology, and restoration and conservation questions. Diatom biodiversity main drivers and distribution in tropical reservoirs were investigated from the ecological point of view, and their effects on the diatom datasets separated into different rarity categories were evaluated, evidencing the importance of the latter group that constitutes the major part of the diatom community, but that is usually excluded from the statistical analyses.

Current study shows that processes in nature are scale dependent and that dispersion limits may be more important on small scales (< 100 km). In addition, the unaccented study area can be considered an important factor for determination of the relative contribution of the geographic components to the diatom community structure. Finally, it was indicated that high levels of hydrological connectivity favor the organisms exchange through a passive dispersion, thus increasing the similarity of the metacommunities composition and decreasing beta diversity, and consequently demonstrating that hydrological connectivity plays a considerable role in explaining metacommunities dynamics. Consequently, this variable must be not neglected in models that aims at predicting the ecosystem functioning. For conservation purposes, the results suggest large-scale protection, ensuring beta diversity between sites, and conserving total diversity. In addition, development and testing of different biomonitoring approaches are essential to provide information on the current state of freshwater ecosystems.

Key words: bioassessment, β-diversity, phytoplankton, diatom, reservoir, RMSP, surface sediments.

Apresentação

A presente tese sobre a “Comparação da biodiversidade e distribuição das diatomáceas planctônicas e de sedimentos superficiais em seis reservatórios no sul do Estado de São Paulo” será apresentada em oito capítulos, sendo cada um deles redigido na forma de um manuscrito científico individualizado quanto a objetivos, revisão da literatura, métodos de pesquisa, resultados, discussão, conclusões e formatação gráfica, de modo que possam ser submetidos em um periódico acadêmico de interesse independente dos demais artigos ou baseado nos resultados parciais de um artigo anterior. Todos os dados utilizados para elaboração desta tese foram coletados durante o ano 2014, com a colaboração de funcionários e colegas estudantes do Núcleo de Pesquisa em Ecologia do Instituto de Botânica da Secretaria do Meio Ambiente do Estado de São Paulo durante o desenvolvimento do projeto “AcquaSed” financiado pela FAPESP, Fundação de Amparo à Pesquisa do Estado de São Paulo. Por conseguinte, uma seção intitulada “Introdução Geral” apresentará os principais referenciais teóricos e outra chamada “Objetivos” conterá as hipóteses básicas que motivaram e direcionaram o desenvolvimento da tese. Na sequência, o capítulo 1, intitulado “Diatom assemblages (Bacillariophyta) in six tropical reservoirs from southwest Brazil: species composition and variation patterns” e representa o original do trabalho que será submetido à “Acta Limnologica Brasiliensia”. O capítulo 2 intitula-se “Variance partitioning of deconstructed tropical diatom communities in reservoirs cascade” conterá o original do trabalho submetido para apreciação na revista “Acta Limnologica Brasiliensia”. O capítulo 3, “Partitioning patterns of freshwater diatoms in tropical reservoirs: effect of hydrological connectivity” é o original do trabalho a ser submetido à revista “Journal of Limnology”. O capítulo 4 intitula-se “Distance decay as a descriptor of the diatom compositional variation in tropical reservoirs” e é o original do trabalho aceito para publicação em “Marine and Freshwater Research”. O capítulo 5, intitulado “Weighted averaging of species indicator values in tropical reservoirs: the influence of rare taxa richness”, é o original do trabalho que será submetido à “Biotropica”. O capítulo 6 intitula-se “Type analysis of *Achnanthidium minutissimum* and *A. catenatum* and description of *A. tropicocatenatum* sp. nov., a common species (Bacillariophyta) in Brazilian reservoirs” e é o original de um trabalho já aceito para publicação em “Plant Ecology and Evolution”. O capítulo 7, “*Sellaphora tropicomadida* sp. nov., a new freshwater diatom species (Bacillariophyta) from a tropical Brazilian reservoir”, é o original do trabalho já aceito para publicação na revista “Brazilian Journal of Botany”. Por fim, o capítulo 8 é intitulado “Morphology and distribution of *Encyonema angustecapitatum* Krammer species complex (Bacillariophyceae) with description of four new species from São Paulo, Southeast Brazil” e é o original do trabalho já aceito para publicação em “Fottea”.

Após a apresentação dos oito capítulos, uma seção intitulada “Conclusões Gerais” reunirá as principais conclusões da tese e suas implicações para ações de conservação das comunidades e pesquisas futuras.

Introdução geral

Motivo de grande preocupação durante as últimas décadas, a degradação de ecossistemas aquáticos continentais é apontada como um dos grandes problemas ambientais da atualidade (Tundisi 2008, Ribeiro 2008). A falta de planejamento urbano, principalmente, no que tange à disposição de efluentes e resíduos, pode ser apontada como uma das principais causas da atual problemática ambiental (Gralhóz & Nogueira 2006). Assim sendo, para especialistas como Rogers *et al.* (2006) a crise da água no século XXI é muito mais de gerenciamento do que de escassez e estresse. Consequentemente, o grande desafio para o século XXI será gerenciar a água doce para equilibrar as demandas da humanidade e dos ecossistemas, de tal maneira que os últimos possam continuar a prestar outros serviços essenciais ao bem-estar humano (MA 2008).

A eutrofização é, presentemente, um dos mais sérios problemas que afeta a qualidade das águas costeiras e interiores (lagos, rios, represas e águas subterrâneas) (Tundisi 2006). Conforme Tundisi (2003), a eutrofização resulta do enriquecimento artificial por dois tipos de nutrientes, fósforo e nitrogênio, que são despejados de forma dissolvida ou particulada em lagos, represas e rios e transformados em partículas orgânicas e matéria viva vegetal pelo metabolismo das plantas. O referido autor esclareceu ainda a existência de dois processos distintos para o problema, quais sejam: (1) a eutrofização natural, que resulta da descarga normal de nitrogênio e fósforo nos sistemas aquáticos; e (2) a eutrofização cultural, que provém dos despejos de esgotos domésticos e industriais e da descarga de fertilizantes usados na agricultura que, ordinariamente, aceleram o enriquecimento das águas superficiais e subterrâneas.

Até meados do século XX, a eutrofização de lagos e reservatórios na Europa e América do Norte era considerada um problema relacionado à poluição. Atualmente, ela representa um problema mundial e tem causado a deterioração de um sem número de ambientes aquáticos, prejudicando os usos múltiplos da água (Bartram *et al.* 1999, Rocha *et al.* 2006). A importância social e econômica desses ecossistemas é enorme, pois possuem usos múltiplos como abastecimento, irrigação, produção de energia elétrica, recreação, turismo, transporte e regulação de enchentes, entre outros (Tundisi 1999). Apesar disso, efluentes urbanos, industriais e agropecuários são constantemente despejados nesses ecossistemas (Nogueira *et al.* 2005), intensificando os processos de degradação e, em particular, da eutrofização.

A eutrofização afeta diretamente o componente biológico dos recursos hídricos aumentando, de modo acentuado, a biomassa de algas e macrófitas aquáticas, além do componente socioeconômico por gerar compostos nocivos à saúde na água potável (Mehner & Benndorf 1995, Júlio-Júnior *et al.* 2005). Este processo resulta também em dano à qualidade ecológica do ecossistema, podendo levar à perda de biodiversidade e à extinção de espécies (Sayer & Roberts 2001, Bennion *et al.* 2011).

No caso de represas, Bicudo *et al.* (1999) comentaram que estas funcionam como "coletores de eventos" ao interceptarem o fluxo de água de um rio proporcionando, portanto, informação fundamental sobre as bacias hidrográficas, desde que as represas atuam como centros de convergência das várias atividades desenvolvidas na bacia hidrográfica, inclusive de seus usos e aspectos econômicos e sociais. Os referidos autores comentaram, ademais, que a informação introduzida no reservatório (entrada de material em suspensão, nutrientes inorgânicos e orgânicos, poluentes, etc.) interfere nos processos de organização das comunidades planctônicas, perifíticas e de peixes, bem como na composição química da água e dos sedimentos.

No Brasil, as represas desempenham papel ecológico, econômico e social preponderante. Deste modo, o estudo integrado de tais ecossistemas artificiais, bem como a perspectiva de manejo dos mesmos tem grande importância para o país (Henry & Nogueira 1999).

As alterações ecológicas observadas nos reservatórios podem ser detectadas a partir do monitoramento da água e dos sedimentos acumulados, sejam estes superficiais ou depositados em camadas (Birks & Birks 2006). O monitoramento da água acompanhado de métodos de análises físicas, químicas e biológicas constitui uma base consistente para a avaliação ecológica do ambiente, pois permitem o conhecimento instantâneo das condições da água, ou seja, no momento em que são feitas as medições (análises físicas e químicas), enquanto que as informações de efeitos prolongados (análises biológicas) são capazes de refletir estados não mais existentes no momento da verificação (Lobo *et al.* 2002).

Nos sedimentos aquáticos, as informações fornecidas são de efeitos ainda mais prolongados, desde que este compartimento constitui um arquivo de informação de natureza biogeoquímica através das camadas de deposição que são temporais e se encontram sequencialmente acumuladas (Mozeto 2004). Por conseguinte, os sedimentos superficiais representam uma amostra espacial e temporalmente integrada dos eventos que acumularam no passado recente, bem como dos organismos provenientes de diversos hábitats do ecossistema (Bennion 1995).

Índices para avaliar as condições ambientais têm incorporado indicadores biológicos como peixes, macrófitas, invertebrados e algas (Bellinger *et al.* 2006). Entre as algas, as diatomáceas ganham destaque especial, pois apresentam alguns atributos primordiais, como: (*1*) ampla distribuição geográfica; (*2*) algumas espécies são muito sensíveis às mudanças ambientais, enquanto outras são tolerantes; (*3*) respondem às mudanças ambientais em curtos e longos prazos; (*4*) facilidade de coleta; (*5*) suas valvas encontram-se, em geral, bem preservadas nos sedimentos, pois seu envoltório celular constituído de sílica polimerizada é resistente à decomposição por bactérias, dissolução química e ruptura física permitindo sua correta identificação taxonômica; (*6*) o material oxidado pode ser preservado e mantido em preparações permanentes, possibilitando seu reexame e intercâmbio com outros laboratórios; e (*7*) é conhecida uma grande quantidade de informação sobre a ecologia de diversas espécies, especialmente para as regiões de clima temperado (Bennion 1995, Lobo *et al*. 2002, Smol 2008).

Consequentemente, as diatomáceas são utilizadas como bioindicadoras em vários programas de gerenciamento por todo o Globo, com destaque para os países europeus (“European Council Water Framework Directive” WFD, European Union 2000), onde se busca estabelecer metas que possam garantir o bom estado ecológico dos corpos d’água (Rocha 2012). Entretanto, apesar do grave problema da eutrofização dos corpos d’água no Brasil, não existe ainda um programa nacional de gerenciamento da qualidade de água, sobretudo que utilize as diatomáceas (Rocha 2012).

Segundo Poulickova *et al*. (2004), estudos utilizando diatomáceas como indicadoras ambientais datam do início do século XX e foram desenvolvidos na Alemanha, quando Kolwitz & Marsson (1909) desenvolveram um sistema de sapróbios a partir das preferências ecológicas e das tolerâncias de espécies em rios daquele país.

Atualmente, os estudos têm buscado a resposta das comunidades de diatomáceas a uma série de variáveis ambientais como, por exemplo, as alterações de salinidade, pH e nutrientes orgânicos e inorgânicos (Bellinger *et al*. 2006). Os resultados obtidos das análises de sedimentos (ex. Bennion *et al*. 2001, Blinn & Bailey 2001, Dalton *et al.* 2005, Smol 2008, Taukulis & John 2009) vêm possibilitando a reconstrução do histórico da eutrofização, da salinidade, do clima e da evolução dos impactos antropogênicos e suas dimensões ecológicas e sócio-econômicas, além da elaboração de modelos de função de transferência que permitem estimar, quantitativamente, as condições ambientais passadas através do estudo das comunidades fósseis. Particularmente o modelo de transferência diatomáceas-fósforo permite resgatar os níveis pretéritos de fósforo na água, sendo possível estabelecer níveis de referência que possibilitam traçar metas mais reais de recuperação (Bennion *et al*. 2001, Smol 2008).

No Brasil, os trabalhos que utilizam diatomáceas como ferramenta na bioindicação se concentram na região sul do país, onde os ambientes lóticos têm sido alvo de mais pesquisas. Estudos com tal contexto foram realizados, primeiro, por Torgan & Aguiar (1974) para o rio Guaíba, onde as diatomáceas mais frequentes foram relacionadas com a saprobidade. Outros trabalhos enfocaram a avaliação do estado trófico (ex. Lobo *et al*. 2004a, 2004b, 2004c, 2004d, Salomoni *et al*. 2006, Lobo *et al*. 2006, Hermany *et al*. 2006, Düpont *et al*. 2007), além de estudos de comparação entre ecossistemas distintos (Lobo *et al*. 2004b, 2004c, 2004d, Hermany *et al*. 2006). Porém, apesar do número significativo de trabalhos, jamais foram realizadas abordagens enfatizando, especificamente, a influência do hábitat na distribuição e bioindicação das diatomáceas.

Para o Estado de São Paulo, contribuições para ambientes lóticos avaliaram a comunidade de diatomáceas e sua associação com as variáveis ambientais do rio Monjolinho e seus tributários (Souza 2002, Bere & Tundisi 2011a, 2011b). Especificamente para represas, Silva (2008) analisou a distribuição das diatomáceas dos sedimentos superficiais e sua associação com os níveis de fósforo dos reservatórios em cascata do rio Paranapanema (reservatórios Capivara, Chavantes, Jurumirim, Rosana e Salto Grande); e Ferrari (2010) avaliou a estrutura e a dinâmica das diatomáceas planctônicas e perifíticas em reservatórios oligotrófico e hipereutrófico do Parque Estadual das Fontes do Ipiranga, na cidade de São Paulo. Além destes, pesquisas que visaram à reconstrução paleolimnológica da represa Guarapiranga e ao diagnóstico atual da qualidade da água e dos sedimentos de mananciais situados na RMSP, Região Metropolitana de São Paulo vêm sendo realizados e estão vinculadas a um projeto mais amplo cognominado AcquaSed. Entre esses, estudos avaliando a biodiversidade e a distribuição das diatomáceas do plâncton e dos sedimentos superficiais foram realizados por Wengrat (2011) em ambiente sob grande interferência antropogênica (complexo Billings), Silva (2012) nas represas Pedro Beicht e Cachoeira da Graça (sistema Alto Cotia) e Nascimento (2012) para represa Jaguari-Jacareí (sistema Cantareira). Este último é considerado ambiente-referência em termos de qualidade da água. Ainda, Rocha (2012) avaliou a biodiversidade e a distribuição das diatomáceas em diferentes hábitats (perifítico, planctônico e sedimentos superficiais) de seis reservatórios com estados tróficos distintos na sub-bacia do Alto rio Tietê. Há também duas publicações de estudos eminentemente paleolimnológicos, quais sejam, uma avaliação do histórico da eutrofização de uma represa urbana rasa hipertrófica (Lago das Garças, São Paulo) com base na bioestratigrafia das diatomáceas nos últimos 100 anos (Costa-Böddeker *et al*. 2012) e o histórico da eutrofização da represa Guarapiranga, a segunda represa mais importante para abastecimento público de água de São Paulo (Fontana *et al.* 2013).

O presente estudo integra informação física, química e biológica da água e dos sedimentos superficiais (recentes) e fornece dados sobre a autoecologia das diatomáceas de reservatórios tropicais e, mais especificamente, de cinco reservatórios da bacia Ribeira do Iguape e Litoral Sul (reservatórios Jurupará, Cachoeira do França, Serraria, Cachoeira da Fumaça e Salto do

Iporanga) e um pertencente à bacia do Alto rio Paranapanema (reservatório Paineiras). O projeto está inserido em outro maior, interdisciplinar, intitulado "Reconstrução paleolimnológica da represa Guarapiranga e diagnóstico da qualidade atual da água e dos sedimentos de mananciais da RMSP com vistas ao gerenciamento do abastecimento – AcquaSed"

Finalmente, o trabalho inclui o uso das diatomáceas de diferentes compartimentos (plâncton e sedimentos superficiais) na avaliação da qualidade ecológica de seis reservatórios (Paineiras, Jurupará, Cachoeira do França, Serraria, Cachoeira da Fumaça, Serraria e Salto do Iporanga), além de contribuir para o conhecimento da biodiversidade e da autoecologia de diatomáceas de regiões tropicais e, principalmente, das áreas de mananciais da RMSP ou próximas a estas; além disso, subsidiará o projeto maior fornecendo informação autoecológica com vistas à elaboração de um banco de dados regional sobre as diatomáceas da RMSP e a futura aplicação em um modelo de função de transferência diatomáceas-fósforo.

Objetivos

Objetivo geral

Avaliar a biodiversidade e a organização espacial e temporal das diatomáceas em diferentes compartimentos (planctônico e sedimentos superficiais) de seis reservatórios (Paineiras, Jurupará, Cachoeira do França, Serraria, Cachoeira da Fumaça e Salto do Iporanga), visando a contribuir para a caracterização da qualidade ecológica desses sistemas, à avaliação do potencial dessas comunidades na bioindicação e à ampliação do conhecimento sobre a autoecologia de diatomáceas de represas tropicais.

Objetivos específicos

- ✓ Conhecer a biodiversidade de diatomáceas planctônicas e dos sedimentos superficiais de seis reservatórios das bacias do Ribeira do Iguape e Litoral Sul (Jurupará, Cachoeira do França, Serraria, Cachoeira da Fumaça e Salto do Iporanga) e do Alto rio Paranapanema (Paineiras).
- ✓ Analisar a distribuição da comunidade de diatomáceas em escala espacial (intra e interrepresas) e temporal (períodos inverno e verão).
- ✓ Avaliar os fatores direcionadores e reguladores da organização estrutural das comunidades de diatomáceas.
- ✓ Avaliar as associações de espécies de diatomáceas que possam ser entendidas como indicadoras de eutrofização.
- ✓ Avaliar a qualidade ecológica das represas em relação ao estado trófico de cada uma, considerando os compartimentos da água e os sedimentos superficiais.

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Capítulo 1

Submetido em **Acta Botanica Brasiliensia**

Diatom assemblages (Bacillariophyta) in six tropical reservoirs from southwest Brazil: species composition and variation patterns

Assembléias de diatomáceas (Bacillariophyta) em seis reservatórios tropicais no sudoeste do Brasil: composição de espécies e padrões de variação

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Abstract

Abstract: Aim: Relationships between species composition and variation patterns in diatom assemblages over six tropical reservoirs located in Southeast Brazil were explored. **Methods:** Surface-sediment and phytoplankton diatom assemblages were determined and Canonical Correspondence Analysis was used to verify the set of environmental variables that best explain the species composition variation among sites. **Results:** A total of 29 diatom taxa representing 20 genera were identified using light and scanning electron microscopy. Information on their ecological preferences was also provided. *Humidophila biscutella* is reported for the first time in Brazil whereas *Sellaphora sassiana* and *Humidophila brekkaensis* are reported for the first time in São Paulo State. Three groups of potential water quality indicators were delineated: the first suggests oligotrophic conditions, the second is related to cold waters with low luminosity in a mixing regime, and the third is a small group of tolerant species occurring in water with high conductivity, pH and total phosphorus conditions. **Conclusion:** present study provided the first insight into the general diatom communities over six tropical reservoirs in Southeast Brazil and provided information on their ecology and distribution aiming bioassessment.

Keywords: bioassessment; diatoms; multivariate analyses; São Paulo

Resumo: Objetivo: As relações entre composição de espécies e padrões de variação nas comunidades de diatomáceas foram exploradas em seis reservatórios tropicais localizados no sudeste do Brasil. **Métodos:** Diatomáceas fitoplanctônicas e de sedimentos superficiais foram determinadas e a Análise de Correspondência Canônica foi utilizada para identificar o conjunto de variáveis ambientais que melhor explica a variação da composição das espécies entre as unidades amostrais. **Resultados:** O total de 29 táxons de diatomáceas representando 20 gêneros foi identificado utilizando microscopia de luz e eletrônica de varredura; e foram fornecidas informações sobre suas preferências ecológicas. *Humidophila biscutella* é reportada pela primeira vez no Brasil e *Sellaphora sassiana* e *Humidophila brekkaensis* foram registradas pela primeira vez no Estado de São Paulo. Foram delineados três grupos de potenciais indicadores da qualidade da água que sugerem condições oligotróficas, águas com baixa luminosidade e em regime de mistura além de um pequeno grupo de espécies tolerantes ocorrentes em águas com elevada condutividade, pH e total fósforo total. **Conclusão:** O presente estudo forneceu uma primeira visão sobre as comunidades de diatomáceas em geral de seis reservatórios tropicais do sudeste do Brasil e providenciou informação sobre sua ecologia e distribuição, contribuindo para o conhecimento das diatomáceas tropicais com vista à bioavaliação.

Palavras-chave: análise multivariada; bioindicação; diatomáceas; São Paulo

Introduction

Currently, diatoms are routinely used in environmental status assessment, because of their importance in food webs and biochemical linkages and due to their sensitivity to physical, chemical and biological disturbances (Stenger-Kovács et al., 2007; Bolla et al., 2010; Chételat et al., 2010; Kireta et al., 2012; B-Béres et al., 2014). Diatoms respond predictably to many water chemistry variables besides having a relatively well known ecology (Soininen et al., 2007). Factors such as the choice of sampling site, the methods for preparing and processing the sample and identifying the taxa can be crucial to the assessment results (Besse-Lototskaya et al., 2006) as their use as environmental indicators requires high taxonomic precision (Birks, 1994).

The composition of diatom communities reflects an entire complex of ecological parameters at a particular site (van Dam et al., 1994; Resende et al., 2005). In this sense, floristic surveys support not only applied research projects, such as water quality monitoring, but also a wide range of basic research issues from taxonomic revisions and monographs, phylogenetic reconstruction, biogeographical studies, as well as ecological, physiological, restoration and conservation biology research programs (Kociolek, 2006).

Although some floristic diatom surveys have been published in Brazil (e.g. Ferrari & Ludwig, 2007; Cavalcante et al., 2014; da Silva et al., 2016), they are mostly concentrated in the South and Southwest regions of the country (e.g. Moura & Bittencourt-Oliveira, 2004; Bertolli et al., 2010; Faria et al., 2010; Souza-Mosimann et al., 2011; Bes et al., 2012; Bartozek et al., 2013; Nardelli et al., 2014; Marra et al., 2016). Specifically for São Paulo state, studies are mainly a result of the Biota-FAPESP, an important project that aims to identify the local phycological flora (e.g. Carneiro & Bicudo, 2007; Rocha & Bicudo, 2008; Marquardt & Bicudo, 2014; Wengrat et al., 2015).

In addition, ecological (e.g. Wengrat & Bicudo, 2011; Bicudo et al., 2016) and paleolimnological (e.g. Costa-Böddeker et al., 2012; Fontana et al., 2014; Faustino et al., 2016) surveys are also available under the Acqua-Sed Project (Base line diagnosis and reconstruction of anthropogenic impacts in the Guarapiranga Reservoir, focusing on the sustainability in water supply and water quality management in reservoirs of the Alto Tietê and surrounding basins), providing a huge contribution to the knowledge of tropical diatom aiming bioassessment. This project has also led to the development of a range of taxonomic surveys (e.g. Almeida & Bicudo, 2014; Almeida et al., 2015, 2016; Marquardt et al., 2016; Wengrat et al., 2016). However, there are still considerable sample gaps in the state, in which the size and diversity of habitats can be considered one of the reasons for the large number of unexplored areas.

The aim of this study was to provide the first insight into the general diatom assemblages over the six tropical reservoirs in Southwest Brazil by investigating their species composition as well as distribution and ecological tendencies.

Material and Methods

Study area

Reservoirs studied are located at two different basins in southwestern São Paulo: Ribeira do Iguape/Litoral Sul and Alto Paranapanema. Three reservoirs (Cachoeira do França, Cachoeira da Fumaça and Serraria) are connected with the same water course (Juquiá river), whereas the other three (Jurupará, Salto do Iporanga and Paineiras) are located in three different rivers (Rio dos Peixes, Assungui and Turvo river, respectively) (Figure 1). Mean elevation range varied from 17 to 996 m a.s.l. All study sites were located in well preserved areas, most of them located in areas of the PEJU State Park (Parque Estadual do Jurupará). Presently, reservoirs were classified mostly oligotrophic to mesotrophic (Lamparelli, 2004) with no obvious signs of human impact. The area protects a representative Atlantic forest area of the Atlantic Plateau Paulista, which were recorded 77 species of fauna and flora currently endangered and 182 endemic species besides more than 300 new species records (SMA, 2010). However, the diatom flora is virtually unknown in this area.

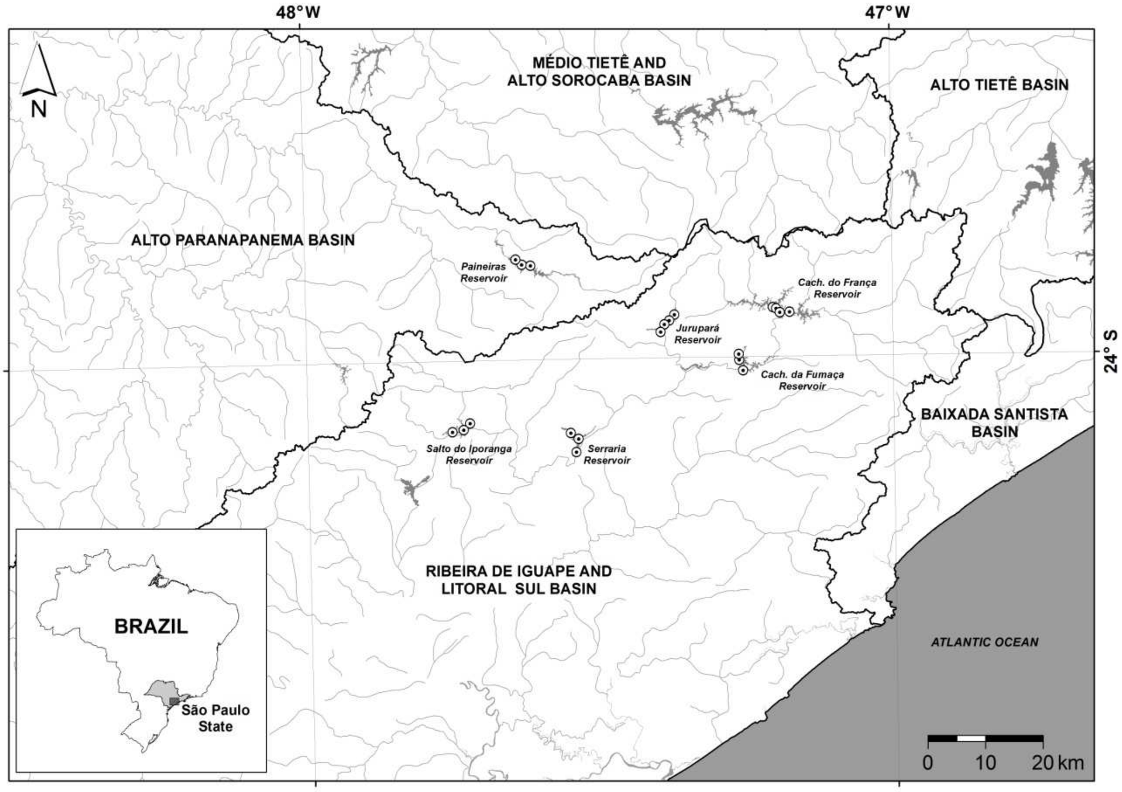

Figure 1. Study area and sampling sites.

Sampling

The sampling covered two distinct habitats (plankton and surface sediment) from 20 sampling sites distributed along the reservoirs during austral summer and winter in 2014. Plankton samples were obtained with Van Dorn water sampler along a vertical profile of the reservoirs. Surface sediment was taken with a gravity core (UWITEC) and the top 2 cm sections were saved for analyses.

Environmental parameters (temperature (°C), pH and conductivity) were measured in the field using a multiparameter probe (Horiba U-53) and the analytical procedure for dissolved oxygen, ammonium, nitrate, soluble reactive silica, total nitrogen and total phosphorus, free carbon dioxide and bicarbonate ions followed Standard Methods (APHA, 2005). Chlorophyll-a, corrected for phaeophytin, was measured using 90% ethanol (Sartory & Grobbelaar, 1984) (Table 1). The Trophic State Index (TSI) was calculated according to Lamparelli (2004).

Table 1. Means and standard deviation of abiotic variables in the six studied reservoirs. The other variables were eliminated because of collinearity. Abbreviations: Sec (Secchi depth), pH, Cond (Conductivity), TN (Total nitrogen), TP (Total phosphorus), SRS (Soluble reactive silica), Chlo (Chlorophyll-a), TSI (Trophic State Index). FR (Cachoeira do França), FU (Cachoeira da Fumaça), SE (Serraria), JP (Jurupará), SI (Salto do Iporanga), PI (Paineiras). Numbers refer to sample units.

	Sec	pH	Cond	TN	TP	SRS	Chlo	TSI (Anual Mean)
FR1	1.7±8.5	7.3±0.9	24.8±0.7	151.8±162.9	5.5±2.2	2.4±1.3	5.4±4.8	Oligotrophic
FR2	1.9±0.2	7.3±1.3	24.8±0.75	191.1±45	10.0±7.5	2.39±1.2	4.12±2	Oligotrophic
FR3	1.7±0.005	7.9±0.6	23.5±2.1	231.2±21.2	6.6±3.7	2.3±1.4	20.2±26.5	Oligotrophic
FR4	1.7±0.1	8.6±0.6	24.5±3.5	280.0±153.7	6.8±2.5	2.2±1.3	22.8±29.7	Oligotrophic
FU1	2.6±1.7	6.5±0	26.5±0.7	228.6±13.7	5.1±1.6	2.5±1	1.7±0.4	Ultraoligotrophic
FU2	2.8±1.5	6.8±0.7	26.0±2.8	176.5±66.1	8.4±2.1	2.4±1	3.4±1.1	Oligotrophic
FU3	2.1±0.3	6.6±0.4	26.0±1.4	180.1±71.7	5.8±2.7	2.3±1	3.3±2.3	Oligotrophic
SE1	4.0±1.8	7.4±1.1	21.0±9.9	180.3±64.1	8.1±2.9	4.4±1.2	1.8±0.7	Oligotrophic
SE2	3.7±1.4	7.3±0.6	25.0±2.8	174.7±80	8.0±1.5	3.9±1.5	4.6±2.2	Oligotrophic
SE3	4.2±1.4	6.6±0.2	28.5±2.1	216.0±147.7	8.5±3.7	3.6±1.5	7.6±0.7	Oligotrophic
JP1	1.5±0.8	6.4±1.3	24.0±2.8	464.3±89.4	19.5±4.2	3.9±0.3	16.8±1.8	Mesotrophic
JP2	1.9±0.4	6.6±0.1	23.5±3.5	351.4±201.4	16.6±0.3	3.8±0.3	10.1±0.7	Mesotrophic
JP3	2.2±0.2	6.6±0.1	24.0±2.8	453.3±8.2	13.5±0.7	3.7±0.3	7.1±1.2	Mesotrophic
JP4	2.1±0.4	6.8±0.2	23.5±3.5	434.1±103.8	13.3±1.9	3.94±0.32	6.6±3.7	Oligotrophic
SI1	1.5±0.2	8.0±2.1	50.0±9.9	415.9±143.2	32.1±3.7	5.1±1.6	36.7±37.6	Mesotrophic
SI2	1.9±0.4	7.8±2	43.5±7.8	315.9±28.4	23.1±1.5	4.9±0.7	17.4±15.3	Mesotrophic
SI3	1.7±0.6	7.9±2.1	43.0±11.3	304.4±22.4	25.6±3.8	4.1±1.5	36.8±44	Mesotrophic
PI1	0.7±0.7	6.7±0.4	39.0±2.8	310.8±119.3	20.3±0.3	4.6±0.4	9.8±5.7	Mesotrophic
PI2	1.3±0.5	6.9±0.6	38.5±2.1	258.4±258.4	16.2±0.4	4.6±0.8	6.9±2.7	Mesotrophic
PI3	1.3±0.3	7.1±0.5	38.0±2.8	289.4±218.8	16.3±2	4.5±0.8	4.8±3.1	Oligotrophic

Slide preparation and counting

Diatom samples were processed by hot digestion using hydrogen peroxide (H_2O_2) and HCl (37%). Through a series of dilutions, the peroxide and acid were removed. Subsequently, the samples were dried onto cover glass and mounted in Naphrax ™ (R.I. = 1.74) according to Battarbee (1986) and examined with a Zeiss Axio Imager A2 light microscope (LM) equipped with DIC and a digital camera model AxioCamMR5. SEM images were taken in three different research centers: Luxembourg Institute of Science and Technology (LIST), Universidade Federal do Paraná (UFPR) and Instituto de Botânica de São Paulo (IBt). For scanning electron microscope (SEM) observations the cleaned samples were filtered with additional deionized water through a 3-µm isopore polycarbonate membrane filter (Merck Millipore). Filter was mounted on aluminium stubs and coated with platinum using a Modular High Vacuum Coating System BAL-TEC MED 020 (BAL-TEC AG, Balzers, Liechtenstein). An ultrahigh-resolution analytical field emission (FE) scanning electron microscope Hitachi SU-70 (Hitachi High-Technologies Corporation, Japan) operated at 5 kV and 10 mm working distance was used for the analysis. SEM images were taken using the lower (SE-L) and upper (SE-U) detector signal at the LIST. Also, a subsample of the oxidized material was placed on aluminum stubs and coated with gold at 1 kV for 5 min in a Balzers Sputtering/SDC030 sputter coater and SEM observations were made with a JEOL JSM 6360LV, operated at 15 kV at 8 mm distance at the UFPR as well with a Phillips 20XL operated at 10 kV at the IBt. Micrographs were digitally manipulated and plates containing light and scanning electron microscopy images were created using CorelDraw X7. Morphometric information is provided for all taxa (D: diameter (µm); L: length (µm); W: width (µm); SH: semi cell height (µm); S: striae in 10 µm; A: areolae in 10 µm).

Taxonomy and nomenclature followed classic works and new publications (e.g., Krammer, 2000; Metzeltin et al., 2005; Lange-Bertalot et al., 2011) and the on-line catalogue of valid names (California Academy of Sciences, 2011). The classification systems followed Medlin & Kaczmarska (2004) for supra-ordinal taxa and Round et al. (1990) for subordinal taxa, except for genera published subsequently to this work. To account for the species distribution in Brazil and the state of São Paulo, literature with illustration or sufficient taxonomic description of the species was considered.

Diatom quantification was made at 1000× magnification using a Zeiss Axioskop 2 microscope. At least 400 valves were counted per slide (Battarbee, 1986) with a minimum sampling efficiency of 90% (Pappas & Stoermer, 1996). Species abundances were calculated expressed as a percentage of the total diatom counts in each sample.

All slides used for diatom identification and enumeration were deposited at the “Herbário Científico do Estado Maria Eneyda P. Kauffmann Fidalgo” (SP), São Paulo State Department of Environment, Brazil.

Statistical analysis

We used the unimodal-based method based on Detrended Correspondence Analysis (DCA) by checking the length of the first DCA axis (length of gradient 2.5) (Birks, 2010). Final ordination was based on a canonical correspondence analysis (CCA) with stepwise function selection to obtain a subset of explanatory variable from the set of all variables available for the constrained ordination. We further performed a PERMANOVA two-way for two different groups: i) reservoirs and ii) habitats (surface sediment and phytoplankton for both seasons).

We downweighted our data of rare species by applying a 2% cut off on the relative abundance and eliminated those taxa occurring in less than two samples because individual samples with rare species may distort the results of the analyses (Birks, 2010). Also, much of the observed species were rare and composed singletons, making the species richness very high and hinder the taxonomic identification. The environmental variables were standardized and species abundances were Hellinger-transformed. Hellinger distance is the recommended measure for clustering or ordinating species abundance data (Legendre & Gallagher, 2001). Diatom names were coded according to the OMNIDIA software (Lecointe et al., 1993).

Ordination techniques were performed in software R v. 3.0.2 (R Core Team, 2014) with the vegan package (Oksanen et al., 2016).

Results

Taxonomy

A total of 29 species of diatoms belonging to 20 genera were identified for the planktonic and surface sediment communities of the six sampled reservoirs. The identification was carried out to species level in 26 cases and to genus level in 3 cases. Morphometric data for the studied diatom species are presented in Table 2 and illustrated in Figures 4-135. *Humidophila* cf. *biscutella* is reported for the first time in Brazil (Table 1, preceded by an asterisk **). *Sellaphora sassiana* (Metzeltin & Lange-Bertalot) C.E.Wetzel and *Humidophila brekkaensis* (J.B.Petersen)

R.L.Lowe et al. were reported for the first time in São Paulo State (Table 1, preceded by an asterisk *).

Table 2. List of diatom taxa identified from the reservoirs (>2% relative abundance). Morphometric and meristic limits and ecology according to the literature. D: diameter (µm); L: length (µm); W: width (µm); SH: semi cell height (µm); S: striae in 10 µm; A: areolae in 10 µm; LN: lineolae in 10 µm.

Taxon	Figures	Code	Metric (µm) & meristic (in 10 µm) limits
AULACOSEIRACEAE			
Aulacoseira ambigua (Grunow) Simonsen	364-37, 109	AAMB	SH: 12.7-14.4; D: 5.4-5.5; A: 14-16
A. granulata var. *angustissima* (O.Müller) Simonsen	38-39, 108	AUGA	SH: 16.6-18.7; D: 2.8-3; S: 16
A. granulata (Ehrenberg) Simonsen var. *granulata*	34-35	AUGR	SH: 18.4-20,3; D: 7.3-7.6; A: 12
A. pusilla (F.Meister) Tuji & Houki	4-8, 105-106	AUPU	SH: 3.3 ; D: 5.2-6.1
A. tenella (Nygaard) Simonsen	9-12, 100-101, 103-104	AUTL	SH: 2-2.6; D: 6.6-6.9
STEPHANODISCACEAE			
Cyclotella cf. *meneghiniana* Kützing	17-21	CMEN	D: 6.9-13.2; S: 14
Cyclotella sp.	30-33	CYCsp	D: 14-17.3; S: 11-15; A: 32
Discostella stelligera (Cleve & Grunow) Houk & Klee	13-16, 97-98	DSTEL	D: 7.2-11.7; S: 12
Spicaticribra kingstonii J.R.Johansen, Kociolek & R.L.Lowe	22-26, 99,102	SKIN	D: 8.1-16.2
MELOSIRACEAE			
Melosira varians C.Agardh	27-29	MVAR	SH: 12.7-13.7; D: 21.1
FRAGILARIACEAE			
Fragilaria billingsii Wengrat, C.E.Wetzel & E.Morales	40-42	FRsp2	L: 56.2-62.7 ; W: 2.5-2.7; S: 21
F. fusa (R.M.Patrick) Wengrat, C.E.Wetzel & E.Morales	43-44	FRsp	L: 78.8-87.1 ; W: 2.8-3; S: 17-18
F. longifusiformis (Hains & Sebring) Siver et al.	45-47, 110	FLONG	L: 58.1-90.6; W: 2.5-2.7; S: 35
Staurosira construens Ehrenberg	54-56, 122	SCON	L: 18-19.1; W: 9.7-10.7; S: 14-16
ACHNANTHIDIACEAE			
Achnanthidium tropicocatenatum Marquardt, C.E.Wetzel & Ector	62-64, 117-119	ACsp	L: 17.7-18.2; W: 2.7; 3.1; S: 38; A: 50
**Achnanthidium* sp.	57-61, 113-116	ACsp2	L: 12-17.5; W: 3.1-3.5; S: 32 ; A: 50
Cocconeis cf. lineata (Ehrenberg) Van Heruck	52-53, 120	CPLAA	L: 29.7-35.2; W: 15.4-15.9; S: 16
EUNOTIACEAE			
Eunotia waimiriorum C.E.Wetzel	48-51, 121	EWAI	L: 16.7-36.2; W: 1.5-2.2; S: 20
CYMBELLACEAE			
Encyonema neomesianum Krammer	90-93, 132	ENNE	L: 36.9-30; W: 7.9-8.4; S: 11-13; A: 32
Encyonema sp.	87-89, 130-131	ENsp	L: 19.8-20.9; W: 5.3-4.7; S: 10-12; A: 40
Encyonopsis subminuta Krammer & E.Reichardt	84-86, 126	ESUM	L: 20.1-22.5; W: 4; S: 25-26; A: 40
GOMPHONEMATACEAE			
Geissleria punctifera (Hustedt) Metzeltin et al.	76-77, 127	GPTF	L: 20.8-26.2; W: 6.2-6.6; S: 16-18
Gomphonema naviculoides W.Smith	94-96	GONAV	L: 41.7-43.1; W: 7.9-8.2;

			S: 14-15
**Humidophila brekkaensis* (J.B.Petersen) R.L.Lowe et al.	111-112	HBRE	L: 9.8-15.2; W: 3.1-3.3; S: 34-35
H. biscustella (Gerd Moser et al.) Lowe et. al.	65-69, 107	HUCO	L: 11.3-11.7; W: 2.4-2.7; S: 44
BRACHYSIRACEAE			
Brachysira microcephala (Grunow) Compère	73-75, 123	BMIC	L: 24.1-26.8; W: 5.1-5.3; S: 37; A: 30
AMPHIPLEURACEAE			
Frustulia crassinervia (Brébisson ex Smith) Lange-Bertalot & Krammer	70-72, 125, 128	FRCS	L: 45.9-48; W: 9.9-10.1; S: 34; A: 36
Navicula notha J.H.Wallace	78-79, 129	NNOT	L: 27.4-33.1; W: 5.4-5.7; S: 10; LN: 24
SELLAPHORACEAE			
**Sellaphora sassiana* (Metzeltin & Lange-Bertalot) C.E.Wetzel	80-83, 124	NDSA	L: 14-14.5; W: 4.6-4.7; S: 14-20; A: 35

Only three species (*Discostella stelligera* (Cleve & Grunow) Houk & Klee, *Aulacoseira tenella* (Nygaard) Simonsen and *Spicaticribra kingstonii* J.R.Johansen, Kociolek & R.L.Lowe were considered dominant (>50% in relative abundance) (Figures 2 A, B and C).

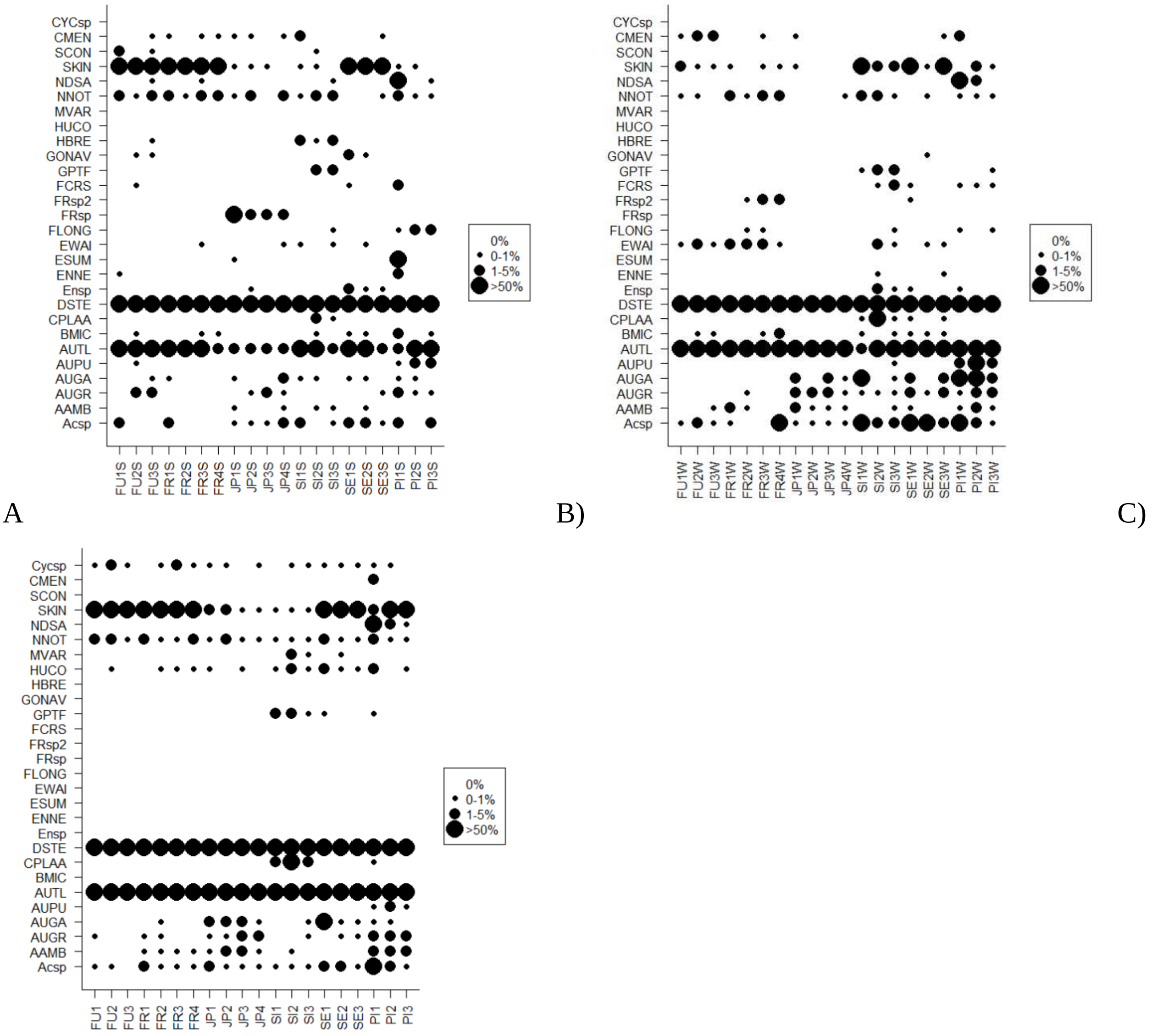

Figure 2. Distribution in samples and relative abundance of diatoms from Cachoeira do França (FR), Cachoeira da Fumaça (FU), Juruparä (JP), Paineiras (PI), Salto do Iporanga (SI) and Serraria (SE) reservoirs. **(A)** Phytoplankton (Summer), **(B)** Phytoplankton (Winter) and **(C)** Surface Sediment.

Canonical correspondence analysis

The CCA biplot with the selected variables are illustrated in Figure 3. PERMANOVA two-way analysis showed significant among-group differences both among reservoirs and habitats (Pr (>F) < 0.001).

The stepwise selection of environmental variables retained six significant variables, in decreasing importance, which influenced diatom distribution: TP, conductivity, pH, SSR, Secchi and TN. The variables account for about 36% of the biological variation in the first two CCA axes. The intraset correlations indicated that TP and conductivity were the most significant contributors to axis 1 whereas pH and Secchi were the most significant contributors to axis 2.

There was a trend in the separation of the sites according to the hydrological connectivity between the rivers of the systems (Figure 3A) and the ordination plot distinguished four main groups: the first group was represented by reservoirs located at the same watercourse (Cachoeira do França, Serraria and Cachoeria da Fumaça reservoirs). This group had the highest abundances of *Spicaticribra kingstonii*, *Staurosira construens* Ehrenberg, *Cyclotella* cf. *meneghiniana* Kützing, *Discostella stelligera*, *Brachysira microcephala* (Grunow) Compère, *Gomphonema naviculoides* W.Smith, *Achnanthidium tropicocatenatum* Marquardt, C.E.Wetzel & Ector, *Encyonema* sp., *Fragilaria billingsii* Wengrat, C.E.Wetzel & E. Morales, *Eunotia waimiriorum* C.E.Wetzel and *Aulacoseira tenella*, correlated to the Secchi vector, to the plankton during the summer and to the surface sediment habitats.

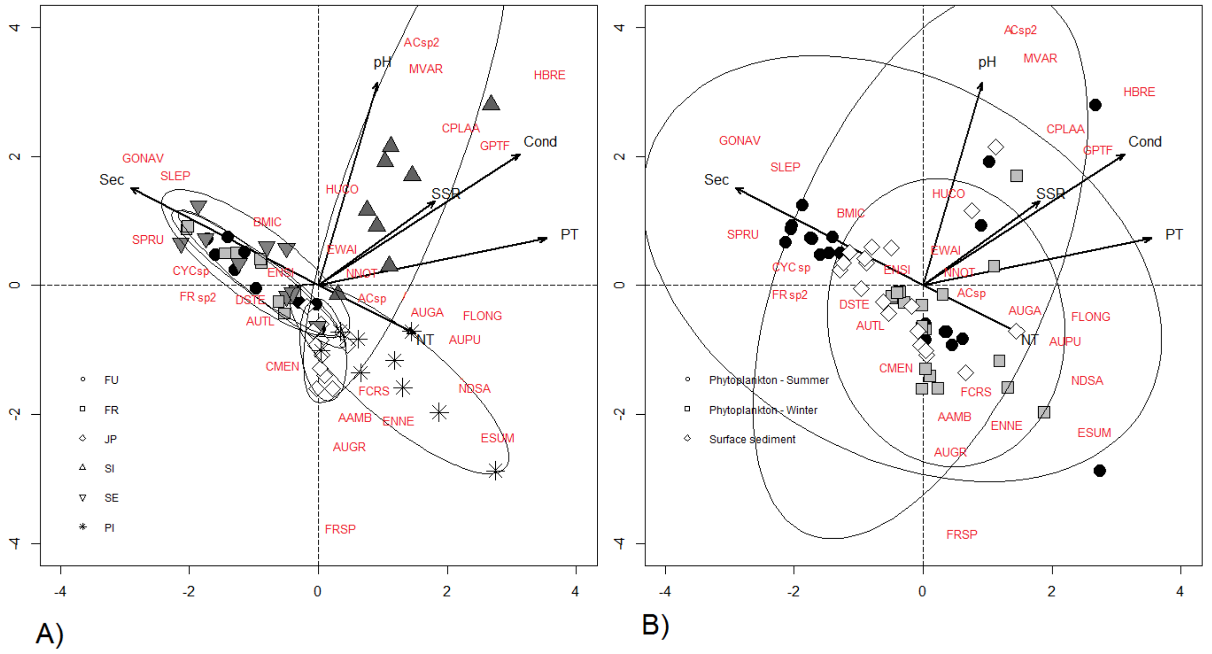

Figure 3. CCA ordination biplots of the Cachoeira do França (FR), Cachoeira da Fumaça (FU), Jurupará (JP), Paineiras (PI), Salto do Iporanga (SI) and Serraria (SE) reservoirs (A) and phytoplankton (summer and winter) and surface sediment (B).

The second group was composed of the Salto do Iporanga reservoir and associated especially with the highest values of conductivity, TP, pH and soluble reactive silica (SRS). Regardless of the habitat, the most correlated species in this group were the benthic ones: *Achnanthidium* sp., *Cocconeis* cf. *lineata* (Ehrenberg) Van Heruck, *Humidophila brekkaensis, H.* cf. *biscutella (Gerd Moser, Lange-Bertalot & Metzeltin) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová, 2014, Navicula notha* J.H.Wallace, *Melosira varians* C.Agardh and *Geissleria punctifera* (Hustedt) Metzeltin, Lange-Bertalot & García-Rodríguez.

Finally, the third group featured in the biplot consisted mainly of the Jurupará and Paineiras reservoirs, which were correlated to the total nitrogen (NT) vector and lowest conductivity and Secchi values. The associated species in this group were *Cyclotella* cf. *meneghiniana*, *Aulacoseira ambigua* (Grunow) Simonsen, *A. granulata* (Ehrenberg) Simonsen, *A. pusilla* (F.Meister) Tuji & Houki, *A. granulata* var. *angustissima* (O.Müller) Simonsen, *Sellaphora sassiana*, *Encyonopsis subminuta* Krammer & E.Reichardt, *Encyonema neomesianum* Krammer, *Fragilaria longifusiformis* (Hains & Sebring) Siver et al., *Fragilaria fusa* (R.M.Patrick) Wengrat, C.E.Wetzel & E.Morales and *Frustulia crassinervia* (Brébisson ex Smith) Lange-Bertalot & Krammer. These species are known for their planktonic habit and they showed the greatest relative abundance during the winter, in which the water column is in a mixing regimen.

Discussion

Studies about limnological characteristics for the studied region are extremely rare, especially those considering seasonal and spatial influence. Most of them are restricted to reports on water sources that focus on the characterization of the conservation unit (CETEC, 2000) and to the PEJU Management Plan (Fundação Florestal do Estado de São Paulo), which covers the water sources inserted in the Park area. Among others, there are some studies regarding the surface water quality (Cetesb, Companhia Ambiental Do Estado De São Paulo) besides reports organized by the Companhia Brasileira de Alumínio (CBA), which operates in eight plants in the Ribeira de Iguape basins. Concerning the Paineiras reservoir, only one survey was found: an unpublished doctoral dissertation whose theme is the adaptation of the fish assembly index in reservoirs along the Turvo river (SP) (Ferreira, 2011).

The currently measured limnological characteristics of the reservoirs indicate that the systems are under relatively protected conditions, with low anthropogenic impact, promoted by their insertion in the PEJU reserve. The low concentrations of nutrients in their dissolved forms as well as the values of TN and TP measured in this study are characteristic of oligotrophic and mesotrophic environments (Tundisi, 2006; Vercellino & Bicudo, 2006). However, despite the nutrients played an important role in diatom community variation, the hydrological connectivity role should not be neglected.

According to the currently calculated TSI, the reservoirs in the same watercourse included in the first group showed a better water quality (ultraoligotrophic and oligotrophic) when compared to the second and third groups (mostly mesotrophic) (Table 1). However, it is important to note that there was not a large trophic gradient in the study area, and these eutrophic sites are probably a result of the system anthropic management during the sampling period.

Regarding ecological preference trends of species, our results agree with those found in the literature, with most of the species having been reported in Brazilian reservoirs and oligotrophic environments. Occurring on Cachoeira do França, Cachoeira da Fumaça and Serraria reservoirs (first group), *Achnanthidium tropicocatenatum* has been found in alkaline waters of good quality and with low electrolyte content (Marquardt et al., 2017). Because of the wide morphological variation, the *Spicaticribra kingstonii* species complex might be a species flock (Tuji et al., 2012). Also, *S. rudis* is frequently reported to the Brazilian southern, southeastern and northeastern regions, found in high temperatures and low conductivity waters (Ludwig et al., 2008); this species occurred in the Paraitinga mesotrophic reservoir (Rocha, 2012), and it was abundant in the ultra-oligotrophic Jaguari reservoir (Nascimento, 2012) as well those located along the Paranapanema river (Fontana & Bicudo, 2009). Also, *Staurosira construens* is considered an alkaliphilous and meso-eutraphentic taxon (van Dam et al., 1994; Morales, 2006), registered in the plankton and periphyton in rivers from the Southern regions of Brazil (e.g. Flôres et al., 1999; Landucci & Ludwig, 2005) besides an oligotrophic reservoir in São Paulo State (Barbosa, 2012). A further species related to the first group, *Discostella stelligera* is considered tolerant to the nutrient enrichment (Stoermer, 1978). In Brazilian reservoirs, the species was recorded by Faustino et al. (2016) in the Guarapiranga reservoir in mesotrophic to super-eutrophic conditions in 37% of samples. Recent studies have suggests that this taxon is part of a key group of diatoms that are frequently dominant members of phytoplankton communities in low- to moderate-productivity lakes and that processes that alter light availability (such as water clarity and water column stability) and nutrient concentrations are likely to play a major role in controlling the growth of small centric diatoms in Arctic lakes (Saros & Anderson, 2015). In addition, *Gomphonema naviculoides* is particularly prevalent in the Tropics and also in North America. has been So far the species has been identified as *G. gracile* Ehrenberg (Reichardt, 2015). Accordin go the latest revision of the complex around *Gomphonema gracile*, the species tolerates low salt concentrations (Reichardt, 2015). *Aulacoseira tenella*, typically associated with oligotrophic and oligo-mesotrophic reservoirs (Bicudo et al., 2016) have been recorded in Brazil in lotic ecosystems (Landucci & Ludwig, 2005), with low conductivity reservoirs (Raupp et al., 2006; Eskinazi-Leça et al., 2010; Silva et al., 2010; Nascimento, 2012), as well as in acidophilic (Camburn & Charles, 2000) and oligotrophic environments (Siver & Kling, 1997). *Fragilaria billingsii* is commonly found in São Paulo State reservoirs, living in slightly acidic waters with moderate to high nutrient enrichment (Wengrat et al., 2016). Finally, *Eunotia waimiriorum* is a common colonial planktonic diatom from oligotrophic pristine rivers and streams in the Amazon hydrographical basin (Wetzel et al., 2010).

Among the diatoms related to the Salto do Iporanga reservoir (second group), *Navicula notha* is considered as a cosmopolitan species, present in oligotrophic environments, with low conductivity, slightly acid to near neutral (Lange-Bertalot, 2001). *Geissleria punctifera* was considered tolerant to the pollution conditions on rivers and streams in Maringá (Paraná State, Brazil) and was an abundant periphytic diatom in three streams with different anthropic influences (Moresco et al., 2011). Centric diatoms as *Melosira varians* prefer low light availability conditions. This species is frequent and abundant in environments with vertical column mixing water (Reynolds et al., 2002) and have a high nutrient requirements and high disturbance tolerance (B-Béres et al., 2014).

Humidophila brekkaensis is reported for the first time in São Paulo State and had strong correlation to the pH and conductivity vectors. This species was registered in lotic environments from southern Brazil (Oliveira et al., 2002).

Mostly taxa correlated to the Juruparà and Paineiras reservoirs (third group) were 'araphid' and 'centric' ones. Our studies corroborate Bicudo et al. (2016) in an ecological study focused on *Aulacoseira* from São Paulo State. In this study, species such as *Aulacoseira ambigua* and *A. granulata* were also associated to the water mixing and low light conditions (Houk, 2003; Taylor et al., 2007) showing higher abundances in colder (winter), slightly acid waters. In the same way, *A. granulata* var. *angustissima* was associated to environments with higher concentrations of TN. The araphid *F. longifusiformis* is distributed in freshwater localities in at least two continents from the northern hemisphere, including ponds, lakes, reservoirs and rivers over a wide range of trophic conditions (Morales & Manoylov, 2006). In USA, the taxa was lacking from the most acidic habitats in both regions and it was most often observed in relatively dilute waters with a specific conductivity below 200 µS cm^{-1} (Morales & Manoylov, 2006). Finally, *Frustulia crassinervia*, considered a species that occurs in oligotrophic environments (van Dam et al., 1994) is a characteristic member of phytobenthos in acid, peaty waters (such as *Sphagnum* bogs), and many ephemeral habitats (Krammer & Lange Bertalot, 1986; Round et al., 1990; Veselá et al., 2012). *Encyonema neomesianum*, widely reported in São Paulo State (Marquardt & Bicudo, 2014) is considered an oligotrophic taxon, with an optimum in alkaline waters (Moro & Fürstenberger, 1997).

Conclusions

This study provided new information on the ecology and distribution of tropical diatoms. Three potential water quality indicator diatom groups were outlined, which indicated oligotrophic conditions, water mixing with low light conditions and species with higher nutrient requirements, occurring in higher conductivity and pH waters, in agreement with those reported in the literature. We reinforce the PEJU importance for the ecological quality maintenance of the reservoirs and reference conditions for the Metropolitan Region of São Paulo water sources.

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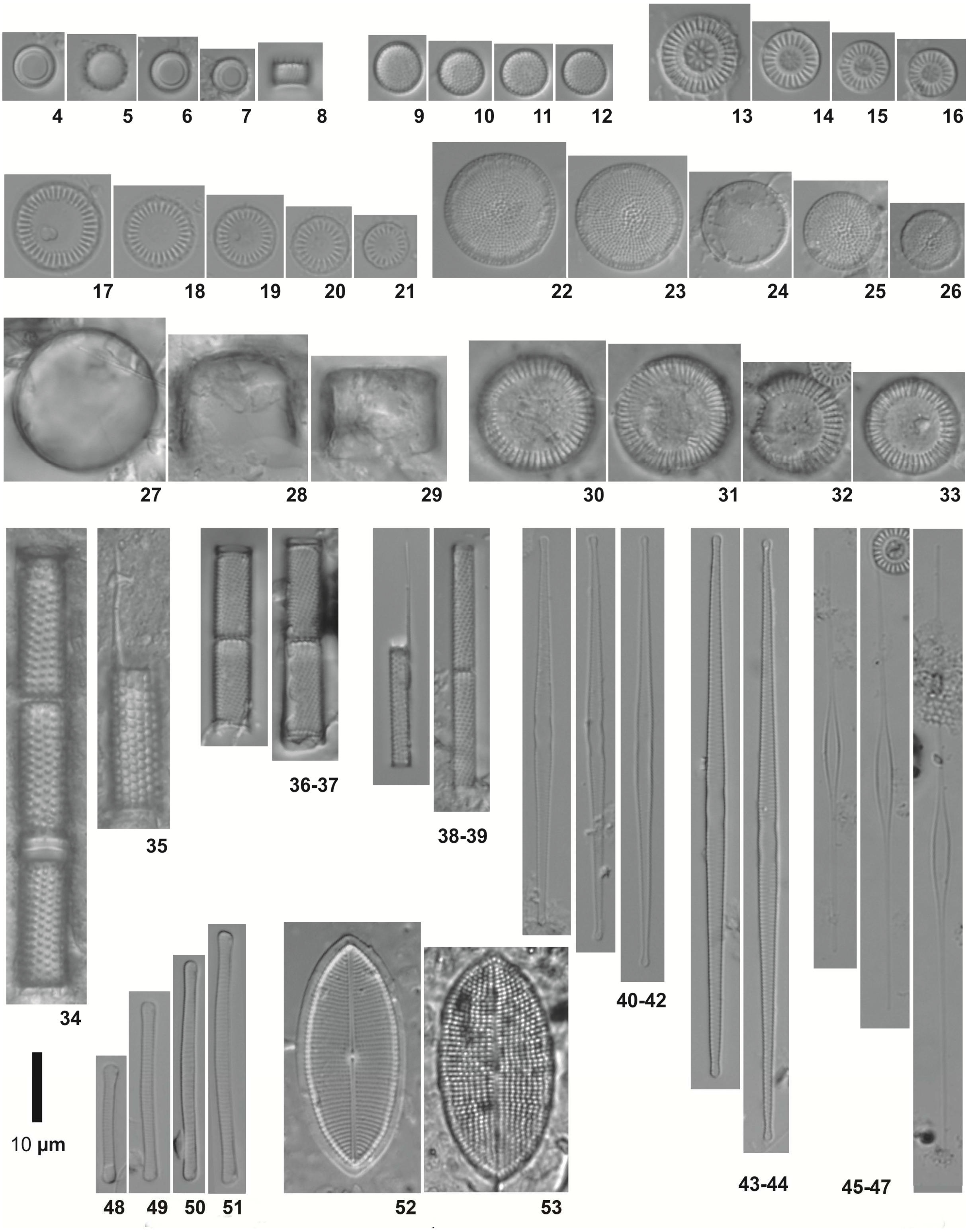

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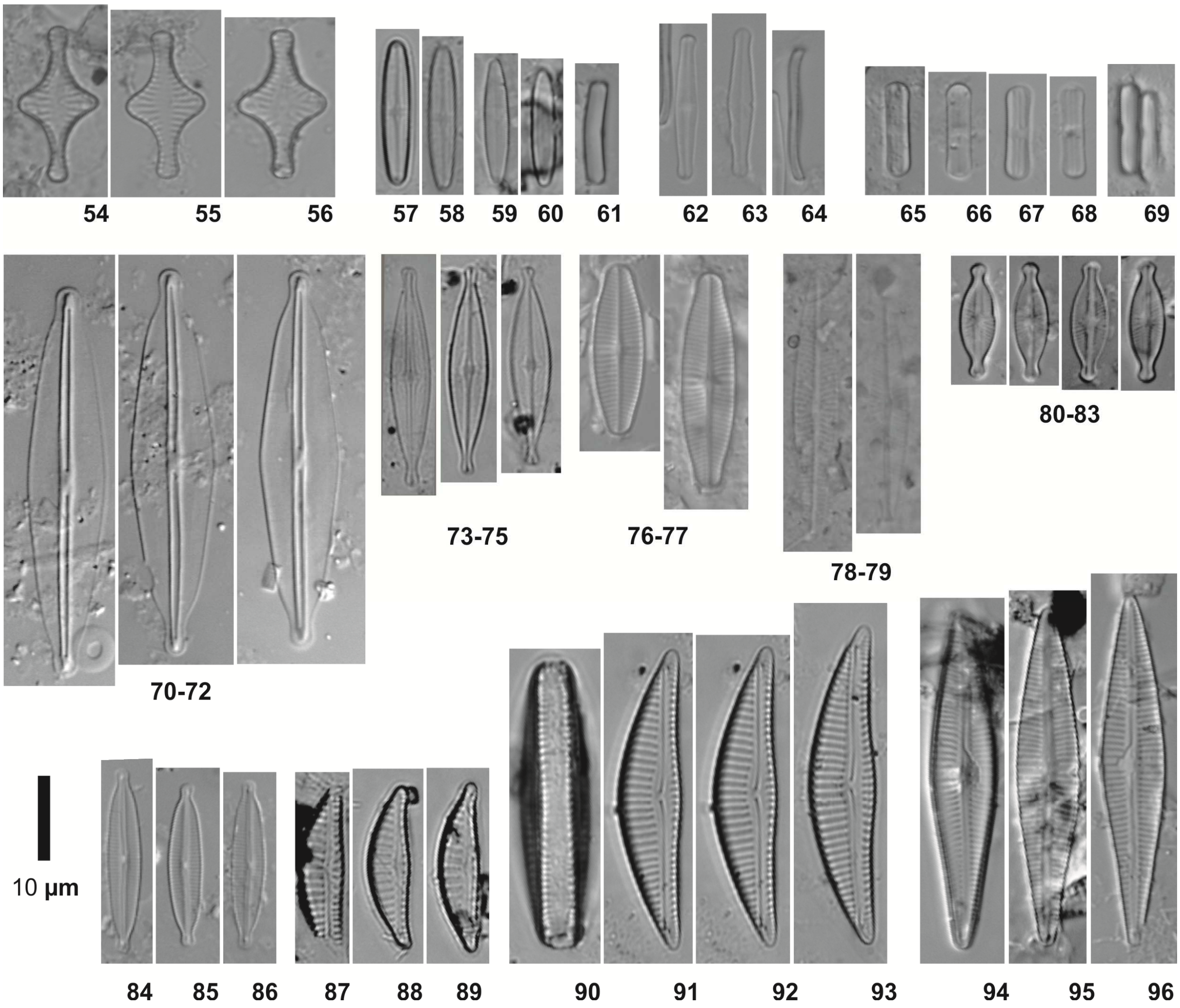

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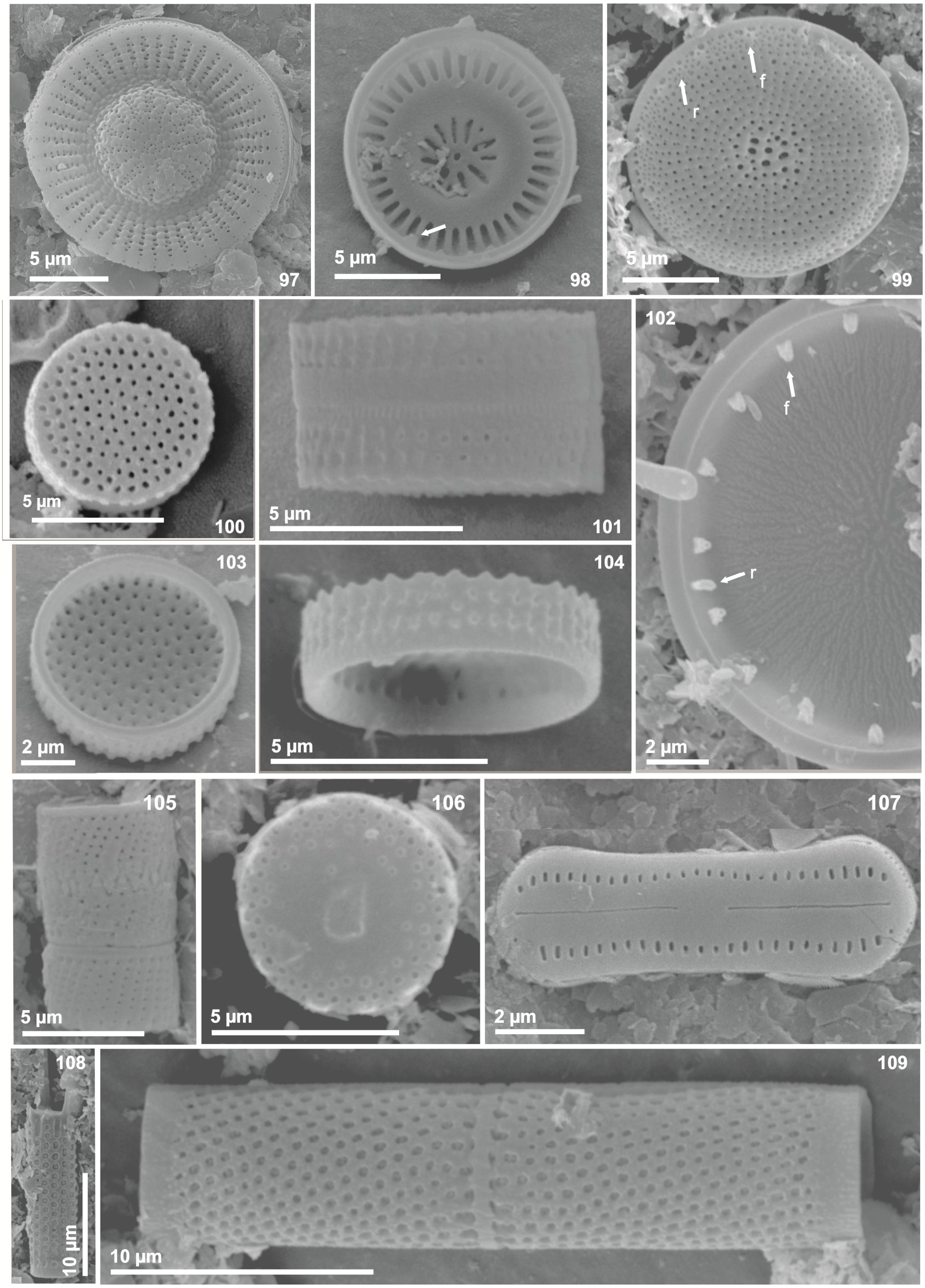

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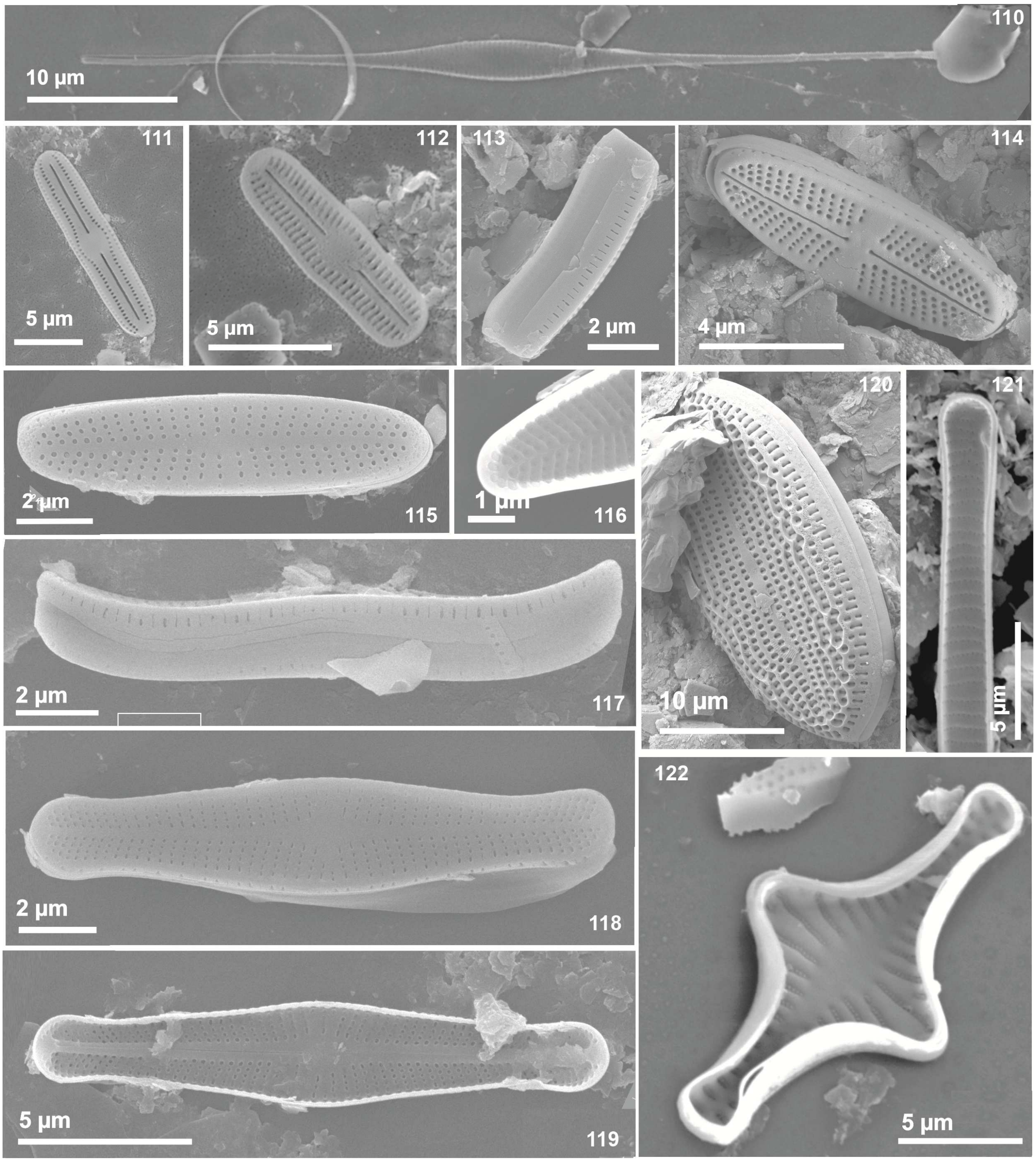
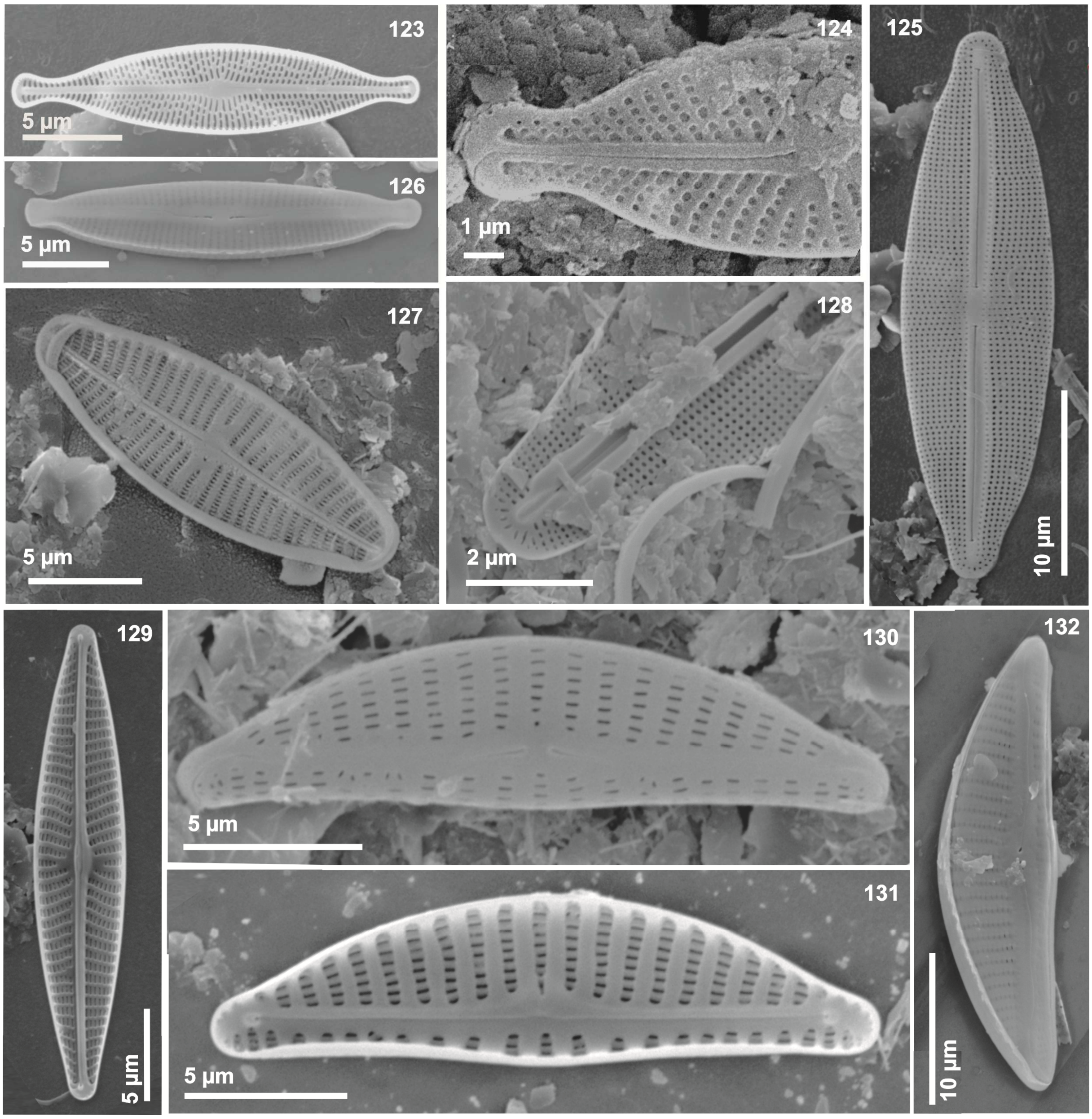


Figures 110-122. Diatom assemblages (Bacillariophyta) in six tropical reservoirs (São Paulo, Brazil). SEM. 110. *Fragilaria longifusiformis*. Internal valve view. 111-112. *Humidophila brekkaensis*. 111. Internal valve view. 112. External valve view. 113-114. *Achnanthidium* sp. 113. Girdle view. Fig. 114. External view of raphe valve. Fig. 115. External view of rapheless valve. Fig. 116. Internal view of rapheless valve. 117-119. *Achnanthidium tropicocatenatum*. 117. Girdle view. 118. External view of rapheless valve. 119. Internal view of raphe valve. 120. *Cocconeis* cf. *lineata*. External view of rapheless valve. Fig. 121. *Eunotia waimiriorum*. Internal valve view. 122. *Staurosira construens*. Internal valve view. Scale bars: Figs 110, 120: 10 µm;

Figs 111, 112, 119, 121, 122: 5 µm; Figs 113, 115, 117, 118: 2 µm; Fig. 114: 4 µm; Fig. 116: 1 µm.



Figures 123-132. Diatom assemblages (Bacillariophyta) in six tropical reservoirs (São Paulo, Brazil). SEM views. 123. *Brachysira microcephala*. Internal valve view. 124. *Sellaphora sassiana*. External valve view. 125, 128. *Frustulia crassinervia*. 125. External valve view. 128. Internal valve view. 126. *Encyonopsis subminuta*. External valve view. 127. *Geissleria punctifera*. Internal valve view. 129. *Navicula notha*. Internal valve view. 130-131. *Encyonema* sp. 132. *Encyonema neomesianum.* External valve view. Scale bars: Figs 123, 126-127, 129, 130-131: 5 µm; Fig. 124: 1 µm; Figs 125, 132: 10 µm; Fig. 128: 2 µm.

Capítulo 2

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Variance partitioning of deconstructed tropical diatom communities in reservoirs cascade

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ABSTRACT

We used variation partitioning to evaluate significance of local environment, spatial structure and hydrological connectivity in the diatom community variation in phytoplankton and superficial sediments of six reservoirs of southeast Brazil. Common and rare diatom species were represented by different data sets, according to the species relative abundance and frequency of occurrence. To clarify the connectivity effect on the metacommunity organization, analyses were performed with and without a hydrological connectivity matrix as predictor. Results for rare and common species were similar, but explanation power depended on the habitat and the climatic season. The hydrological connectivity predictor proved to play an important role toward explaining the diatom metacommunity dynamics. Consequently, this landscape feature should not be neglected in ecological models of managed rivers.

KEYWORDS
connectivity, occurrence frequency, rare species, relative abundance, reservoir

INTRODUCTION

Community assembly is likely shaped by an interacting effect of stochastic, neutral processes and deterministic, niche-based processes (Royo & Ristau, 2012). Assessing their relative significance and understanding such perspectives have been a long-standing challenge in ecological studies (Tilman, 2004; Cottenie, 2005; Heino *et al.*, 2015). The main analytical approach involves partitioning variation in community composition at regional extents (Capers *et al.*, 2010; O'Hare *et al.*, 2012; Alahuhta *et al.*, 2014) into environmental and spatial components (Padial *et al.*, 2014; Vilmi *et al.*, 2016; Wojciechowski *et al*., 2017).

An advance of this perspective is the comparison of patterns generated by common and rare taxa (e.g. Siqueira *et al.*, 2012; Spitale *et al.*, 2012; Alahuhta *et al.*, 2014); Natural rarity seems to be a general species trait, and a number of species are spatially or numerically constrained as a consequence of their evolutionary strategy as "specialists", in contrast to "generalists" that often have a wide distributional range (Grime 2001, Spitale *et al*. 2012). For example, specialist species are expected to benefit from environments that are relatively homogeneous (in space and/or time) and tend to be mainly determined by environmental filtering due to local factors, whereas generalist species should benefit from environments that are heterogeneous and mainly determined by dispersal limitation reflected by spatial constrains (Kassen, 2002; Marvier *et al.*, 2004; Östergård & Ehrlén, 2005).

Knowledge about these patterns in tropical reservoirs is still very scarce, especially considering communities in different habitats and also due to the fact that reservoirs are usually organized in cascade along river basin. Such regulation in hydrological connectivity may be central to determine variation in species composition. Indeed, a key mechanism to explain differences in community composition predictability in aquatic systems seems to be the connectivity among communities (Mouquet & Loreau 2003; Heino *et al.*, 2015; Vilmi *et al.*, 2016). Connectivity is likely to affect dispersal rates and, hence, the degree to which species sorting may be observed in nature (Vilmi *et al.*, 2016). In the metacommunity concept, species sorting results in a matching between the environmental gradients and the taxa composition, and is impeded by either too low or too high dispersal rates (van der Gucht *et al.*, 2007). Also, in many ecological systems dispersal is likely to be influenced not only by the geographic distance, but also by its direction (Moritz *et al.*, 2013). Relatedly, species cross-site similarity is predicted to decline logarithmically with increasing geographical distance when migration rate is low according to the neutral theory (Chave & Leigh, 2001; Hubbell, 2001).

In this study, we used phytoplankton and surface sediment diatom datasets from six reservoirs in southeast Brazil to evaluate species cut-off in the importance analysis of local environment and space in the community variation, and also to clarify the effect of hydrological connectivity *per se* after controlling for spatial structuring. While phytoplankton show extreme sensitivity to changes in the biotic, physical and chemical structure of aquatic environments (Reynolds, 1980; 1998; Wojciechowski *et al.*, 2017), the surface sediment communities integrates habitats from across an entire lake and throughout time (Winegardner *et al.*, 2014). We thus aimed at answering the following questions: (*1*) what are the main drivers of diatom metacommunities in tropical reservoirs? (*2*) Did we observe different results among climatic seasons or habitats (phytoplankton and superficial sediment)? (*3*) Do common and rare species have similar metacommunity correlates? (*4*) Does the hydrological connectivity play a central role in structuring diatom metacommunities?

Diatoms are considered ubiquitous and their community composition is dominantly determined by species sorting by the environment (Wetzel *et al.*, 2012). However, there is a plain contrast between this assessment and the recent diatom literature, which contains many claims of endemism. The combination of very large number of species and relatively rapid dispersal in diatoms is inconsistent with some versions of the "ubiquity hypothesis" of protest biogeography, and appears paradoxical (Mann & Vanormelingen, 2013). We expect that diatoms in highly-connected systems are not limited by dispersal. Furthermore, diatom assemblages within surface sediments are assumed to integrate small-scale temporal and spatial perturbations into more defined assemblages. Consequently, they are assumed to be more accurate indicators of general environmental conditions and may respond mainly to local environmental filtering (Hassan *et al.*, 2008). Besides, we believe that climate controls species distributions and local abundances by producing, for example, physiological stress (Root, 1988). High temporal variability of the environment selects for increased dispersal ability (Gandon & Michalakis, 2001), which allows organisms to track their best preferred habitat (Jocque *et al.*, 2010). Finally, we predict that variation in local environmental conditions would significantly influence community composition mainly for specialists (Rodil *et al.*, 2017).

MATERIAL AND METHODS

Study area

Reservoirs studied are located at two different basins in southwestern São Paulo: Ribeira do Iguape/Litoral Sul and Alto Paranapanema. Three reservoirs (Cachoeira do França, Cachoeira da Fumaça and Serraria) are connected with the same water course (Juquiá river), whereas the other three (Jurupará, Salto do Iporanga and Paineiras) are located in three different rivers (Rio dos Peixes, Assungui and Turvo river, respectively) (Fig. 1).

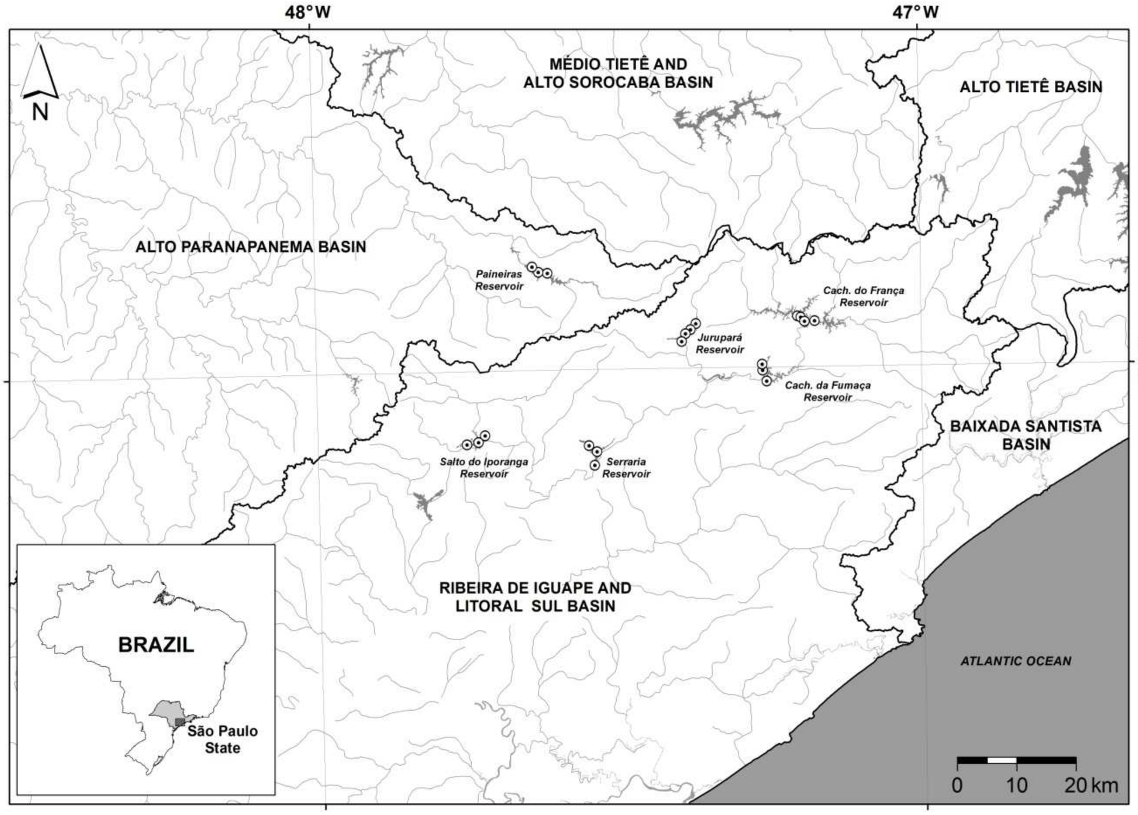


Figure 1. Study sites showing the 20 sampling areas over the six studied reservoirs.

Based on their current water chemistry, reservoirs widely vary according to environmental conditions ranging from ultraoligotrophic to eutrophic according to determination of the Trophic State Index (TSI) for reservoirs (Lamparelli, 2004). Eutrophic sampling units are probably a result of the anthropogenic management of the system related to the automation of the hydroelectric plant that occurred during the sampling period. Information about the reservoirs is summarized in table 1.

Table 1. Means and standard deviation of abiotic variables in the six reservoirs studied. Sec (Secchi depth; m), Temp (Temperature; ºC), Cond (Conductivity; µS cm^{-1}), Alk (Alkalinity; mEq L^{-1}), DO (Dissolved oxygen; mg L^{-1}), TN (Total nitrogen; µg L^{-1}), TP (Total phosphorus; µg L^{-1}), SRS (Soluble reactive silica; mg L^{-1}), Chlo (Chlorophyll *a*; µg L^{-1}). FR (Cachoeira do França), FU (Cachoeira da Fumaça), SE (Serraria), JP (Jurupará), SI (Salto do Iporanga), PI (Paineiras). Numbers refer to the sample units.

	Sec	Temp	pH	Cond	Alk	DO	TN	TP	SRS	Chlo
FR1	1.7±8.5	23.1±6.8	7.3±0.9	24.5±0	0.2±0	7.5±0	151.8±162.9	5.5±2.2	2.4±1.3	5.4±4.8
FR2	1.9±0.	24.2±8.1	7.3±1.3	24±0	0.2±0	7.43±0.9	191.1±45	10.0±7.5	2.39±1.2	4.12±2
FR3	1.7±0	24.1±8.3	7.9±0.6	23.5±2.1	0.2±0	7.3±1.6	231.2±21.2	6.6±3.7	2.3±1.4	20.2±26.5
FR4	1.7±0.1	24.0±7.9	8.6±0.6	24.5±3.5	0.2±0	8.2±1.6	280.0±153.7	6.8±2.5	2.2±1.3	22.8±29.7
FU1	2.6±1.7	24.8±7.3	6.5±0	26.5±0.7	0.2±0	6.9±2.2	228.6±13.7	5.1±1.6	2.5±1	1.7±0.4
FU2	2.8±1.5	24.3±7	6.8±0.7	26.0±2.8	0.2±0	8.3±0.5	176.5±66.1	8.4±2.1	2.4±1	3.4±1.1
FU3	2.1±0.3	24.8±6.4	6.6±0	26.0±1.4	0.2±0	7.5±0.1	180.1±71.7	5.8±2.7	2.3±1	3.3±2.3
SE1	4.0±1.8	26.3±7.2	7.4±1.1	21.0±9.9	0.2±0	8.3±1.4	180.3±64.1	8.1±2.9	4.4±1.2	1.8±0.7
SE2	3.7±1.4	26.2±7	7.3±0.6	25.0±2.8	0.2±0	7.9±0.4	174.7±80	8.0±1.5	3.9±1.5	4.6±2.2
SE3	4.2±1.4	25.6±6.2	6.6±0.2	28.5±2.1	0.2±0	7.5±1.2	216.0±147.7	8.5±3.7	3.6±1.5	7.6±0.7
JP1	1.5±0.8	24.1±6.7	6.4±1.3	24.0±2.8	0.2±0	7.8±0.8	464.3±89.4	19.5±4.2	3.9±0.3	16.8±1.8
JP2	1.9±0.4	24.6±6.5	6.6±0.1	23.5±3.5	0.2±0	6.7±1.3	351.4±201.4	16.6±0.3	3.8±0.3	10.1±0.7
JP3	2.2±0.2	25.4±6.9	6.6±0.1	24.0±2.8	0.2±0	7.5±0.3	453.3±8.2	13.5±0.7	3.7±0.3	7.1±1.2
JP4	2.1±0.4	25.0±7.1	6.8±0.2	23.5±3.5	0.2±0	7.1±1.1	434.1±103.8	13.3±1.9	4.0±0	6.6±3.7
SI1	1.5±0.2	24.0±2.6	8.0±2.1	50.0±9.9	0.3±0	6.7±1.9	415.9±143.2	32.1±3.7	5.1±1.6	36.7±37.6
SI2	1.9±0.4	23.7±2.7	7.8±2	43.5±7.8	0.3±0	6.2±2.9	315.9±28.4	23.1±1.5	4.9±0.7	17.4±15.3
SI3	1.7±0.6	23.6±2.7	7.9±2.1	43.0±11.3	0.3±0	7.4±2.2	304.4±22.4	25.6±3.8	4.1±1.5	36.8±44
PI1	0.7±0.7	22.5±6.4	6.7±0.4	39.0±2.8	0.3±0	6.6±0.3	310.8±119.3	20.3±0.3	4.6±0.4	9.8±5.7
PI2	1.3±0.5	22.7±6.3	6.9±0.6	38.5±2.1	0.3±0	6.3±0.2	258.4±258.4	16.2±0.4	4.6±0.8	6.9±2.7
PI3	1.3±0.3	23.1±6.1	7.1±0.5	38.0±2.8	0.3±0	6.4±0.3	289.4±218.8	16.3±2	4.5±0.8	4.8±3.1

Sampling

Water and diatom communities were obtained in 20 sampling sites with a van Dorn water sampler along the vertical profile of reservoir during the austral summer and winter (2014) in which the hydrological regime is characterized, respectively, by a wet and a dry season. Surface sediments were collected only during the winter with an UWITEC gravity core and the top 2 cm were used for analyses.

In the field, temperature (°C), pH and conductivity (µS cm^{-1}) were measured with a Horiba U-53 multiparameter probe, and water transparency with a Secchi disk. The analytical procedure for dissolved oxygen (mg L^{-1}), ammonium (µg L^{-1}), nitrate (µg L^{-1}), soluble reactive silica (mg L^{-1}), total nitrogen and total phosphorus (µg L^{-1}), free carbon dioxide (mg L^{-1}), and bicarbonate ions (mg L^{-1}) followed Standard Methods (APHA, 2005). Chlorophyll *a* (µg L^{-1}) corrected for phaeophytin was measured in 90% ethanol (Sartory & Grobbelaar, 1984).

Diatom samples were prepared with standard techniques (Battarbee, 1986). Permanent slides were mounted with Naphrax followed by taxonomic identifications performed in a binocular Zeiss Imager A2 equipped with Differential Interference Contrast (DIC) and an AxioCam MR5 digital camera (magnification 1000x). Relative abundance was estimated with three quantification limit criteria: (*1*) species rarefaction curve (Bicudo, 1990), (*2*) counting of at least 400 valves in total, and (*3*) efficiency count of at least 90% (Pappas & Stoermer, 1996).

Statistical analyses

In this study, community matrices were composed of common and rare diatom species, represented by different data sets that were deconstructed according to their relative abundance (at least 1%, 2% and 5%) and the frequency of occurrence (rare < 25% and common > 75%).

In order to eliminate disparity between values, all diatom data sets, including “all species” matrix, were Hellinger-transformed. Variation partitioning was used to determine the contribution of environmental, spatial and hydrological connectivity predictors to the structure of the community variation of all diatom data sets, both within phytoplankton and superficial sediment and between climatic seasons. If species composition patterns solely vary along environmental gradients, it would indicate that the underlying mechanism is environmental filtering; if only spatial structure affects, variation in community patterns may arise from dispersal limitations (Arnan *et al.*, 2015). In this sense, the total metacommunity variation was partitioned into pure and shared effects: (*1*) environmental variables, accounting processes related to environmental filtering of species and, therefore, related to the grinnellian niche concept; (*2*) spatial variables represented processes related to the stochastic dispersion and, therefore, related to the neutral theory concepts; and (*3*) hydrological connectivity variables, representing another facet of the species dispersion not related to geographical distance, but to the ecosystems' connectivity at different scales. Therefore, matrices of local communities (sampling × species) were used as a response matrix in separate analyses and three predictor matrices were generated. The first is a matrix of environmental variables, comprising the limnological variables described above. Environment variables considered during present analyses were selected after screening collinear variables. The second matrix is composed of spatial variables that were generated to represent probable dispersion routes. For this purpose, we used the *Asymmetric Eigenvector Maps* approach, proposed by Blanchet *et al*. (2008a). As a result, spatial filters were generated by eigenvalue analysis, a *Principal Coordinates of Neighbor Matrices* (PCNM, Borcard & Legendre, 2002) generalization, considering the water flow directionality, an inherent characteristic of aquatic ecosystems connected in the watercourse. Only variables with positive and significant spatial correlation (determined with Moran's I) were selected. The hydrological connectivity was represented by a matrix of ordinal variables grouping the sampling stations at different scales. In this case, four variables were formulated as follows: one with similar values for stations within a reservoir; another with similar values for stations in reservoirs of the same river; another with similar values for stations in reservoirs in two directly connected rivers; and the last one with similar values for stations in reservoirs of the same basin (Fig. 2). We also performed the same procedure with Forward Selection procedure (Blanchet *et al*. 2008b, as implemented in Dray, 2007).These results are available as Supplementary Material (Annex S1).

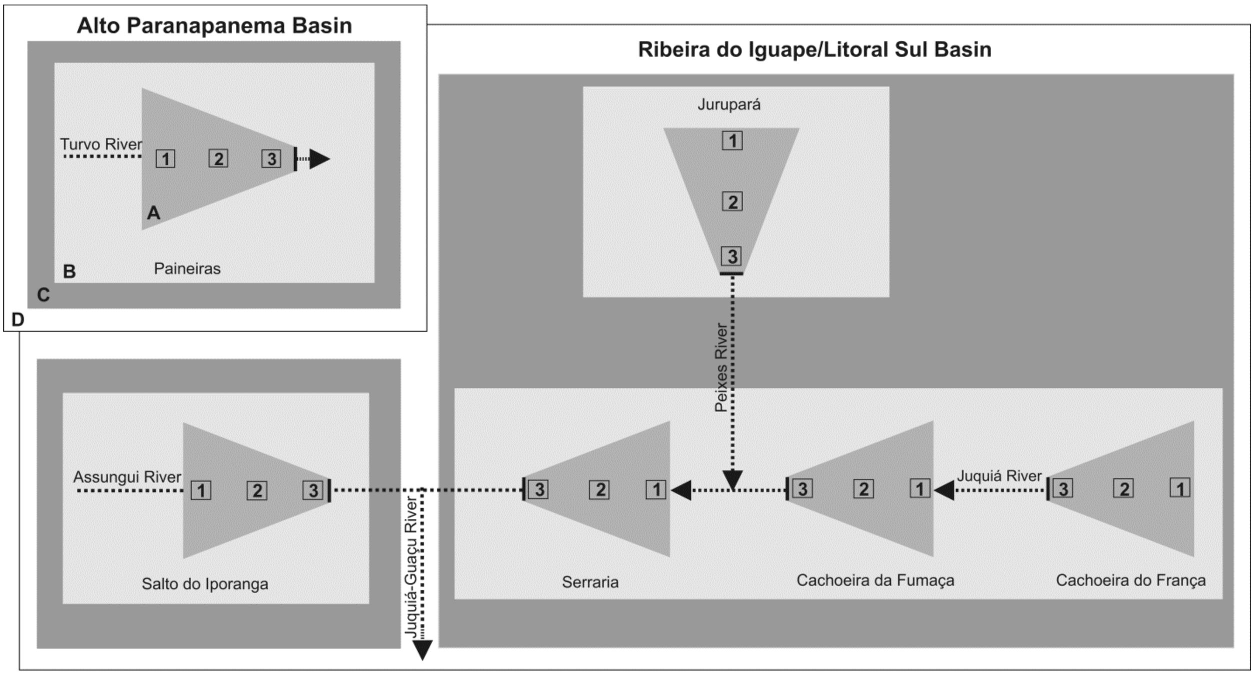


Figure 2. Schematic representation of the ordinal variables used as predictor matrix for hydrological connectivity in the study area. (A) similar values for sample units (numbers) within a reservoir (trapezium); (B) similar values for sample units in reservoirs of the same river (light gray area); (C) similar values for sample units in reservoirs in two directly connected rivers (dark gray area); and (D) similar values for sample units in reservoirs of the same basin (white area). Rivers and their watercourse are represented by dotted arrows.

Predictor matrices and their individual and shared contribution were estimated by partial redundancy analysis (pRDA) (Borcard *et al.,* 1992). In this, eight explanation components were generated: (*1*) pure environmental, ***E***: fraction of variation in the community structure explained by environmental variables that are neither spatially nor hydrologically connected; (*2*) pure spatial, ***S***: species data in spatial patterns that are independent of any hydrological connectivity or environmental predictors; (*3*) pure hydrological, ***H***: hydrological connectivity patterns that are independent of any spatial or environmental predictors; (*4*) pure spatial component of environmental predictors, ***SE***: the spatial variation component that is linked to one/some environmental variable(s); (*5*) pure hydrological of environmental components, ***HE***: fraction of (non-spatial) variation explained by hydrological connectivity and environmental variables; (*6*) pure combined spatial/hydrological connectivity component, ***SH***: fraction of the species data variation that is not related to environmental variables, but that can be attributed to pure combined spatial-hydrologic patterns; (*7*) combined spatial/hydrological connectivity of environmental component, ***SHE***: variation fraction that can be explained by combined action of spatial, hydrological connectivity and environmental variables; and (*8*) unexplained, ***U***: this component is the remainder in the analysis that cannot be explained neither by the spatial coordinates, nor by the hydrological connectivity variables or the environmental variables included in the analysis.

To highlight the connectivity effect in the metacommunity organization, the same analysis was performed without the hydrological connectivity matrix. In this case, four components are generated: ***S***, ***E***, ***SE*** and ***U***. Both analyses' results were compared and the connectivity effect was implied by the change in the total explained variance and the variance explained by environmental or spatial variables. Fractions significances were tested using 999 random permutations. Analyses were performed in software R v. 3.0.2 (R Core Team, 2014) with 'varpart' function of the vegan package (Oksanen *et al.,* 2016).

RESULTS

Diatom community composition

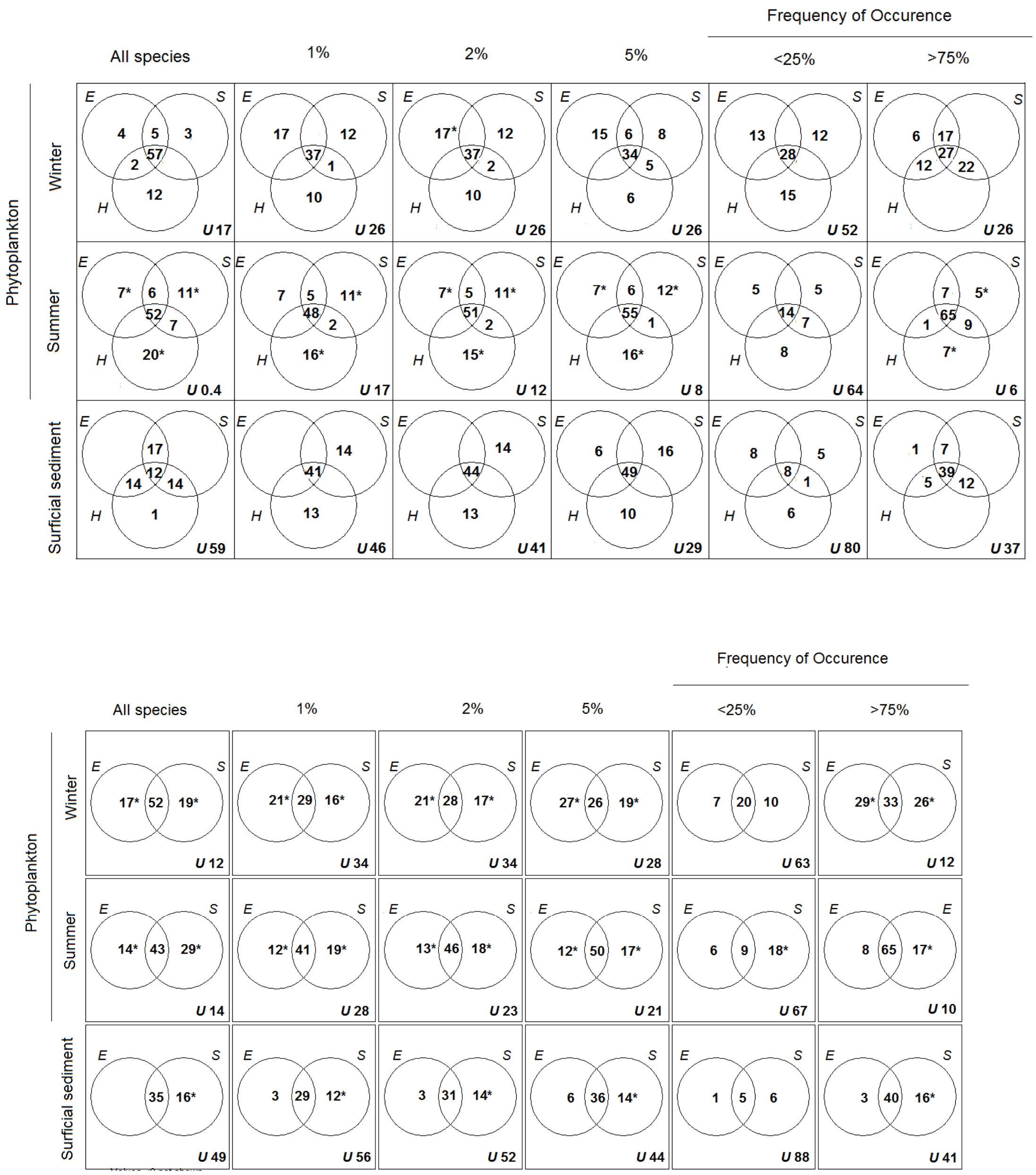
A total of 447 diatom taxa belonging to 71 genera were identified within the 60 samples from the studied reservoirs. 186 taxa occurred in the phytoplankton during the summer, 125 taxa occurred in the phytoplankton during the winter, and 334 occurred in the surface sediments.

Several taxa showed high abundances in the samples, including *Aulacoseira tenella* (Nygaard) Simonsen, *Discostella stelligera* (Cleve & Grunow) Houk & Klee and *Spicaticribra kingstonii* Johansen, Kociolek & Lowe. However, the singletons are anomalously high in most samples.

Variance partitioning

The matrices resulting from the VIF Function and Forward Selection showed similar results and tendencies. Hence, the latter results are showed as supplementary material (Annex S1).

The explanatory capacity of predictor variables was usually high and although part of the variability in the diatom community structure remained unexplained, this fraction containing residuals [***U***] was quite low in all performed analyses (Fig. 3). The metacommunity variation explanation depended on the predictor matrices and the rarity categories employed, especially when rarity was defined as occurrence frequency (Fig. 3).



A)

B)

Figure 3. Results from partial redundancy analysis (pRDA). Shown are the relative contributions (% of explanation) environmental (***E***), spatial (***S***), hydrological connectivity (***H***, only in A) variables and the shared components explaining variation in abundance of diatom metacommunities. ***U*** = unexplained component. Significance of the pure components (***E***, ***S*** and ***H***) was tested using random permutations. Significant values ($p < 0.05$) are represented by "*".

Inclusion of the hydrological connectivity matrix resulted not only in increase of the explained total variance, but also in change in the effect of environmental and spatial variables (Fig. 3A). In this case, we observed the lowest values obtained for the > 75% data set in virtually all partitions.

The ***S*** component explained a significant portion of the total variation in the several group structures. This component was particularly high for the summer phytoplankton data set, when the hydrological connectivity matrix is not considered, especially for the “all species” data set. The lowest (and non-significant) ***S*** component was obtained for the surface sediments diatom communities with the hydrological connectivity predictor, especially for the “all species” and > 75% data sets, without any explicability considering the three predictors. The ***E*** component was significantly accounted for part of the variation in all groups’ community structure, except for that of the surface sediments. Also, environmental predictor results were not significant to almost all the frequency occurrence data sets. The environmental variables were also higher especially for the phytoplankton data set during the winter.

When the hydrological matrix was considered, ***S***, ***H*** and ***E*** shared fraction was the highest component explaining variation in the all groups community structure, especially the ones recorded for the summer phytoplankton (Fig. 3A). Other shared fractions were low or absent.

DISCUSSION

We innovate by showing that the relative importance of environmental, spatial and hydrological connectivity predictors for explaining the species composition and diatoms assemblage distribution varied not only among phytoplankton, superficial sediments and climatic seasons, but also among the different cut-offs. A very tiny portion of the community variation remained unexplained and we showed that better representing hydrological connectivity is central to disentangle relative roles of different mechanisms in community assembly in a system of reservoirs cascade.

Despite the important role that the environment consistently plays in the diatom community structure, they have a strong spatial component, showing a clear geographic structure. These findings are highly congruent with the number of recent studies unraveling the spatial structure of unicellular organisms in general (Potapova & Charles, 2002; Reche *et al.,* 2005; Soininen, 2007). Environmental heterogeneity and dispersal limitation were likely the main factors determining diatom beta diversity in tropical reservoirs (Zorzal-Almeida *et al.,* 2017).

Environmental gradients may be barely evident at small scales. At large spatial scales, adaptation to specific regional environmental regimes provides support for a niche-based perspective. However, at small and intermediate spatial scales, the covariance of environment and geographic distance has made it more challenging to distinguish the likely effects of niche versus neutral processes on community composition (Gilbert & Lechowicz, 2004). In these cases, causes of the low percentage explanation is credited, in general, to the non-inclusion of variables relevant to the community structuring (Soininen *et al.,* 2007; Padial *et al.,* 2014; Heino *et al.,* 2015) and stochastic colonization and extinctions, ecological drift as well as biological interactions (Vilmi *et al.,* 2016).

Spatial factors may indicate dispersal-related mechanisms, such as mass effects and dispersal limitation (Székely & Langenheder, 2014). When considering the hydrological connectivity predictors, total variation percentages explained by the spatial and shared effects were greatly reduced. Therefore, we suggest that representation of hydrological connectivity in key in studies carried out in reservoirs cascade, given that only PCNM techniques may not represent how connections may affect community assembly. Independent on how representing hydrological connectivity, it has proven to be an important factor in forming communities along riverine dendritic networks (Besemer *et al.,* 2013; Freimann *et al.,* 2015) and tropical reservoirs (Zorzal-Almeida *et al.,* 2017). A high dispersal rate facilitated by high connectivity between sites was the most probable explanation for the diatom community patterns observed by Vilmi *et al*. (2016), in which high dispersal rates between sites are likely to interfere with environmental filtering and may result in clear spatial structures in the index values used in bioassessment. These results were dependent on the size and connectivity of the study system. Currently, the fraction predicted by the combined action of spatial, hydrological connectivity and environmental variables contributed with most of the data explanation.

We observed that results from the application of the 1%, 2% and 5% cut-offs based on relative abundances do not greatly differ from those observed pattern in the "all species" data set. It suggests that applying these cut-offs on data does not imply a significant information loss provided by rare species. Although authors used different criteria of rarity, Siqueira *et al.* (2012) found similar results, even though their rare species definition as the 25% and 50% less abundant species produced different results when compared to common or non-significant species. However, common and rare species sets react to similar major environmental gradients and that the rare species also respond to processes that operate at a more fine-grained spatial scale, particularly biotic interactions. Yet, the authors emphasize that rarity is a complex concept, and finding a useful and objective rarity definition is difficult (Rabinowitz, 1981; Gaston, 1994; Siqueira *et al.,* 2012). Our results are also in accordance with those by Checon & Amaral (2016) in which inclusion of only a few abundant species or of all species would lead to the same results. However, latter authors emphasized that the rare species influence was related to the metrics applied to the analysis. Similar responses of common and rare species to ecological gradients also suggest that common species assemblages may be used to describe biodiversity-environment relationships. Common species can potentially indicate various environmental changes in lakes and, hence, there is support for the practice of eliminating rare species from certain analyses leading to the development of environmental indicators (Cao & Larsen, 2001). Conversely, Nijboer & Schmidt-Kloiber (2004) found that an European multimetric index of biotic integrity was depressed by removing species defined as spatially rare, and was elevated by removing taxa with low abundances, suggesting that certain types of rarity describe different responses to environmental conditions (Arscott *et al.,* 2003).

Although recent studies supported the idea that both common and rare species are mainly affected by environmental factors (e.g. Jetz & Rahbek, 2002; Siqueira *et al.,* 2012), local environment explained very little about the diatom species compositional changes among reservoirs, especially in regard to the surface sediments habitat. However, when the hydrological connectivity matrix was included, results from this compartment were not significant, despite following the same phytoplankton compartment tendencies, with prevalence of the spatial predictors to explain the diatom community variation. Due to its degree of temporal integration, surface sediments are expected to provide longer intervals for the immigration, emigration and colonization of taxa than do water-column samples, being more powerfully explained by environmental gradients (Winegardner *et al.,* 2015). Inference models generally assume that dead diatom assemblages faithfully reflect the environmental conditions prevailing at the sampling station. Sedimentary diatom assemblages, however, are the result of ecological processes affecting the distribution of living diatoms along environmental gradients plus taphonomic processes that alter dead frustules after deposition, so that their distribution within a locality may not represent their living habitat with high fidelity (Hassan *et al.,* 2008). Nevertheless, environmental variables were better predictors than the spatial and hydrological connectivity for the winter phytoplankton data sets. The winter period is the dry season, which possibly changes the environment connectivity, and consequently the dispersal capacity of the local assemblies. Also, the trophic gradient was more heterogeneous in this period with sample units previously classified as oligotrophic and mesotrophic becoming mesotrophic and eutrophic. In this sense, abiotic variables might have an outstanding influence on the diatom community structure reflecting mainly the minor changes in water chemistry within a watershed.

CONCLUSION

In this study, we clearly demonstrate that importance of the different predictors in structuring tropical diatoms metacommunities was similar for the different cut-offs, but dependent on the climatic season and habitat. Primarily, the diatom distribution in phytoplankton and superficial sediment was explained mainly by the spatial structure and during the summer, contrary to recent studies that support the environmental factors as the main predictor. During the winter, the environmental component was preponderant only for the phytoplankton diatom communities. More important, hydrological connectivity proved to be central in dispersion processes on the community structure, playing an important role in the explanation of the diatom metacommunities variation. We conclude that this variable should never be neglected in the models that predict the functioning aquatic ecosystems.

ACKNOWLEDGEMENTS

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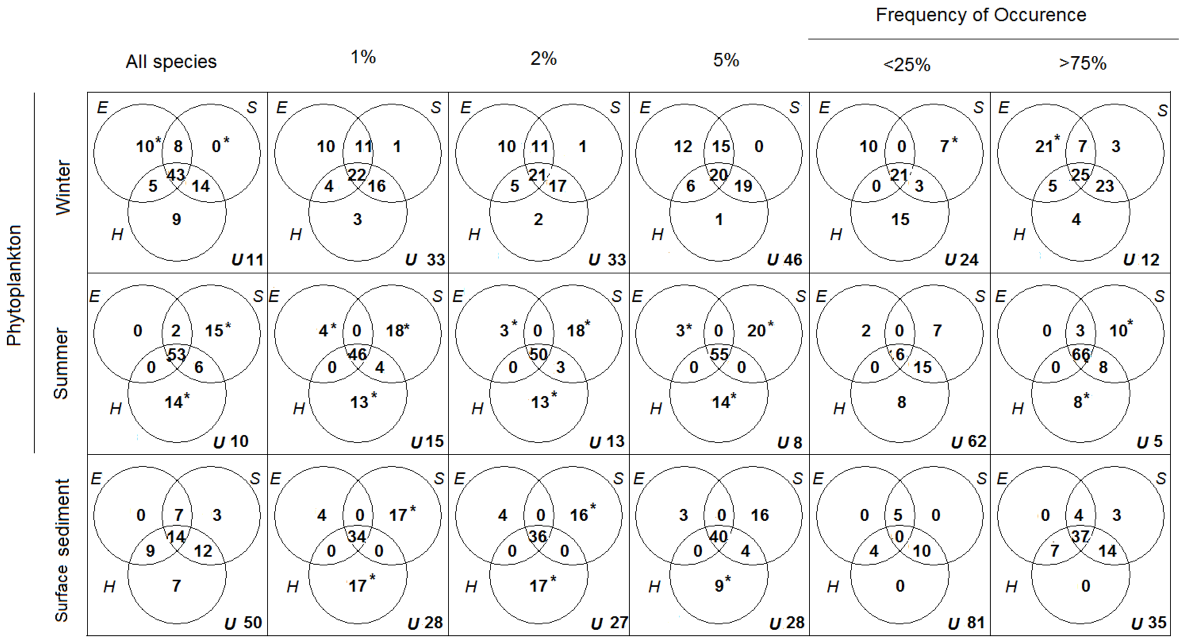
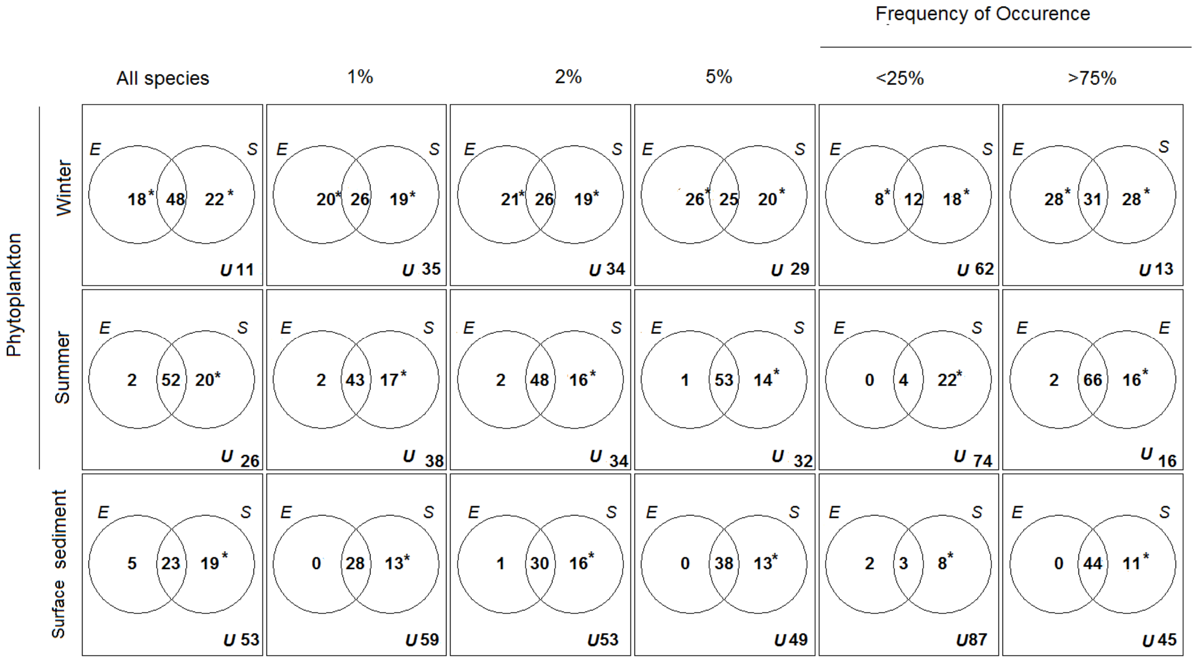
Appendix S1 (Anexo S1). Partitioning variation results considering the Forward Selection procedure (Blanchet et al. 2008).



A)



B)

Figure 1. Results from partial redundancy analysis (pRDA). Shown are the relative contributions (% of explanation) of environmental (***E***), spatial (***S***), hydrological connectivity (***H***, only in A) variables and the shared components explaining variation in abundance of diatom metacommunities. ***U*** = unexplained component. Significance of the pure components (***E***, ***S*** and ***H***) was tested using random permutations. Significant values are represented by "*".

Capítulo 3

A ser submetido à **Journal of Limnology**.

Partitioning patterns of freshwater diatoms in tropical reservoirs: effect of hydrological connectivity

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ABSTRACT

Detection of diversity spatial patterns varies according to the sampling scale, that is, the pattern observed in a local community may be different than that found in larger areas. We used two additive partitioning of total species richness and a null model to evaluate whether cascade reservoirs located at the same river differ on the partition of diversity at different scales than reservoirs located in different rivers. Besides this, beta diversity among reservoirs was estimated by Sørensen index and was partitioned in a component related to turnover and nestedness, to assess whether reservoirs with direct connection by watercourse have lower beta diversity than connectionless reservoirs. Residues of linear regression of beta diversity indices with the geographical distance of reservoirs were used to test the beta diversity of reservoirs connected directly to the watercourse. We found that high levels of hydrological connectivity favors the exchange of organisms through passive dispersal, increasing similarity in the metacommunity species composition and decreasing beta diversity. For conservation purposes, present results suggest a protection in a large scale, ensuring beta diversity between sites and conserving total diversity.

Keywords Additive diversity partitioning, alpha and beta diversity, null hypothesis, spatial scales

Introduction

Urban reservoirs are considered important artificial aquatic ecosystems formed by damming one or more rivers (Räsänen 1986). These artificial systems are important points of convergence of the different activities developed in the basin, their uses, and socio-environmental and economic aspects, so that water quality reflects the multiple human activities and the impacts resulting from these activities (Rebouças 1999).

Assessing the ecological quality of aquatic ecosystems and their stressors has, therefore, become a central theme of discussion. This assessment is commonly performed based on ecological indicators that provide tools to guide actions of environmental managers (Bicudo et al. unpublished manuscript). In this sense, diatoms have been extensively used in water quality monitoring (Round 1991).

Diatoms are single-celled organisms (class Bacillariophyceae) that typically possess siliceous cell walls or frustules that are well preserved in most aquatic sediments (Dixit & Smol 1994). Hence, sedimentary diatoms have also been increasingly used in environmental monitoring and assessment studies (Dixit et al. 1992, Charles et al. 1994) as they provide an extended temporal record of environmental conditions, far beyond those that are possible by current monitoring conditions (Dixit et al. 1992, 1998). Diatoms exist in a wide range of ecological conditions and colonize almost all suitable habitats (Stevenson & Bahls 1999, Omar 2010). Depending on the species concept, conservative estimates suggest that the total number of diatom species could be as high as two hundred thousand (Mann & Droop 1996, Wetzel et al. 2012). However, such global estimates are confused because most studies are focused on understanding the patterns of diversity in a particular diatom genus at a local or regional scale (Malviya et al. 2016).

Species diversity is heterogeneously distributed among habitats, landscapes and regions. To account for different spatial scales, Whittaker (1977) proposed a hierarchical nomenclature in which alpha diversity refers to that within-habitat, beta to diversity among-habitat differentiation in a landscape, gamma diversity to total within-landscape diversity, delta diversity to among-landscape differentiation in a region, and epsilon diversity to total within-region diversity (Tuomisto 2010).

Many authors have stated in the past few years that decomposition of the total diversity into alpha and beta community diversity should be additive (e.g. Allan 1975, Pielou 1975, Veech et al. 2002, Ricotta 2003), where the regional diversity is the sum of alpha and beta diversity, when alpha is the average diversity within the sampling units in the region and beta is diversity between sampling units (Allan 1975, Lande 1996). Diversity components within (alpha) and between (beta) communities are expressed in the same units so that they can be directly compared (Diekötter et al. 2007). Differences in abiotic conditions among the geographic location of communities, existence of predators and competitors, differences in species adaptations, and dispersal ability might all influence the species distribution among communities (Veech 2005).

Recently, Baselga (2010) proposed that Sørensen dissimilarity, a monotonic transformation of strict sense beta diversity (e.g. gamma/alpha) could be decomposed into two additive components (Si et al. 2015). Then, regardless of the beta diversity concept used there are two basic patterns that lead to variation in species composition: (i) *turnover* refers to the replacement of some species by other species from site to site, independent of potential differences in species richness between the sites (e.g. Koleff et al. 2003, Baselga 2010, Chase et al. 2011) and (ii) *nestedness* component accounts for the differences in composition when there is no species replacement from one site to the other. These differences are derived from differences in species richness between nested assemblages, e.g. the species composition of the poorest site is a subset of that of the richest site (Baselga et al. 2010, Baeten et al. 2012).

Partitioning biological diversity across spatial scales has been focused on in numerous ecological studies for several decades since Whittaker (1960, 1972) pioneering works. Thus, additive partitions have been used to analyze hierarchical patterns of species diversity in agricultural landscapes (e.g. Wagner et al. 2000, Fournier & Loreau 2001), tropical forests (e.g. De Vries et al. 1997, 1999, De Vries & Walla 2001, Cabra-García et al. 2010, Beck et al. 2012), temperate forests (e.g. Gering et al. 2003, Summerville et al. 2003) and several studies have been conducted to evaluate diversity partitioning in rivers (Ligeiro et al. 2010, Hepp et al. 2012, Hepp & Melo 2013), lakes (Flach et al. 2012), and wetlands (Ávila et al. 2011, Maltchik et al. 2012). However, additive partitioning analyses in tropical reservoirs (e.g. Molozzi et al. 2013) and especially involving diatoms (e.g. Wetzel et al. 2012) are rare. Also, studies evaluating the effects of hydrological connectivity on the components of diversity within and between communities have been scarce (e.g. Diekötter et al. 2007, Molozzi et al. 2013). Besides this, the surface sediment compartment has been neglected.

Specifically, present study addressed two major objectives. First, we used additive partitioning of diversity to examine whether cascade reservoirs in the same river differ on the partition of diversity at different scales from reservoirs located in different rivers. Second, we determined whether reservoirs with direct watercourse connection have lower beta diversity than connectionless reservoirs. We disentangled the contributions of spatial turnover and nestedness to beta diversity patterns and their changes via species loss and gain over the study period with the quantitative framework recently proposed by Baselga (2010). We expected that in strongly connected habitats, dispersal rates can be so high that lead to homogenization through mass effects compared to non-connected reservoirs, based on the assumption that high levels of hydrological connectivity favors the exchange of organisms through passive dispersal, increasing similarity in metacommunity species composition and decreasing beta diversity (Lopes et al. 2014).

Methods

Study area

Fieldwork was carried out along the six reservoirs studied as part of the AcquaSed Project (Base line diagnosis and reconstruction of anthropogenic impacts in the Guarapiranga Reservoir, focusing on the sustainability in water supply and water quality management in reservoirs of the Alto Tietê and surrounding basins). Reservoirs are located at two different basins: Ribeira do Iguape and Litoral Sul, and Alto Paranapanema (Fig. 1) with a mean elevation ranging from 17 to 996 m a.s.l. Main reservoirs are for power generation, but they are also used for fishing and recreation (Votorantim Energia, *personal communication*). All study sites were located in well preserved areas, most of them located in areas of the PEJU State Park (Parque Estadual do Jurupará). Presently, reservoirs were classified mostly oligotrophic to mesotrophic (Lamparelli 2004) with no obvious signs of human impact.

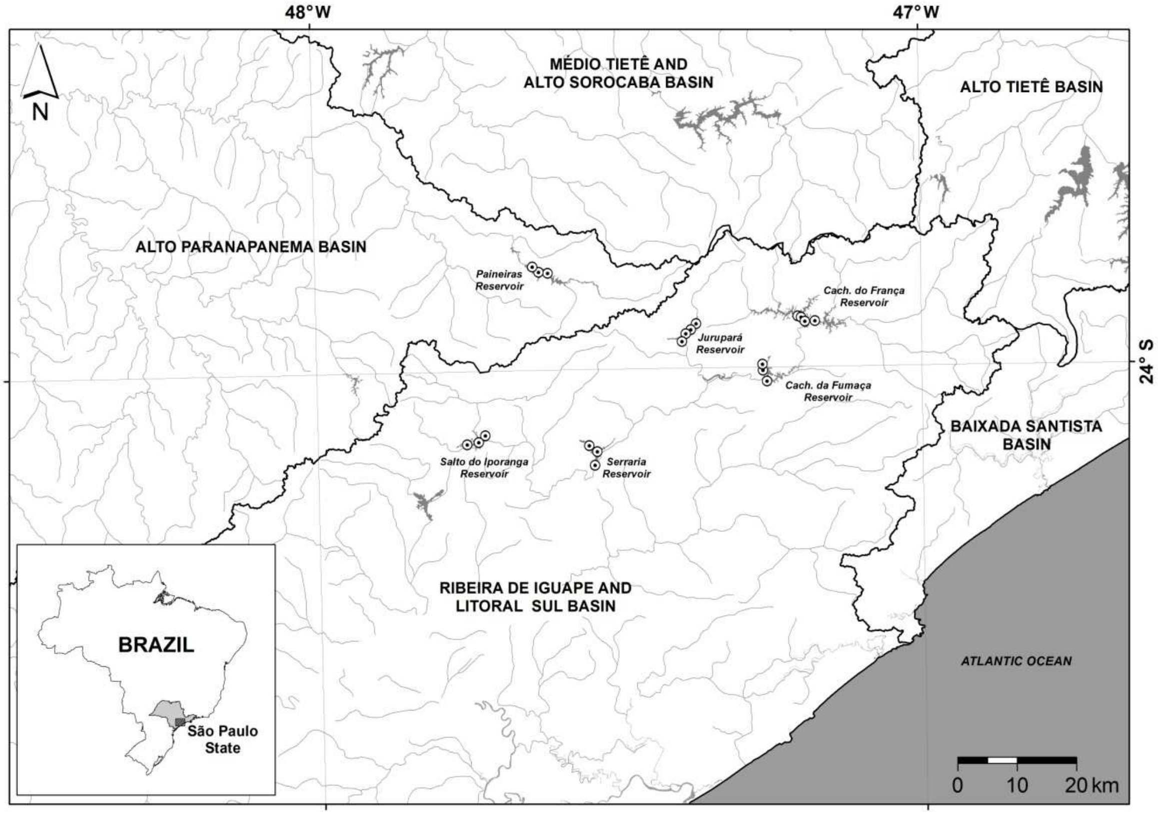

Figure 1. Sampling sites located in the six studied reservoirs along the Ribeira do Iguape e Litoral Sul and Alto Paranapanema basins.

This area supports enormous biological diversity and high degree of endemism within the region and species of fauna and flora presently threatened with extinction (SMA 2010). Recently, a number of diatom species have been described (e.g. Marquardt et al. 2017a, b; in press). Therefore, understanding of the scaling of diversity is important to enhance conservation and management strategies for ecosystems under future environmental changes.

Sampling and slides preparation

Water and diatom assemblages were sampled during the rainy (January to February) and dry seasons (June to September) of 2014 in 20 sampling sites distributed along the six reservoirs studied. Bathymetric maps were not available for the sampled reservoirs, and maximum depths (Z_{max}) were measured using a depth meter. Water samples were collected with a van Dorn sampler at three depths along the water column (subsurface, mean depth, and 1 m above the sediments). Temperature (°C), pH and electrical conductivity (μS cm^{-1}) were measured in the field at every 50 cm depth with a multiparameter probe (Horiba U-53), and the water transparency with a Secchi disk. The following water variables were also measured: alkalinity

(Golterman & Clymo 1969), free CO_2^-, HCO_3^-, CO_3 and ammonium (N-NH_4) (Solorzano 1969), nitrate (N-NO_3) and nitrite (N-NO_2) (Mackereth et al. 1978), dissolved oxygen (DO) (Winkler modified by Golterman et al. 1978), soluble reactive phosphorus (SRP) and total dissolved phosphorus (TDP) (Strickland & Parsons 1965), soluble reactive silica (SRS) (Golterman et al. 1978), and total nitrogen (TN) and total phosphorus (TP) (Valderrama 1981). Chlorophyll-a concentration corrected for phaeophytin was measured with 90% ethanol as the organic solvent (Sartory & Grobbelaar 1984) (Table 1).

Table 1. Means and standard deviation of limnological variables in the six studied reservoirs. Variables were eliminated because of collinearity. Abbreviations: Sec (Secchi depth; m), Temp (Temperature; ºC), Cond (Conductivity; µS cm^{-1}), Alk (Alkalinity; mEq L^{-1}), DO (Dissolved oxygen; mg L^{-1}), TN (Total nitrogen; µg L^{-1}), TP (Total phosphorus; µg L^{-1}), SRS (Soluble reactive silica; mg L^{-1}), Chlo (Chlorophyll-a; µg L^{-1}), TSI (Trophic State Index). FR (Cachoeira do França), FU (Cachoeira da Fumaça), SE (Serraria), JP (Jurupará), SI (Salto do Iporanga), PI (Paineiras). Numbers refer to the sample units.

	Sec	**Temp**	**pH**	**Cond**	**Alk**	**DO**	**TN**	**TP**	**SRS**	**Chlo**	**TSI**
FR1	1.7±8.5	23.1±6.8	7.3±0.9	24.5±0	0.2±0	7.5±0	151.8±162.9	5.5±2.2	2.4±1.3	5.4±4.8	49.2
FR2	1.9±0.	24.2±8.1	7.3±1.3	24±0	0.2±0	7.43±0.9	191.1±45	10.0±7.5	2.39±1.2	4.12±2	50.4
FR3	1.7±0	24.1±8.3	7.9±0.6	23.5±2.1	0.2±0	7.3±1.6	231.2±21.2	6.6±3.7	2.3±1.4	20.2±26.5	51.0
FR4	1.7±0.1	24.0±7.9	8.6±0.6	24.5±3.5	0.2±0	8.2±1.6	280.0±153.7	6.8±2.5	2.2±1.3	22.8±29.7	51.6
FU1	2.6±1.7	24.8±7.3	6.5±0	26.5±0.7	0.2±0	6.9±2.2	228.6±13.7	5.1±1.6	2.5±1	1.7±0.4	46.8
FU2	2.8±1.5	24.3±7	6.8±0.7	26.0±2.8	0.2±0	8.3±0.5	176.5±66.1	8.4±2.1	2.4±1	3.4±1.1	49.9
FU3	2.1±0.3	24.8±6.4	6.6±0	26.0±1.4	0.2±0	7.5±0.1	180.1±71.7	5.8±2.7	2.3±1	3.3±2.3	48.5
SE1	4.0±1.8	26.3±7.2	7.4±1.1	21.0±9.9	0.2±0	8.3±1.4	180.3±64.1	8.1±2.9	4.4±1.2	1.8±0.7	48.2
SE2	3.7±1.4	26.2±7	7.3±0.6	25.0±2.8	0.2±0	7.9±0.4	174.7±80	8.0±1.5	3.9±1.5	4.6±2.2	50.4
SE3	4.2±1.4	25.6±6.2	6.6±0.2	28.5±2.1	0.2±0	7.5±1.2	216.0±147.7	8.5±3.7	3.6±1.5	7.6±0.7	51.9
JP1	1.5±0.8	24.1±6.7	6.4±1.3	24.0±2.8	0.2±0	7.8±0.8	464.3±89.4	19.5±4.2	3.9±0.3	16.8±1.8	56.5
JP2	1.9±0.4	24.6±6.5	6.6±0.1	23.5±3.5	0.2±0	6.7±1.3	351.4±201.4	16.6±0.3	3.8±0.3	10.1±0.7	54.8
JP3	2.2±0.2	25.4±6.9	6.6±0.1	24.0±2.8	0.2±0	7.5±0.3	453.3±8.2	13.5±0.7	3.7±0.3	7.1±1.2	53.3
JP4	2.1±0.4	25.0±7.1	6.8±0.2	23.5±3.5	0.2±0	7.1±1.1	434.1±103.8	13.3±1.9	4.0±0	6.6±3.7	52.8
SI1	1.5±0.2	24.0±2.6	8.0±2.1	50.0±9.9	0.3±0	6.7±1.9	415.9±143.2	32.1±3.7	5.1±1.6	36.7±37.6	59.0
SI2	1.9±0.4	23.7±2.7	7.8±2	43.5±7.8	0.3±0	6.2±2.9	315.9±28.4	23.1±1.5	4.9±0.7	17.4±15.3	56.5
SI3	1.7±0.6	23.6±2.7	7.9±2.1	43.0±11.3	0.3±0	7.4±2.2	304.4±22.4	25.6±3.8	4.1±1.5	36.8±44	57.7
PI1	0.7±0.7	22.5±6.4	6.7±0.4	39.0±2.8	0.3±0	6.6±0.3	310.8±119.3	20.3±0.3	4.6±0.4	9.8±5.7	55.1
PI2	1.3±0.5	22.7±6.3	6.9±0.6	38.5±2.1	0.3±0	6.3±0.2	258.4±258.4	16.2±0.4	4.6±0.8	6.9±2.7	53.7
PI3	1.3±0.3	23.1±6.1	7.1±0.5	38.0±2.8	0.3±0	6.4±0.3	289.4±218.8	16.3±2	4.5±0.8	4.8±3.1	52.6

Two different sets of samples (surface sediment and phytoplankton) were collected. Subsurface phytoplankton diatoms were sampled by using a van Dorn sampler and samples of each vertical profile were incorporated in the laboratory into a sample of equal volume from each sampling station. This procedure was executed in order to represent the planktonic diatom community as a whole along the vertical profile. Due to its integrative character, sediment diatoms were sampled only during the winter with an UWITEC gravity core. Small homogenized aliquots from both surface sediment and phytoplankton samples were heated to 90°C with concentrated hydrogen peroxide (H_2O_2 35%) and hydrochloric acid (HCl 37%) for at least 4 h to oxidize the organic matter and clean the frustules. Next, the clean material was diluted with deionized water and mounted on permanent slides with Naphrax as mounting medium. Slides were examined under a Zeiss microscope (Imager A2) at 1000x magnification under oil immersion. Relative abundances were estimated following the counting technique described by Pappas & Stoermer (1996) with a minimum of 400 valves counted per slide on up to six random transects until reach the efficiency of at least 90% besides the species and the rarefaction curve (Bicudo 1990).

Statistical analyses

As sampling within the reservoirs were performed at different periods (with the exception of the surface sediment diatoms), the gamma diversity of the set of reservoirs could be partitioned as follows: (i) the average diversity for sampling unit (alpha), the variation of the diversity within the reservoir (beta 1), (ii) the variation of diversity between reservoirs (beta 2), and (iii) the variation of diversity between seasons (beta 3, only phytoplanktonic diatoms). Therefore, we used two additive partitioning of diversity, being one for the cascade reservoirs and the other one for those located in different rivers. Components of diversity were transformed into percentage of gamma diversity and compared with the values expected by a null model (999 permutations). A comparison of the observed patterns with those predicted by null models suggests a nonrandom ecological process that may disproportionately differentiate total species diversity (Crist et al. 2003, Sasaki et al. 2012). A small proportion, for example, prop exp > obs < 0.05 indicates that diversity observed is significantly greater than expected by chance. Likewise, a value of > 0.95 indicates that the observed diversity is significantly lower than expected by chance (Crist et al. 2003). Intermediate values indicate that diversity is similar to that expected by chance.

The alpha diversity at a given scale is the sum of the alpha and beta diversity at the next lowest scale (e.g. alpha 2 = alpha 1 + beta 1; Allan 1975, Lande 1996) so, the overall diatom diversity in our study was described by the following formula: alpha 1 + beta 1 + beta 2 + beta 3. For these analyses, we used the ‘adipart’ function of the ‘vegan’ package (Oksanen et al. 2016) of the free software Rv. 3.0.2 (R Core Team, 2014). This function implements a statistical approach developed by Crist et al. (2003) for testing null hypotheses that observed that partitions of species richness differed from those expected by chance. A rejection of the null hypothesis indicates the non-random distribution of individuals due to intraspecific aggregation, resource partitioning and community saturation.

Furthermore, the beta diversity among reservoirs was estimated by the Sørensen index. In this case, the occurrence of species of different sampling units was combined in the occurrence of species for each reservoir. In addition, the beta diversity was partitioned in a component related to *turnover* and *nesting* as proposed by Baselga (2010), and implemented by the ‘nestedbeta’ function of the ‘vegan’ package (Oksanen et al. 2016). Specifically, this framework consists of decomposing the pair-wise Sørensen dissimilarity index (total beta diversity) into two additive components accounting for pure spatial turnover and nestedness (Leprieur et al. 2011). Since there probably is spatial autocorrelation in species composition, a linear regression of the beta diversity indices was performed against the reservoirs’ geographical distance.

Residues of such regression were used to test the beta diversity of the reservoirs directly connected by the watercourse. The basic idea is that if the simple linear model is appropriate, the residuals should reflect assumptions such as independence, constant variance for different levels of *x* and normal distribution. In this sense, if there is a violation the use of the model must be called into question (Korner-Nievergelt et al. 2015).

Results

Diatom composition and diversity

The total diatom richness recorded for the phytoplankton was 190 and 186 species, and 334 and 187 to surface sediments for the connected and non connected systems, respectively. The species-abundance distribution was characterized by a high percentage of singletons (i.e. species represented by only one individual) and a few dominant species. The studied reservoirs do not greatly differ regarding its TSI (Fig. 2). Limnological variables and chlorophyll-*a* values measured during the study period at the six reservoirs are summarized in table 1.

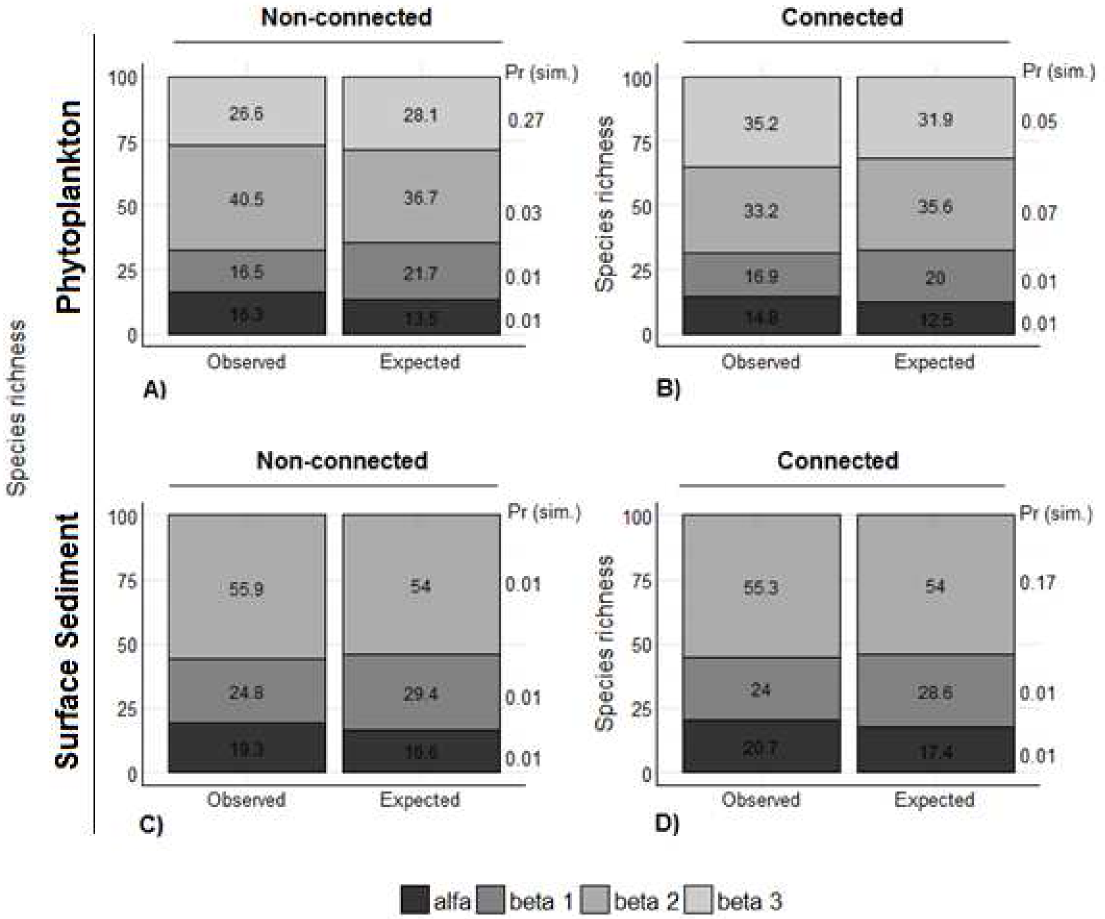

Figure 2. Total diversity of diatoms in six reservoirs of southwest Brazil, partitioned into alpha and beta components. Proportions on the right-hand side of the graph indicate the proportion of individual-based randomized samples containing more species than the observed sample for each partition. A low proportion value (e.g. propexp > obs < 0.025) indicates that the observed richness is significantly higher than the expected, and a high proportion value (e.g. propexp > obs > 0.975) indicates that the observed diversity is significantly lower than the expected. $\alpha 1$ = mean species richness per sampling unit. $\beta 1$ = beta diversity among sampling units within reservoirs. $\beta 2$ = beta diversity among reservoirs within area. $\beta 3$ = beta diversity among seasons.

Additive partitioning

Partitioning of α and β diversity components was similar both for phytoplankton and the benthic diatom communities. The sample unit component (alpha 1) in the model was always greater than expected by chance for both partitionings ($p < 0.01$, Fig. 2). However, there was less variation within the reservoir (beta 1) than it would be expected for the null model, revealing that there was no variation in the diatom community composition at this scale (Fig. 2).

In general, the largest fraction of the total diversity was accounted for by beta 2-diversity. Nevertheless, it is remarkable that for the phytoplankton in cascade reservoirs, variation between reservoirs (beta 2) was not different from what would be expected for the null model, whereas the variation among reservoirs located at different rivers is significantly higher than it would be expected for the null model (Fig. 2). On the other hand, variation between climatic seasons is greater than would be expected for the null model only for cascade reservoirs (Fig. 2).

Concerning the Sørensen index, turnover and nestedness, all of them were significantly greater among reservoirs without a direct connection by the watercourse except for the nestedness component, which showed lower values for the surface sediment in non-connected systems and higher values in connected ones (Fig. 3).

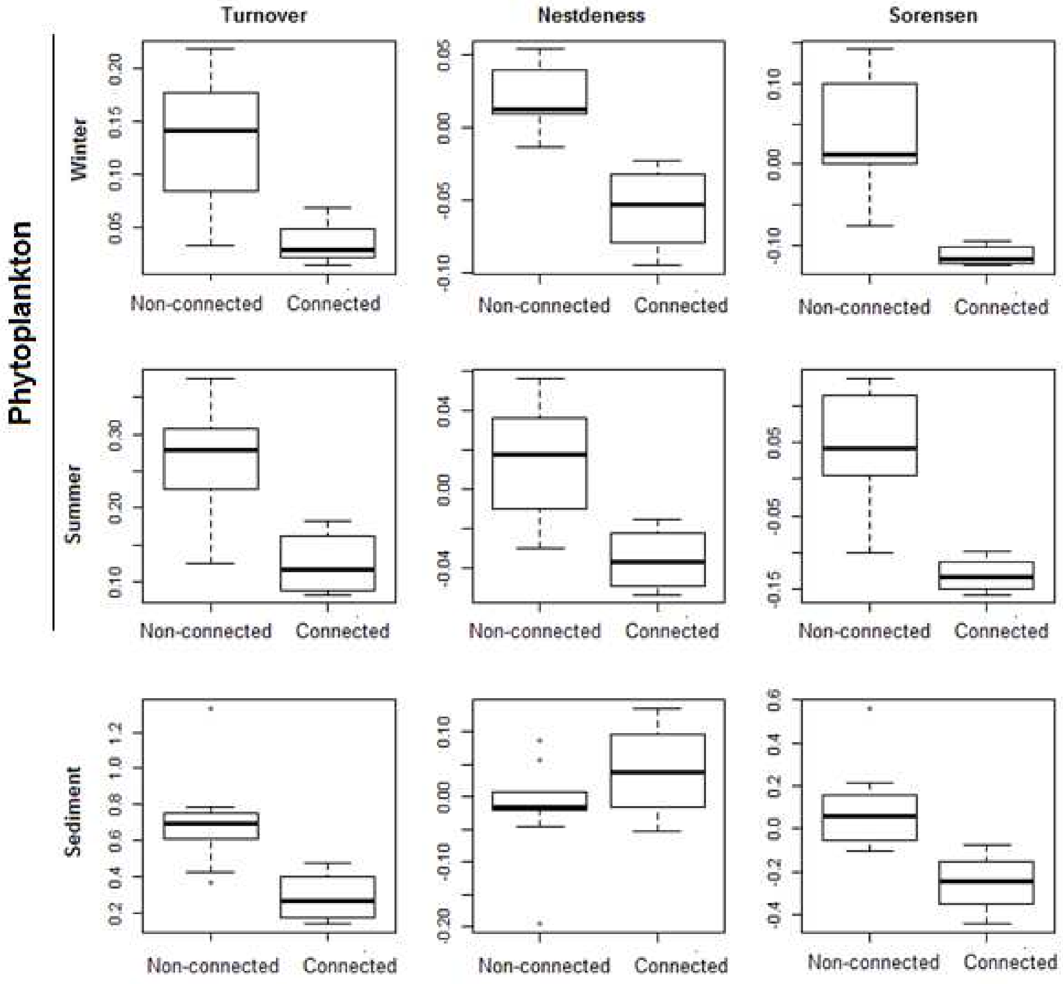

Figure 3. Total diversity of diatoms in six reservoirs in southwestern Brazil, partitioned into turnover, nestedness and Sørensen components among planktonic (summer and winter) and surface sediment diatom communities.

Discussion

The partitioning of species richness in the reservoirs studied showed that richness at the smallest scale (alpha, within a sample unit) was greater than that expected under the null model for both phytoplankton and surface sediment diatom communities. This high taxonomic richness indicates a strong dissimilarity in species composition. Indeed, it has long been known that tropical communities typically contain disproportionately more specialized and rare species than do assemblages at higher latitudes (Klopfer & MacArthur 1961, Hubbell 1979, 2008, Brown 2014). In addition, high dispersal (mass-effects) may lead to greater alpha-diversity since it may sustain populations that have a negative growth rate (Pulliam 1988, Loreau & Mouquet 1999). This process can be observed in highly connected systems.

Ecologists have been using the concept of connectivity for already a number of years as a critical property in the persistence of spatially structured populations (Metzger & Decamps 1997, Bracken & Croke 2007). Hydrologic connectivity (*sensu* Pringle 2001) is used here in an ecological context to refer to water-mediated transfer of matter, energy and/or organisms within or between elements of the hydrologic cycle (Pringle 2003, Freeman et al. 2007, Bracken et al. 2013). Highly connected communities (e.g. by hydrological connections and smaller distance between habitats) may have lower beta diversity due to the higher exchange of individuals between these communities via active and passive dispersal. In this study, the observed taxonomic beta 1-diversity (samples within the reservoir) is actually lower than expected by chance for both connected and non-connected systems. In this sense, dispersal rates are obviously a function of the connectivity between habitats which, in turn, are dependent upon geographic distances and the presence of corridors or dispersal barriers (Ricketts 2001, Verleyen 2009). Then, movements between spatial units (e.g. dispersal, migration) act as a homogenizing force, which tend to reduce the beta diversity component, but at the same time it increases the alpha component because immigration contributes to maintaining local coexistence (Loreau & Mouquet 1999, Loreau 2000).

Regarding the beta 2-diversity (among reservoirs diversity), it was higher than expected by chance only in non-connected reservoirs. High levels of beta-diversity may arise from the ecological effects of habitat heterogeneity and dispersal limitation (Condit et al. 2002, Cotennie 2005, Legendre et al. 2005, Crist et al. 2006). Moreover, enhancement of beta-diversity mostly occurs because the increasing intraspecific aggregation increases differences among communities in species composition (e.g. species turnover) but not differences in species richness (Veech 2005). We believe however, that intraspecific aggregation in this study is not due to deterministic factors, such as niche differentiation and competition. It is important to note that there was no high dissimilarities regarding the nutrient content and trophic state among the studied reservoirs (Table 1).

As the evaluated connected systems, communities within the same reservoir (beta 2) do not experience any dispersion limitation, and previous studies have shown that increased connectivity among local habitats can homogenize species composition among localities and simultaneously decrease components of beta-diversity (Harrison 1997, Kneitel & Miller 2003, Diekötter et al. 2007). Lopes et al. (2014) observed that hydrological connectivity affect the zooplankton beta diversity by facilitating dispersal between habitats. This could be explained by the dominance of just a few species at these scales, resulting in the homogenization of the system. Similarly, in the present study the connected systems were dominated by only two species: *Spicaticribra kingstonii* Johansen, Kociolek & Lowe and *Discostella stelligera (*Cleve & Grunow) Houk & Klee, whose relative abundances reached 84.5% in some sample units.

The additive partitioning concerning the seasons (beta 3) showed that beta-diversity in the study area was greater among connected systems. Species turnover occurs not only in space but also in time (e.g. MacArthur & Wilson 1967) and locations with higher temporal turnover will accumulate more species within a given time period than will locations with lower temporal turnover (Chalcraft et al. 2004). In addition, spatial turnover should be driven, in part, by temporal turnover due to the decreased probability of sampling a given species repeatedly when temporal turnover is high (Steiner & Leibold 2004, Stegen et al. 2013).

Also, turnover and nestedness components were higher in non-connected habitats, once beta-diversity was partially out. Although the latter presented a smaller contribution, a high turnover suggests the existence of any barrier or selective differentiation (Baselga 2010).

In metacommunities, the presence of strong nestedness is a clear indication of coupled gradients of site environmental characteristics and species traits (Ulrich et al. 2009, Ulrich & Almeida-Neto 2012). In the same way, Wetzel et al. (2012) found that beta-diversity patterns in phytoplankton and periphyton at the Negro River hydrographical basin was mainly caused by the spatial turnover and a small contribution from nestedness. A high nestedness component of beta diversity was observed only for the true planktonic species, demonstrating the role of different growth forms in the dispersal ability on beta diversity patterns in which dissimilarity is greater in more vagile organisms.

The diatom assemblage captured in the surface sediment reflected the water column assemblage, except for the nestedness component. This component was high only for the surface sediment, indicating that it is largely formed by a subset of species that make up the reservoirs flora, with less evolutionary or ecological differentiation across the basins. The fact that diatom assemblage captured in the surface sediment does not respond exactly in the same way as phytoplankton may be related to the fact that diatoms in this habitat have a cumulative effect through time and space.

Nevertheless, Sørensen dissimilarity index is one of the most used measures due to its dependence on the proportion of species shared between two communities and its linear relationship with Whittaker’s beta (Diserud & Ødegaard 2007, Baselga 2010). It is well known that this measure incorporates both true spatial turnover and differences in richness (Koleff et al. 2003).

Conclusion

The study revealed that hydrological connections were important in the community structuring, suggesting greater dispersal rates among localities in connected environments. For conservation purposes, our results suggest a protection in a large scale, ensuring beta diversity between sites and conserving total diversity.

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Capítulo 4

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Distance decay as a descriptor of the diatom compositional variation in tropical reservoirs

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MARQUARDT et al.: Distance decay in tropical reservoirs

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Abstract

We used the distance decay as a descriptor of the compositional variation in diatom communities' similarity between different habitats (phytoplankton and surface sediment) and seasons (summer and winter, only for phytoplankton) in a geographical gradient of c. 64 km over six reservoirs located at Southeast Brazil. We tested whether the rates of decay in similarity changes with distance in tropical biological communities, and estimated how much dispersal (geographic distance) and niche (habitat association) processes explain variation in the diatom communities. We also evaluate whether the diatom assemblage captured in the surface sediment reflected the water column assemblage events. Distance-decay curves were estimated using linear regressions. Partial Mantel tests were performed to examine significance of the relationships among the biological, environmental and spatial datasets. Similarity decreased significantly with distance between habitats and seasons, but results were not statistically significant for the surface sediment assemblages. All diatom communities were controlled more by limited dispersal than by environmental factors probably as a result of the water-quality and scale of the study area.

INTRODUCTION

The distance decay of similarity, firstly implemented by Tobler (1970), consists of the positive relationship between dispersion limitation and increase of spatial distances between surveyed sites. This pattern can be affected by environmental characteristics (i.e. extent and grain) and characteristics of organisms (i.e. body size and dispersal ability). Recently, it was demonstrated that eutrophication can affect distance–decay relationships by decreasing turnover rates in microorganisms (Vilar *et al.*, 2014).

Although there is no consensus, the general assumptions that supports the distance decay of similarity can be accounted by at least three not exclusive mechanisms: (i) niche-based processes, (ii) spatial configuration and (iii) neutrality (Soininen *et al.*, 2007). The relative role of these processes determines the strength of distance decay in ecological communities and can vary between different ecosystems and organisms (Bahram *et al.*, 2013; Nekola and White, 1999; Soininen *et al.*, 2007).

The distance decay of similarity studies has been applied to a wide range of organisms (e.g. Oliva and González, 2005; Qian *et al.*, 2005; Thompson and Townsend, 2006), geographic gradients (e.g. Astorga *et al.*, 2012; Bahram *et al.*, 2013; Condit *et al.*, 2002), and environments (e.g. Astorga *et al.*, 2012; Bahram *et al.*, 2013). Regarding to tropical environments, most studies are based on plant communities, and the discrimination of the relative importance of environment and niche-based processes (e.g. Costa *et al.*, 2009; Jones *et al.*, 2006; Tuomisto *et al.*, 2003) from spatial configuration and dispersal-based ones (e.g. Andersen *et al.*, 2010; Normand *et al.*, 2006; Vormisto *et al.*, 2004) is still considered a challenge (Andersen *et al.*, 2010; Endara and Jaramillo, 2011; Palmiotto *et al.*, 2004). Moreover, the degree to which the observed patterns can be applied to aquatic communities remains unclear (Heino *et al.*, 2015).

Regardless whether we can evaluate causes, the distance decay of similarity provides a simple descriptor of how biological diversity is distributed and thus has important consequences on understanding ecosystem functioning and management, and biodiversity conservation (Legendre *et al.*, 2005; Nekola and White, 1999). In Brazil, reservoirs have preponderant ecological, economic and social roles since they have multiple uses as supply, irrigation, energy production, recreation, tourism, transport, flood regulation and others (Tundisi, 1999). However, the growing anthropogenic impacts in the last century has led to threats growing to fresh water biodiversity as well as to the largely "unknown" diversity worldwide (Faustino *et al.*, 2016; Strayer and Dudgeon, 2010). Thus, the integrated study of such artificial ecosystems and the management perspective of them have a great importance for the country (Henry and Nogueira, 1999).

Recently, ecological studies have used diatoms as a model system to investigate determinants of species distributions and diversity patterns (Astorga *et al.*, 2012; Bottin *et al.*, 2014; Vilar *et al.*, 2014; Wetzel *et al.*, 2012). In addition, diatom biogeographical patterns have been greatly discussed, supported by the idea that small organisms (less than 1 mm in length) tend to have a cosmopolitan distribution (Fenchel and Finlay, 2004). Currently that view has been questioned with evidence emerging of the importance of spatial patterns (Grenouillet *et al.*, 2008; Heino *et al.*, 2009; Martiny *et al.*, 2006; Wetzel *et al.*, 2012), widespread diatom endemism (Sabbe *et al.*, 2001; Vanormelingen *et al.*, 2008; Vyverman *et al.*, 2010), and historical constraints of global patterns in regional and local diatom diversity (Vyverman *et al.*, 2007).

Because of diatoms fast response to environmental change, they are useful as indicators of stream water quality (Stevenson *et al.*, 1996). Additionally, diatoms (Bacillariophyceae) are good indicators of past limnological conditions because they are usually well preserved in lake sediments, and their remains can be identified at the species level. Hence, they have been widely used to generate transfer functions enabling reconstruction of lakes, pH (Battarbee, 1984), salinity (Fritz, 1990; Fritz *et al.*, 1991), and lake trophic status (Bennion, 1995). As for most other groups of microscopic organisms, however, remarkably little is as yet known about diatom biology, ecology, and the factors driving diatom species diversity and geographic distributions (Chepurnov *et al.*, 2004; Mann, 1999; Vanormelingen *et al.*, 2008).

In this study, we used the distance decay as a descriptor of the compositional variation in diatom community similarity over six reservoirs (Southeastern São Paulo, Brazil) between different habitats (phytoplankton and surface sediment) and season (summer and winter, only for phytoplankton). Our present purposes are: (i) to evaluate whether the rates of decay in similarity changes with distance in tropical biological communities; (ii) to estimate how much dispersal (geographic distance) and niche (habitat association) processes explain variation in the diatom communities, and (iii) to evaluate whether the diatom assemblage captured in the surface sediment reflected the water column assemblage events.

MATERIALS AND METHODS

Study area

The six studied reservoirs are located in southeastern São Paulo State (Brazil), covering a range of latitudes (23°50’35,5”–24°06’33,7”), but they are restricted in longitude (47°09’25.9”–47°44’57.2”) (Fig. 1). The altitude of these reservoirs ranges from 17 to 996 m above sea level with annual mean air temperature of 6–46°C, and rainfall of 150–521.5 mm (Votorantim Energia, personal communication). Cachoeira do França, Cachoeira da Fumaça and Serraria reservoirs form a cascade in the same river while the Juruparà, Salto do Iporanga and Paineiras reservoirs are located in different rivers (Fig. 1). The areas of the reservoirs vary from 0.42 to 12.7 km^2, with water depth of 2.5–35.1 m at the sampled sites. Built between 1912 and 1989, the reservoirs were presently classified from ultraoligotrophic to eutrophic according to the Trophic State Index (TSI) for reservoirs (Lamparelli 2004). They are mainly used for power generation, but also for fishing, fish farming (Juruparà), irrigation and recreation (Votorantim Energia, personal communication). The area protects a representative Atlantic forest area of the Atlantic Plateau Paulista, where it is been recorded a large number of fauna and flora currently endangered and endemic species, besides the new species occurrence records (SMA, 2010).

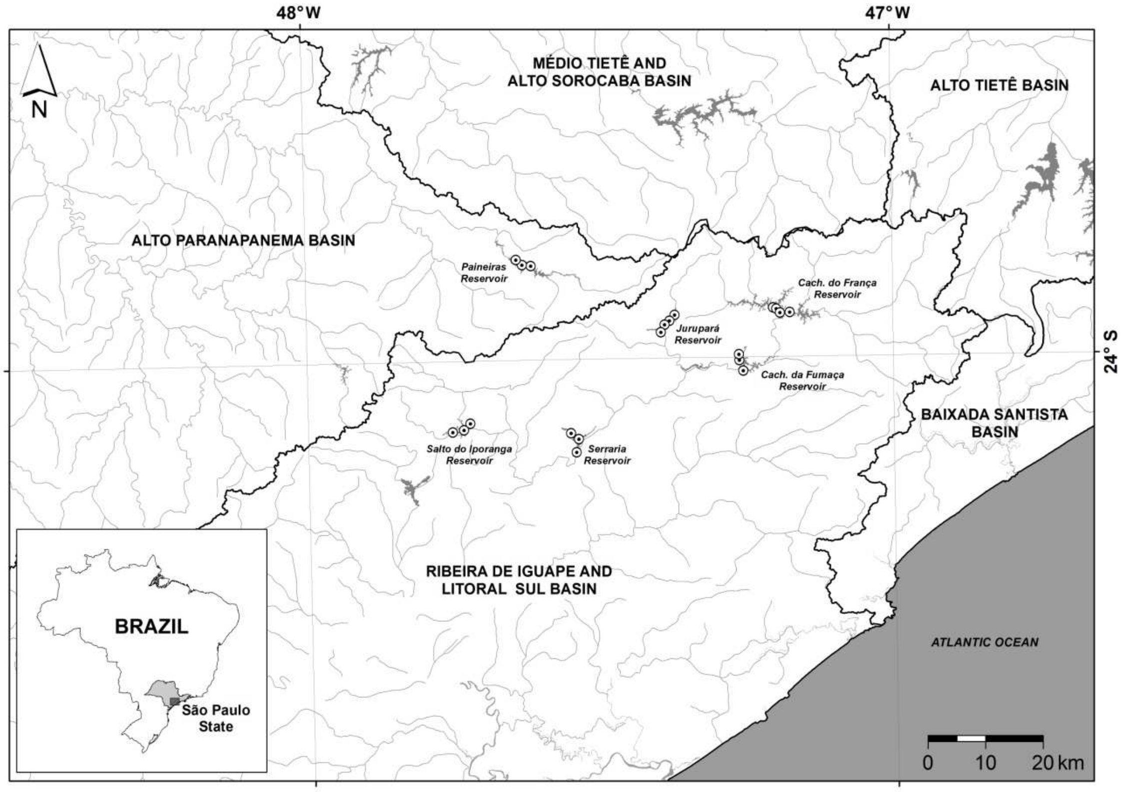

Fig. 1. Study area showing the six reservoirs and sample units.

Environmental variables

Water samples were collected during the austral winter and summer of 2014 with a van Dorn sampler in 20 sampling sites in three different depths along the water column (subsurface, mean depth and 1 m above the sediments). Temperature (°C), pH and electrical conductivity (µS cm^{-1}) were measured in the field at every 50 cm depth with a multiparameter probe (Horiba U-53), whereas water transparency was measured with a Secchi disk. The following water variables were also measured according to Standard Methods (APHA, 2005): alkalinity, free CO_2^-, HCO_3^- and CO_3, ammonium (N-NH_4), nitrate (N-NO_3) and nitrite (N-NO_2), dissolved oxygen (DO), soluble reactive phosphorus (SRP) and total dissolved phosphorus (TDP), soluble reactive silica (SRS), total nitrogen (TN) and total phosphorus (TP). Chlorophyll-a concentration corrected for phaeophytin was measured with 90% ethanol as the organic solvent.

Diatom communities

Phytoplankton diatoms were sampled (winter and summer) with van Dorn sampler, and samples of each vertical profile were incorporated in the laboratory into a sample of equal volume from each sampling station. This procedure was performed in order to represent the planktonic diatom community as a whole along the vertical profile. Sediment diatoms (top 2 cm) were sampled only in winter with a gravity core (UWITEC). Small homogenized aliquots from both sediment and phytoplankton samples were heated to 90°C with concentrated H_2O_2 (30%) and hydrochloric acid (HCl) for at least 4 hr to oxidize the organic matter and clean the frustules (Battarbee *et al.*, 2001). Next, the cleaned material was diluted with deionized water and mounted on permanent slides with Naphrax (IR = 1.74) as mounting medium. Diatoms were counted on a Zeiss microscope (Imager A2) at 1000X magnification under oil immersion. Relative abundances were estimated following the counting technique described by Pappas and Stoermer (1996) with a minimum of 400 valves counted per slide on up to six random transects until reaching an efficiency of at least 90% besides the species rarefaction curve (Bicudo, 1990).

Data analysis

The similarity of diatom assemblages among sites was summarized in three matrices, one for the phytoplanktonic diatom community in each season and one for surface sediment diatom community. All species were included in subsequent analyses. Within each group, we calculated assemblage similarity among all site pairs using the Bray–Curtis coefficient. Bray–Curtis is appropriate for the analysis of abundance and presence/absence information of species because it ignores variables that have zeros for both objects (joint absences; Endara and Jaramillo, 2010). Subsequently, we calculated a distance matrix for limnological variables, using the Gower metric, which is appropriate for heterogeneous data since it uses range normalization.

Finally, a third matrix with spatial (geographic) distances between sampling points was computed using the Vicenty (1975) algorithm. The relationship between floristic, limnological and geographical distances was explored using regression analysis, testing linear, exponential, logarithmic, quadratic and Gaussian functions, and the best-fit model was chosen based on R^2 and AIC values.

Statistical analyzes were carried out with PAST Version 3.14 (Hammer *et al.*, 2001).

Distance decay can be expressed as a rate equivalent to the slope of the linear regression between community similarity and either environmental distance (reflecting niche-mediated processes) or geographical distance (reflecting dispersal mediated processes; Astorga *et al.*, 2012; Martiny *et al.*, 2006; Tuomisto *et al.*, 2012; Vilar *et al.*, 2014). The latter also provides a measurement of spatial autocorrelation (Shurin *et al.*, 2009) although the slope of the distance–decay relationship alone is a poor indicator of species spatial turnover (or β-diversity) and total species richness in a landscape (or γ-diversity) (Morlon *et al.*, 2008).

RESULTS

Diatom Composition and Diversity

Within the phytoplankton assemblages, 186 taxa were identified during summer and 125 during the winter surveys, whereas 333 taxa were identified in the surface sediment samples. The most species-rich genera were *Aulacoseira* Thwaites, *Achnanthidium* Kützing and *Discostella* Houk and Klee.

The species-abundance distribution was characterized by a high percentage of singletons (i.e., species represented by one individual) and a few dominant species. The dominant species were *Discostella stelligera* (Cleve and Grunow) Houk and Klee, *Spicaticribra kingstonii* Johansen, Kociolek and Lowe and *Aulacoseira tenella* (Nygaard) Simonsen present in all 20 sites sampled, often with abundances above 50%.

Distance-Decay Relationships

Limnological variables and chlorophyll-a values measured during the study period at the six reservoirs are summarized in Tab. 1.

Tab. 1. Means (and standard deviation) of abiotic variables in the six studied reservoirs. The other variables were eliminated because of collinearity. Abbreviations: Sec (Secchi depth; m), Temp (Temperature; ºC), Cond (Conductivity; µS cm^{-1}), Alk (Alkalinity; mEq L^{-1}), DO (Dissolved oxygen; mg L^{-1}), TN (Total nitrogen; µg L^{-1}), TP (Total phosphorus; µg L^{-1}), SRS (Soluble reactive silica; mg L^{-1}), Chlo (Chlorophyll-a; µg L^{-1}). FR (Cachoeira do França), FU (Cachoeira da Fumaça), SE (Serraria), JP (Jurupará), SI (Salto do Iporanga), PI (Paineiras). Numbers refer to the sample units.

	Sec	**Temp**	**pH**	**Cond**	**Alk**	**DO**	**TN**	**TP**	**SRS**	**Chlo**
FR1	1.7±8.5	23.1±6.8	7.3±0.9	24.5±0	0.2±0	7.5±0	151.8±162.9	5.5±2.2	2.4±1.3	5.4±4.8
FR2	1.9±0.	24.2±8.1	7.3±1.3	24±0	0.2±0	7.43±0.9	191.1±45	10.0±7.5	2.39±1.2	4.12±2
FR3	1.7±0	24.1±8.3	7.9±0.6	23.5±2.1	0.2±0	7.3±1.6	231.2±21.2	6.6±3.7	2.3±1.4	20.2±26.5
FR4	1.7±0.1	24.0±7.9	8.6±0.6	24.5±3.5	0.2±0	8.2±1.6	280.0±153.7	6.8±2.5	2.2±1.3	22.8±29.7
FU1	2.6±1.7	24.8±7.3	6.5±0	26.5±0.7	0.2±0	6.9±2.2	228.6±13.7	5.1±1.6	2.5±1	1.7±0.4

FU2	2.8±1.5	24.3±7	6.8±0.7	26.0±2.8	0.2±0	8.3±0.5	176.5±66.1	8.4±2.1	2.4±1	3.4±1.1
FU3	2.1±0.3	24.8±6.4	6.6±0	26.0±1.4	0.2±0	7.5±0.1	180.1±71.7	5.8±2.7	2.3±1	3.3±2.3
SE1	4.0±1.8	26.3±7.2	7.4±1.1	21.0±9.9	0.2±0	8.3±1.4	180.3±64.1	8.1±2.9	4.4±1.2	1.8±0.7
SE2	3.7±1.4	26.2±7	7.3±0.6	25.0±2.8	0.2±0	7.9±0.4	174.7±80	8.0±1.5	3.9±1.5	4.6±2.2
SE3	4.2±1.4	25.6±6.2	6.6±0.2	28.5±2.1	0.2±0	7.5±1.2	216.0±147.7	8.5±3.7	3.6±1.5	7.6±0.7
JP1	1.5±0.8	24.1±6.7	6.4±1.3	24.0±2.8	0.2±0	7.8±0.8	464.3±89.4	19.5±4.2	3.9±0.3	16.8±1.8
JP2	1.9±0.4	24.6±6.5	6.6±0.1	23.5±3.5	0.2±0	6.7±1.3	351.4±201.4	16.6±0.3	3.8±0.3	10.1±0.7
JP3	2.2±0.2	25.4±6.9	6.6±0.1	24.0±2.8	0.2±0	7.5±0.3	453.3±8.2	13.5±0.7	3.7±0.3	7.1±1.2
JP4	2.1±0.4	25.0±7.1	6.8±0.2	23.5±3.5	0.2±0	7.1±1.1	434.1±103.8	13.3±1.9	4.0±0	6.6±3.7
SI1	1.5±0.2	24.0±2.6	8.0±2.1	50.0±9.9	0.3±0	6.7±1.9	415.9±143.2	32.1±3.7	5.1±1.6	36.7±37.6
SI2	1.9±0.4	23.7±2.7	7.8±2	43.5±7.8	0.3±0	6.2±2.9	315.9±28.4	23.1±1.5	4.9±0.7	17.4±15.3
SI3	1.7±0.6	23.6±2.7	7.9±2.1	43.0±11.3	0.3±0	7.4±2.2	304.4±22.4	25.6±3.8	4.1±1.5	36.8±44
PI1	0.7±0.7	22.5±6.4	6.7±0.4	39.0±2.8	0.3±0	6.6±0.3	310.8±119.3	20.3±0.3	4.6±0.4	9.8±5.7
PI2	1.3±0.5	22.7±6.3	6.9±0.6	38.5±2.1	0.3±0	6.3±0.2	258.4±258.4	16.2±0.4	4.6±0.8	6.9±2.7
PI3	1.3±0.3	23.1±6.1	7.1±0.5	38.0±2.8	0.3±0	6.4±0.3	289.4±218.8	16.3±2	4.5±0.8	4.8±3.1

In all data sets studied, the similarity among plots declined with increasing distance between them (Fig. 2). Regression slopes between environmental vs. geographical distance and also between similarity vs. environmental distance were statistically significant for phytoplankton in both seasons (summer and winter: $P < 0.001$) and surface sediment ($P < 0.001$) (Fig. 2). For community similarity vs. geographical distance relationships, turnover showed significant differences between phytoplankton (summer: $P < 0.0006$, winter: $P < 0.0001$) and surface sediment diatom communities ($P < 0.1033$) (Tab. 2). There were differences in initial similarity (i.e. the similarity at 1 km distance) among habitats. Whereas phytoplanktonic communities showed a high initial similarity, the surface sediment showed a low initial similarity vs. geographic distance.

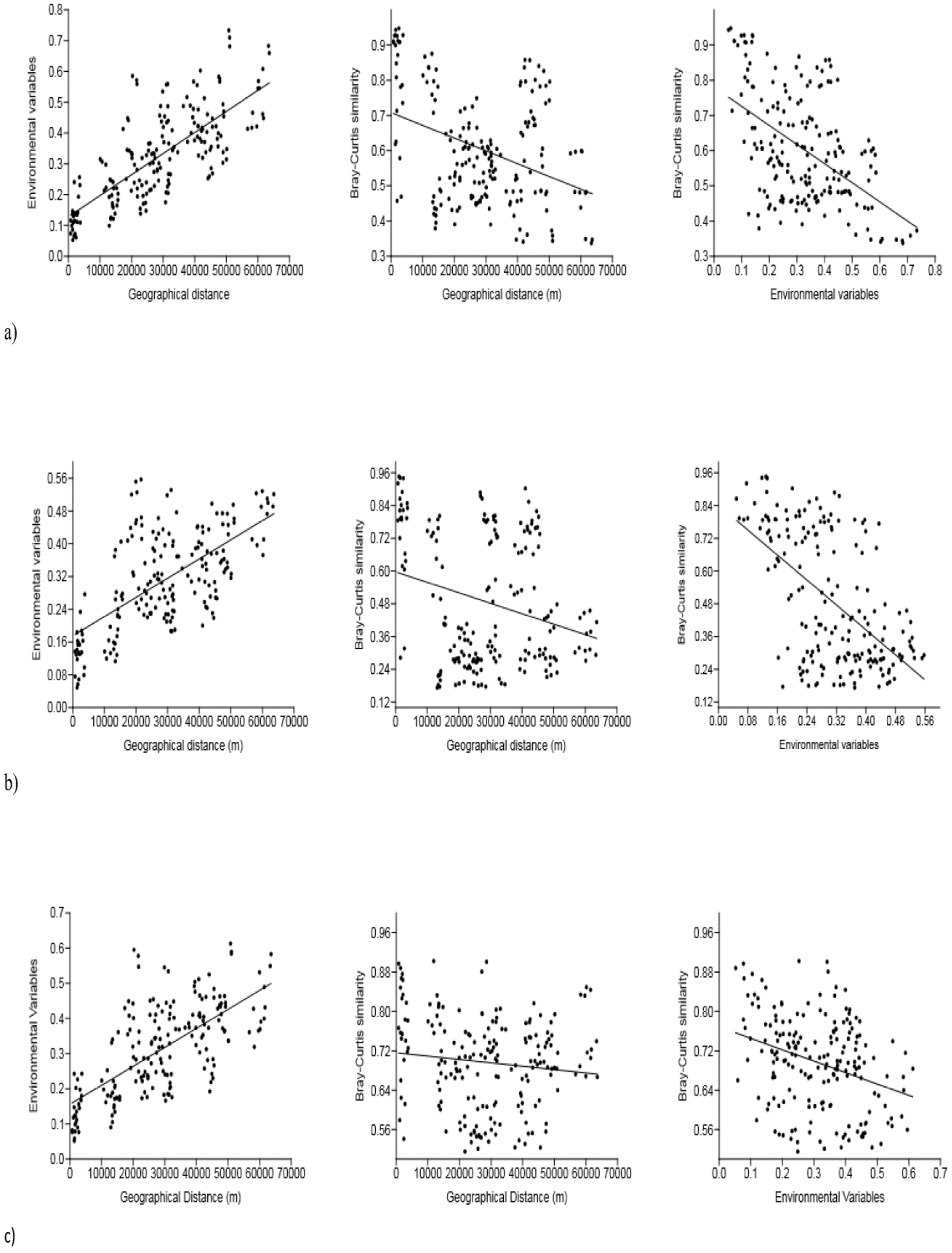

Fig. 2. Relationship between environmental distance (Gower) vs. geographical distance (m), geographical distance vs. community similarity (Bray–Curtis) and community similarity vs. environmental distance and for diatom communities during the (a) winter, (b) summer and (c) surface sediment diatom communities.

Tab. 2. Regression statistics for the decay of similarity with distance for environmental distance vs. community similarity, environmental distance vs. geographical distance and geographical distance vs. community similarity relationships for phytoplanktonic (summer and winter) and surface sediment diatom data sets.

	Environmental distance + Community similarity			
	r^2	***P***	**Intercept**	**Slope**
Summer	0.32865	0.0001	0.84117	-1.1425
Winter	0.26415	0.0001	0.78018	-0.54295
Surface sediment	0.10404	0.0001	0.76859	-2.32E-01
	Environmental distance + Geographical distance			
Summer	0.4124	0.0001	0.17509	4.69E-06
Winter	0.56223	0.0001	0.12826	6.84E-06
Surface sediment	0.45889	0.0001	0.15608	5.39E-06
	Geographical distance + Community similarity			
Summer	0.069257	0.0006	0.59698	-3.83E-06
Winter	0.14219	0.0001	0.70823	-3.63E-06
Surface sediment	0.014066	0.1033	0.71585	-6.79E-07

The highest rate of environmental turnover (measured as the slope of the regressions) was observed for the phytoplankton during the summer, whereas the highest rate of spatial turnover was observed for the surface sediment, but this relationship was not significant.

However, Mantel tests confirmed the variation in compositional dissimilarity is consistently much better explained by geographic distance than by environmental distance for phytoplankton (summer: $P < 0.005599$, winter: $P < 0.0009998$) but not for the surface sediment (Tab. 3). These results are in accordance to dispersal theory. It is important to note that environmental variables were not correlated to geographic distances.

Tab. 3. Partial Mantel correlations (*r*) and *P*-values between community similarity (Bray–Curtis) and environmental distance controlling for geographical distance and vice versa, for phytoplanktonic (summer and winter) and surface sediment diatom communities.

	Environmental + Community		**Environmental + Geographical distance**		**Geographical distance + Community**	
	r	***P***	***r***	***P***	***r***	***P***
Summer	0.02022	0.2803	0.1018	0.1598	0.2632	0.005599
Winter	0.1501	0.12	0.1785	0.03819	0.3771	0.0009998
Surface sediment	0.1647	0.1418	0.1594	0.04739	0.1186	0.1102

DISCUSSION

Traditionally, microbial biogeographical patterns were assumed to follow the Baas-Becking hypothesis, which predicts that ‘everything is everywhere but, the environment selects’ (Baas-Becking, 1934), that is, microorganisms are not dispersal limited and the resulting microbial community is shaped by the local environment (Hazard *et al.*, 2013). Indeed, studies on diatoms have long focused on local environmental factors, since the distribution of diatom species has been considered cosmopolitan. However, the effect of regional factors on the structure of local communities (e.g. Hubbell, 2001) is now acknowledged and the role of historical, biogeographical, and evolutionary aspects were underlined (Mann, 1999; Soininen *et al.*, 2004). Our results agree with recent studies where communities were strongly spatially structured suggesting some degree of dispersal limitation. This view support not only local niche-based processes, but also neutrality (Soininen *et al.*, 2007). Because of dispersal limitation, there is a gradual turnover in regional species pools between distant areas. Adding to this, biotic communities in reservoirs and other lentic habitats may be more spatially structured because of the lesser degree of exchange of settlers between localities.

The study of the relationships between floristic, geographic and environmental distances is a classic paradigm of ecology (Hubbell, 2001); although its application in microbial communities is scarce. Linear regressions provide a direct estimate of the rate of distance decay and the rate of composition change through space (Nekola and White, 1999). A progressive distance-decay in floristic similarity generally indicates a limitation in the dispersive capacity of the species (e.g. Costa *et al.*, 2009; Tuomisto *et al.*, 2003). This limitation is especially intense in complex and fragmented habitats (Eiserhardt *et al.*, 2011), and is observed mainly at short distances (Costa *et al.*, 2009; Endara & Jaramillo 2011; Normand *et al.*, 2006).

Specifically for diatoms, Wetzel *et al.* (2012) provided evidences of high species turnover and biogeographical patterns in Neotropical communities in which the highest initial similarity (i.e., lowest small-scale beta diversity) for true planktonic species (intercept: 0.78) with the expectation of high dispersal abilities (as assumed for true planktonic algae) tend to homogenize communities and to reduce beta diversity in small spatial scales. High initial similarity was also presently observed for the phytoplanktonic communities. In the case of the surface sediment, it may be related to its degree of temporal integration, thus providing a longer interval for the immigration, emigration and colonization of taxa than do water-column samples, assemblages within surface sediment would be more strongly explained by environmental gradients (Winegardner *et al.*, 2015). Also, lower initial similarities are generally related to stronger environmental gradients (Soininen *et al.*, 2007).

A positive relationship between floristic and geographical distances, and the lack of correlation between these and the environmental similarity, confirm that there are effective dispersive limitations in the study area. Within reservoir plankton communities, species migration may have a significant impact on local floristic composition, which is influenced by the ability to disperse exhibited by different species. In our study, although many species currently identified have characteristics of benthic habit, no statistically significant difference was observed when compared to diatoms with a characteristic of phytoplanktonic habit. For the surface sediment, the distribution pattern of diatom abundance in the sediment samples was similar to the water column. However, the sediment record not necessarily reflected the water column characteristics (not statistically significant results). Diatoms from the surface sediment usually represent 1-2 years of deposition (Smol, 2008). As a result, the community does not respond to the same conditions from the moment that phytoplankton communities were sampled. Moreover, diatom communities for both habitats were characterized by a small number of dominant (relative abundance > 50%) and a large number of rare species. Rarer taxa may be more susceptible to dispersal limitation, because the number of chances for a propagule to travel a long distance and establish a new population is reduced (Martiny *et al.*, 2011).

Besides this, the amplitude of environmental gradients has been shown to be an important factor in determining the relative contributions of environmental and geographical components to community structure (Costa *et al.*, 2008). As the degree of eutrophication decreases, environmental factors became less important in explaining changes in diatom turnover while spatial factors imposed a more prominent signature on community composition (Vilar *et al.*, 2014). The limnological characteristics of the reservoirs indicate low anthropogenic impact conditions. Also, the low nutrient concentrations in its dissolved forms are characteristic of oligotrophic and mesotrophic environments, as well as nitrogen and total phosphorus values. This finding is supported by the presence of species such as *A. tenella*, typically associated with oligotrophic and oligo-mesotrophic Brazilian reservoirs (Bicudo *et al.*, 2016), *S. kingstonii*, recorded in environments characterized by high temperatures and low conductivity (Ludwig *et al.*, 2008), and *D. stelligera* widely recorded in oligotrophic environments in São Paulo State reservoirs (AcquaSed Project data). Changes in abundance of the latter species have been recently associated to warming across lakes within regions of North America (Boeff *et al.*, 2016).

Finally, studies involving fungal communities showed that the distance from the equator and the sampling area were the main determinants of the extent of distance decay, and the rate of distance decay was greater in tropical compared to non-tropical ecosystems (Bahram *et al.*, 2013). Tropical countries have more species of most taxa than temperate ones, and small areas in the tropics have a smaller multiple of the number of species than small temperate areas. Where many species are present, abundances tend to be more equal and geographic distributions more spotty (MacArthur, 1969).

CONCLUSIONS

The findings of the present study support that the decay of community similarity should be particularly strong at short spatial distances, determining patterns in diatom metacommunities from the studied reservoirs. Diatoms on surface sediment respond similarly to the results observed in water column, but results were not statistically significant. This is probably due to the integrative character of the sediment, which accumulates 1 to 2 years of reservoir events rather than a single season. Also, processes in nature are scale-dependent and dispersal limitation may be more important at small scales (< 100 km). Besides this, the non-impacted study-area may be considered an important factor in determining the relative contributions geographical components to the diatom community structure.

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Capítulo 5

A ser submetido à publicação em **Biotropica**.

Weighted averaging of species indicator values in tropical reservoirs: the influence of rare taxa richness

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ABSTRACT

We compared planktonic diatom data sets with different rarity categories to verify their responses in the weighted average (WA) approach. To do so, forty diatom-training sets were developed for six reservoirs located at two different basins in Southwest São Paulo, Brazil. A total of 339 diatom taxa were identified and different models were constructed based on their relative abundances (≥1%, ≥2%, ≥5%), occurrence frequencies (≥1%, ≥2%, ≥5%) besides the data set with no species deletions (all species). Furthermore, optimum and tolerance of each taxon were estimated with the WA formula (Zelinka-Marvan weighted averages) according to their abundance in the samples and the pH in the data sets used to infer the environmental conditions based on the sample taxonomic composition. We repeated the procedure first with the complete dataset and then downweighting taxa according to their rarity categories. The predicted pH and measured pH values were compared via regression analysis. The effects of species deletions on the predictive ability of the different models were estimated in terms of the coefficient of determination values (r^2) of the response curves. Manipulation of the different data sets significantly influenced the performance of the WA models. Contrary to expectations, by taking into account the r^2 values, downweighting rare taxa affected negatively the transference functions. However, deleting non-abundant taxa (for relative abundance ≥1% and occurrence frequency ≥2%, ≥5%) had a positive effect on *p* values, providing a robust reconstructive relationship. The work contributes to a better understanding of diatom ecology especially diatoms from tropical reservoirs, supporting the development of a diatom biological monitoring protocol for the study area.

Keywords biological indicator**,** Gaussian curves**,** optimum, tolerance, pH

Introduction

Freshwater ecosystems support unique and complex ecological communities and have a critical role as a human resource. For this reason, ecologists are often asked to assess or monitor the "health", "status" or "condition" of these ecosystems (Bailey et al., 2004).

The application of algae in the environmental assessment of aquatic habitats, particularly lakes and streams, has been well-documented (Dixit and Smol, 1994; Stevenson et al., 1999; Weilhoefer and Pan, 2006). Among algae, diatoms have been widely used in water quality monitoring worldwide over the past 50 years (Round, 1991). Their short life cycle, in conjunction with the narrow ecological tolerances of many taxa, makes them useful water condition indicators (Dixit et al., 1992; Charles and Smol, 1994). In addition, their siliceous frustules are preserved in sediments and can provide information about past environments (Moser et al., 1996; Smol and Glew, 1992).

Indices of water quality using diatoms are considered to provide more precise data compared to chemical and zoological assessment methods (Leclercq, 1988; Omar 2010). However, the choice of a bioindicator group must satisfy certain criteria, such as that each taxon can be identified without equivocation (Céspedes-Vargas et al., 2016; Cox, 1991). Generally, diatom indicators are calculated based on a weighted-averaging (WA) model using relative abundances of all taxa in a sample of a site and the autecological parameters of the taxa (Stevenson et al., 1999). These parameters can be used to predict values of any given environmental variable based on species composition, simply by averaging the indicator values of species that are present (Ellenberg, 1979; Ter Braak and Looman, 1986).

However, whether the goodness of WA -based estimations depends on (1) the shape of the response curves, (2) the definition of each indicator value, and (3) the distribution of the indicator values along the environmental variable (Ter Braak et al., 1986). Nevertheless, the WA approach is considered mathematically very simple and easy to understand. Noteworthy, it has been shown to be equal to or better than other commonly applied methods and is routinely used in environmental paleolimnological reconstructions (Hämäläinen, 2000). For example, for rare species (species with low maximum probability of occurrence and/or narrow tolerance), WA demonstrated to be nearly as efficient as Gaussian logistic regression (GLR, a form of the generalized linear model which fits a

Gaussian-like species response curve to presence-absence data) in most situations (Ter Braak et al., 1986).

Rare species represent the vast majority of the species in an assemblage (Alahuhta et al., 2014; Gaston, 1994; Gillet et al., 2011; Hessen and Walseng, 2008; Kunin and Gaston, 1997; Marchant et al., 1997; Mouillot et al., 2013). However, they are frequently neglected in statistical analyses by setting relative abundance or occurrence criteria prior to the application of a species-based transfer function because their optimal may be uncertain (Bellen et al., 2017). Recently, studies linking rarity to bioassessment have proved that the number of diatoms in some rarity categories can be useful as an indicator of human disturbance in streams and rivers, especially in mountain ecoregions (e.g. Gillet et al., 2011; Potapova and Charles, 2004). However, little has been published addressing tropical environments.

In Brazil, most studies on diatoms as a bioassessment tool have been developed in the southern region of the country (e.g. Düpont et al., 2007; Hermany et al., 2006; Lobo et al., 2004a, 2004b, 2004c, 2004d; Lobo et al., 2006; Salomoni et al., 2005; Torgan and Aguiar, 1974). Most of these works have been carried out in lotic systems with few studies concentrating on reservoirs. In São Paulo State, the WA regression and calibration of benthic diatom assemblages was applied by Bere and Tundisi (2010) to assess the importance of conductivity and pH in structuring benthic diatom communities in streams influenced by urban pollution (São Carlos city, São Paulo State, Brazil). Specifically for reservoirs, diatom research is mostly linked to the AcquaSed project (Base line diagnosis and reconstruction of anthropogenic impacts in the Guarapiranga Reservoir, focusing on the sustainability in water supply and water quality management in reservoirs of the Alto Tietê and surrounding basins, e.g. Zorzal-Almeida et al., 2017), which aims to further create an inferential model based on the quantitative distribution of diatom species in water and recent sediments. Brazilian reservoirs have a predominant ecological, economic and social role; in this regard, the integrated studies of such artificial ecosystems, as well as the perspective of their management are of great importance (Henry and Nogueira, 1999).

In this study, we compared diatom datasets with different rarity categories in terms of relative abundances and occurrence frequencies, to verify the performance of a WA calibration of water pH. The pH concentration is considered a limiting factor for the colonization of aquatic ecosystems by different organisms (Esteves, 1988). This environmental variable is often a major factor influencing species composition of diatom assemblages in freshwater (Battarbee, 1980; Findlay and Shearer, 1992; Round, 1964). In addition, it has demonstrated a strong relationship with diatom distributions (e.g. Birks et al., 1990; Dixit et al., 1992; Weckström et al., 1997). For this, we used a training set composed of forty phytoplanctonic diatom samples from six reservoirs located at the Southwest São Paulo (Brazil). The correlation (r^2) values of the observed-expected values resulting in the different models tested were used to evaluate their relative precision. We expect that, by downweighting rare taxa in the WA formula (Zelinka-Marvan weighted averages), more accurate models can be obtained. Since the choice of various cutoff criteria may affect the predictive abilities of different models (Wilson et al., 1996), we hypothesize that, by downweighting taxa according to their rarity categories, an approximately proportional increase in WA model performance (e.g. increases in r^2) can be obtained. The underlying assumption is that WA models are unable to characterize the optima and tolerances for taxa with low occurences, and that overall model performance may be improved by overriding them (Payne et al., 2006).

Material and Methods

Study area and field work

The six studied reservoirs are located in two different basins: Ribeira do Iguape/Litoral Sul and Alto Paranapanema. We selected the trophic state index (TSI) of Lamparelli (2004), based on Chlorophyll-*a* (Chlo-*a*) and total phosphorous (TP) values as a quantitative measure of reservoirs trophic state. According to the TSI and currently measurements, the reservoirs were considered mostly oligotrophic and mesotrophic (Table 1). Phytoplankton and water were sampled during austral summer and winter in 2014 with a van Dorn water sampler along a vertical profile from 20 sampling sites distributed along the reservoirs. The pH environmental parameter data for the training set was measured in the field concurrently with the phytoplankton sampling with a multiparameter probe (Horiba U-53). This environmental parameter showed a strong significant correlation during stepwise selection in a CCA of diatom assemblages with environmental variables in the study area (Marquardt et al. unpublished manuscript) and was considered a relevant variable from our dataset, with a relatively long gradient. TP analysis followed Standard Methods (APHA, 2005). Chl-*a*, corrected for phaeophytin, was extracted using 90% ethanol (Sartory and Grobbelaar, 1984) (Table 1). Details of the study area and other information of the limnological variables are given by Marquardt et al. “…”).

Table 1. Means and standard deviation of abiotic variables in the six studied reservoirs. Abbreviations: Sec (Secchi depth), pH, TP (Total phosphorus), Chlo (Chlorophyll-a), TSI (Trophic State Index). FR (Cachoeira do França), FU (Cachoeira da Fumaça), SE (Serraria), JP (Jurupará), SI (Salto do Iporanga), PI (Paineiras). Numbers refer to sample units.

	Sec	pH	TP	Chlo	TSI (Anual Mean)
FR1	1.7±8.5	7.3±0.9	5.5±2.2	5.4±4.8	Oligotrophic
FR2	1.9±0.2	7.3±1.3	10.0±7.5	4.12±2	Oligotrophic
FR3	1.7±0.005	7.9±0.6	6.6±3.7	20.2±26.5	Oligotrophic
FR4	1.7±0.1	8.6±0.6	6.8±2.5	22.8±29.7	Oligotrophic
FU1	2.6±1.7	6.5±0	5.1±1.6	1.7±0.4	Ultraoligotrophic
FU2	2.8±1.5	6.8±0.7	8.4±2.1	3.4±1.1	Oligotrophic
FU3	2.1±0.3	6.6±0.4	5.8±2.7	3.3±2.3	Oligotrophic
SE1	4.0±1.8	7.4±1.1	8.1±2.9	1.8±0.7	Oligotrophic
SE2	3.7±1.4	7.3±0.6	8.0±1.5	4.6±2.2	Oligotrophic
SE3	4.2±1.4	6.6±0.2	8.5±3.7	7.6±0.7	Oligotrophic
JP1	1.5±0.8	6.4±1.3	19.5±4.2	16.8±1.8	Mesotrophic
JP2	1.9±0.4	6.6±0.1	16.6±0.3	10.1±0.7	Mesotrophic
JP3	2.2±0.2	6.6±0.1	13.5±0.7	7.1±1.2	Mesotrophic
JP4	2.1±0.4	6.8±0.2	13.3±1.9	6.6±3.7	Oligotrophic
SI1	1.5±0.2	8.0±2.1	32.1±3.7	36.7±37.6	Mesotrophic
SI2	1.9±0.4	7.8±2	23.1±1.5	17.4±15.3	Mesotrophic
SI3	1.7±0.6	7.9±2.1	25.6±3.8	36.8±44	Mesotrophic
PI1	0.7±0.7	6.7±0.4	20.3±0.3	9.8±5.7	Mesotrophic
PI2	1.3±0.5	6.9±0.6	16.2±0.4	6.9±2.7	Mesotrophic
PI3	1.3±0.3	7.1±0.5	16.3±2	4.8±3.1	Oligotrophic

Diatom sample preparation and analysis

Diatom samples were prepared according to Battarbee et al. (2001). The procedure involved hot digestion using hydrogen peroxide (H_2O_2) and HCl (37%). Through a series of dilutions, the peroxide and acid were removed. Subsequently, the samples were dried onto cover glass, mounted in Naphrax ™ (R.I. = 1.74) and examined with a Zeiss Axio Imager A2 light microscope equipped with DIC and a digital camera model AxioCamMR5. A total of 400 diatom valves were counted along random transects at 1000× magnification (Battarbee, 1986) and a minimum sampling efficiency of 90% (Pappas and Stoermer, 1996). Species abundances were calculated and expressed as a percentage of the total diatom counts in each sample. Taxa were identified to the lowest taxonomic level possible based on diatom checklists, specific manuscripts, iconographs (e.g. Krammer, 2000; Lange-Bertalot et al., 2011; Metzeltin et al., 2005) and the on-line catalogue of valid names (site of California Academy of Sciences, 2011). Frequent meetings and discussions including invited diatom taxonomy experts enabled a high level of agreement in diatom identification.

Weighted averaging regression and calibration

Diatom taxa were derived from data collected as part of the AcquaSed project, based upon 40 samples, referred here as training set.

In order to assess the effect of the exclusion of rare taxa on the WA models, we developed several rarity aggregation scenarios ranging from the complete data set (all species), in which there was no deletion criterion, to three rarity categories established according to the relative abundances (≥1%, ≥2% or ≥5% of the whole dataset) and three categories established according to their occurrence frequencies (present in ≥1%, ≥2% or ≥5% of the samples).

Subsequently, optimal values and species tolerances for pH were calculated using the WA approach based on Gaussian response curves of taxa (Ter Brack and van Dam, 1989), in which the value of each environmental variable is weighted based on their abundance in samples and the pH in the dataset (Lepš and Šmilauer, 2003) (regression step). Although alternative unimodal response curves could be fitted, a Gaussian model represents a compromise between ecological realism and simplicity (Ter Braak and van Dam, 1989, Holden et al., 2008).

In order to facilitate more direct comparisons of the taxa tolerances to the reservoir pH, tolerance ranges of the taxa were reescaled within a 0-1 range, these values representing the broadest and narrowest tolerances, respectively, observed within the dataset:

$$\mathrm{S_{tol}} = \mathrm{x} - \mathrm{min}/\mathrm{max} - \mathrm{min}$$

Then, the computed taxon autecological parameters were used to back-calculate pH values based on the taxonomic composition of the sample using the Zelinka-Marvan WA formula (for each sampling station; calibration step):

$$\mathrm{pH} = \sum \mathrm{A.S.V} \Big/ \sum \mathrm{A.V}$$

where the index pH value corresponds to the mean value of the optimum (S) weighted by amplitude (A) and ecological tolerance (S_{tol}).

We repeated the procedure first with the complete dataset and then downweighting taxa according to their rarity categories (see above). Once we had all the predicted pH values, we compared them with the measured pH values via regression analysis. The effects of species deletions on the predictive abilility of the different models were assessed in terms of squared correlation (r^2) values of the observed-expected values.

All analyses were implemented with PAST Version 3.14 (Hammer et al., 2001). Results are given in table 2.

Table 2. Comparative performance among predictive models data sets based on relative abundance and occurrence frequency produced by regression analysis of the predicted and measured pH values.

		Relative abundance			Occurrence frequency		
Correlation	Exp "All species"	Exp >1%	Exp > 2%	Exp > 5%	Exp>1%	Exp>2%	Exp>5%
r:	0.35	0.29	0.23	0.24	0.19	0.28	0.28
r2:	0.12	0.08	0.05	0.06	0.03	0.08	0.08
t:	2.37	1.87	1.50	1.59	1.19	1.84	1.83
p (uncorr.):	0.02	0.06	0.13	0.11	0.23	0.07	0.07
Permutation p:	0.02	0.06	0.13	0.11	0.23	0.07	0.07

Results and discussion

Composition of diatom assemblages

In total, we identified 339 species representing 51 genera. Samples were dominated by centric taxa, occurring in almost all the sites sampled, especially *Discostella stelligera* (Cleve and Grunow) Houk and Klee, *Aulacoseira tenella* (Nygaard) Simonsen and *Spicaticribra kingstonii* J.R.Johansen, Kociolek and R.L.Lowe. Within the study area, their abundance were correlated with the Secchi vector, being considered as indicators of oligotrophic conditions (Marquardt et al., unpublished manuscript).

Species response curves

Most sites were acid to slightly alkaline, with pH values ranging between 5.2 and 7.8 (see table 1). We found that most diatoms exhibited symmetrical, unimodal (bell-shaped) response curves against this variable (Fig. 1), thus making WA a reliable model to infer pH values.

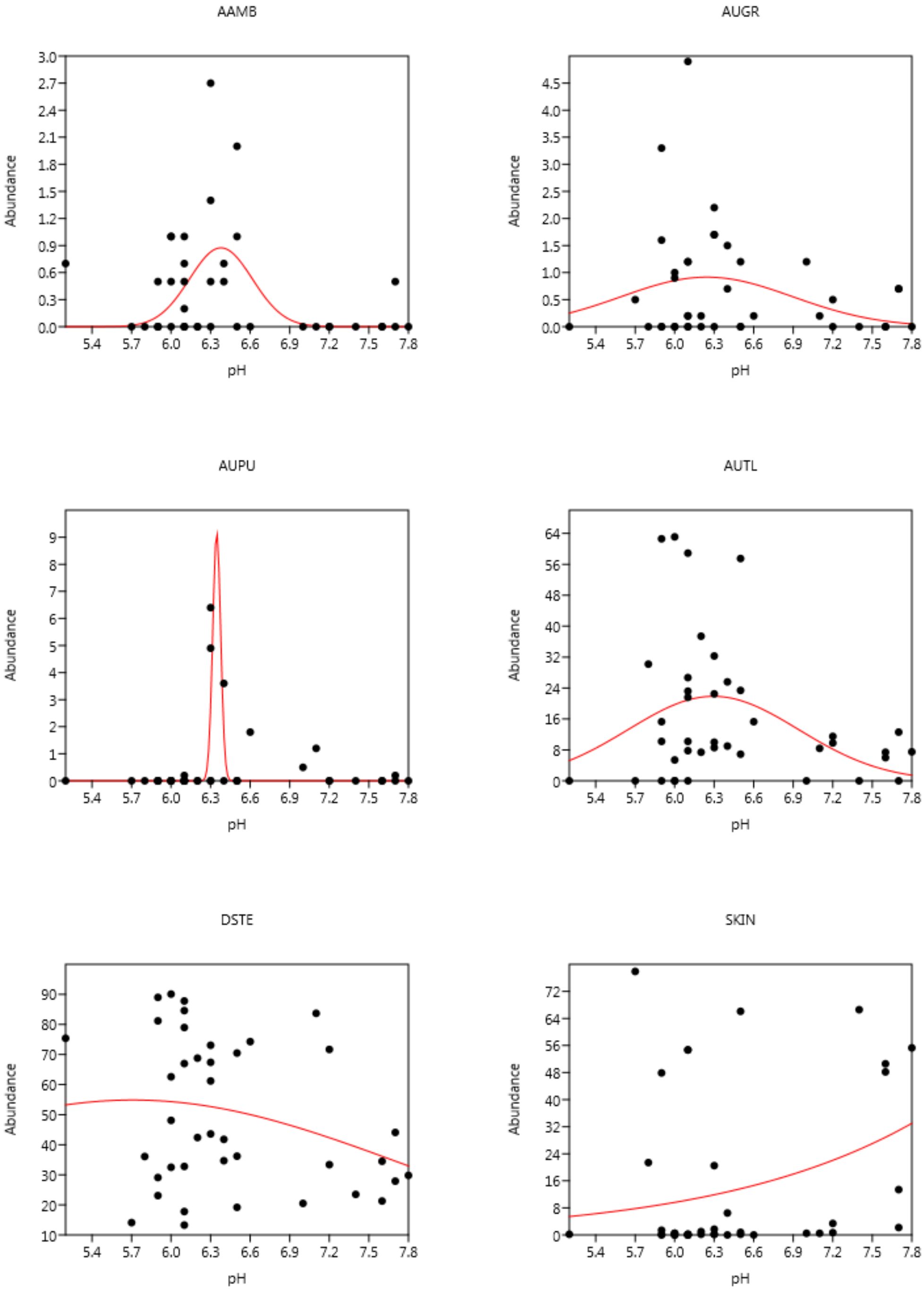
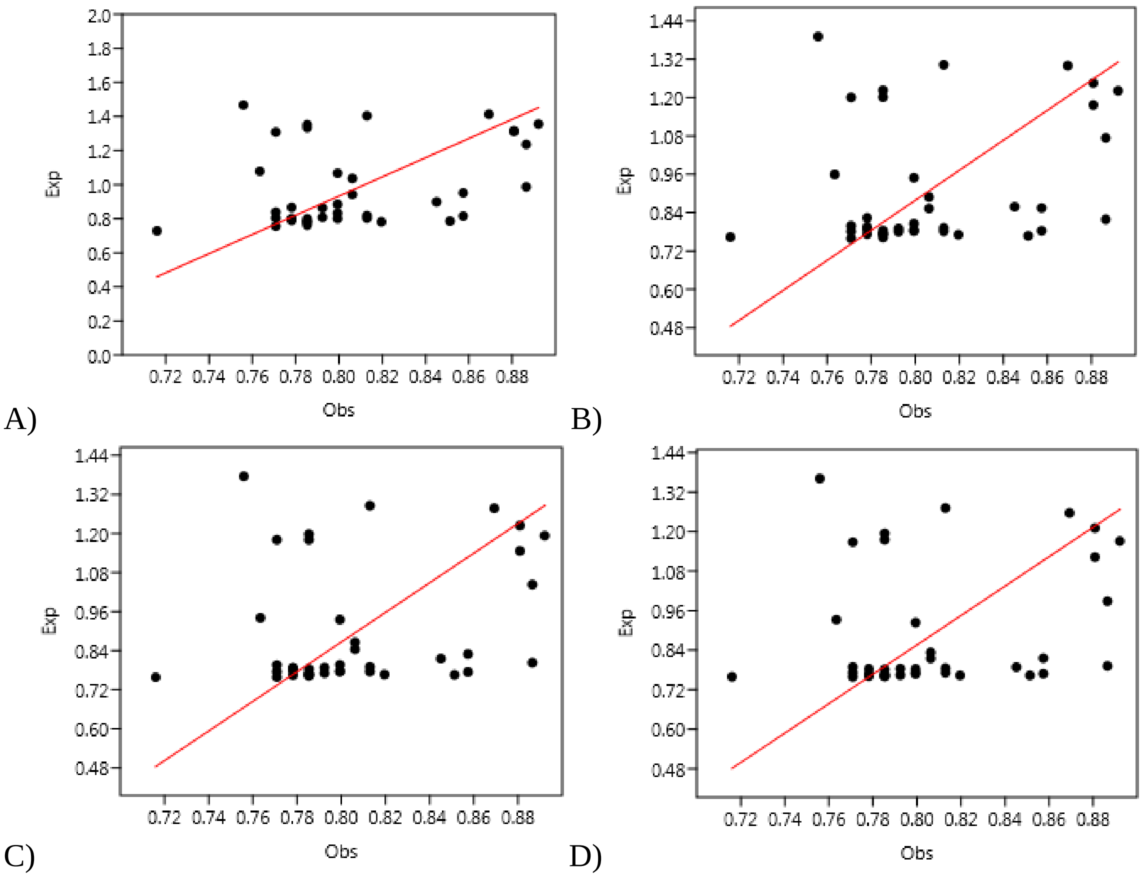


Fig. 1. Probability of occurrence of some contrasting species in relation to pH in the six studied reservoirs, as fitted with logistic regression. The curves can be identified by the code near their optimum indicated by dotted lines. The species arranged in order of their optima are: *Achnanthidium* sp.1 (Acsp); *Aulacoseira ambigua* (AAMB); *A. granulata* (AUGA); *A. pusilla* (AUPU), *Aulacoseira tenella* (AUTL); *Discostella stelligera* (DSTE) e *Spicaticribra kingstonii* (SKIN).

Considering relative abundances, the number of taxa in each model declined from 339 in the “all species” data set, with no deletion criterion, to 56 taxa where the deletion criterion was ≥ 1%, 36 when the deletion criterion was ≥ 2% and 16 taxa where the deletion criterion was ≥ 5%, . When the deletion criterion was based on occurrence frequencies, the number of taxa in each model declined from 61, 50 and to 12 taxa where the deletion criterion was ≥ 1%, ≥ 2% and ≥ 5%, respectively.

The regression models were significant ($p \leq 0.07$) except for ≥2% (p 0.11) and ≥5% (p=0.11) data sets based on relative abundances and for the model ≥1% based on occurrence frequency (p=0.23). On the other hand, the removal of rare taxa from the 40 diatom training set sites according to occurrence frequencies improved the performance of the WA models significantly and the p values decreased from 0.23 (≥1%) to 0.07 (≥2%, ≥5%) (Table 2). However, the model that retained all taxa had the lowest p (0.02) and r^2 (0.12) values (Fig. 2A-G; Table 2).



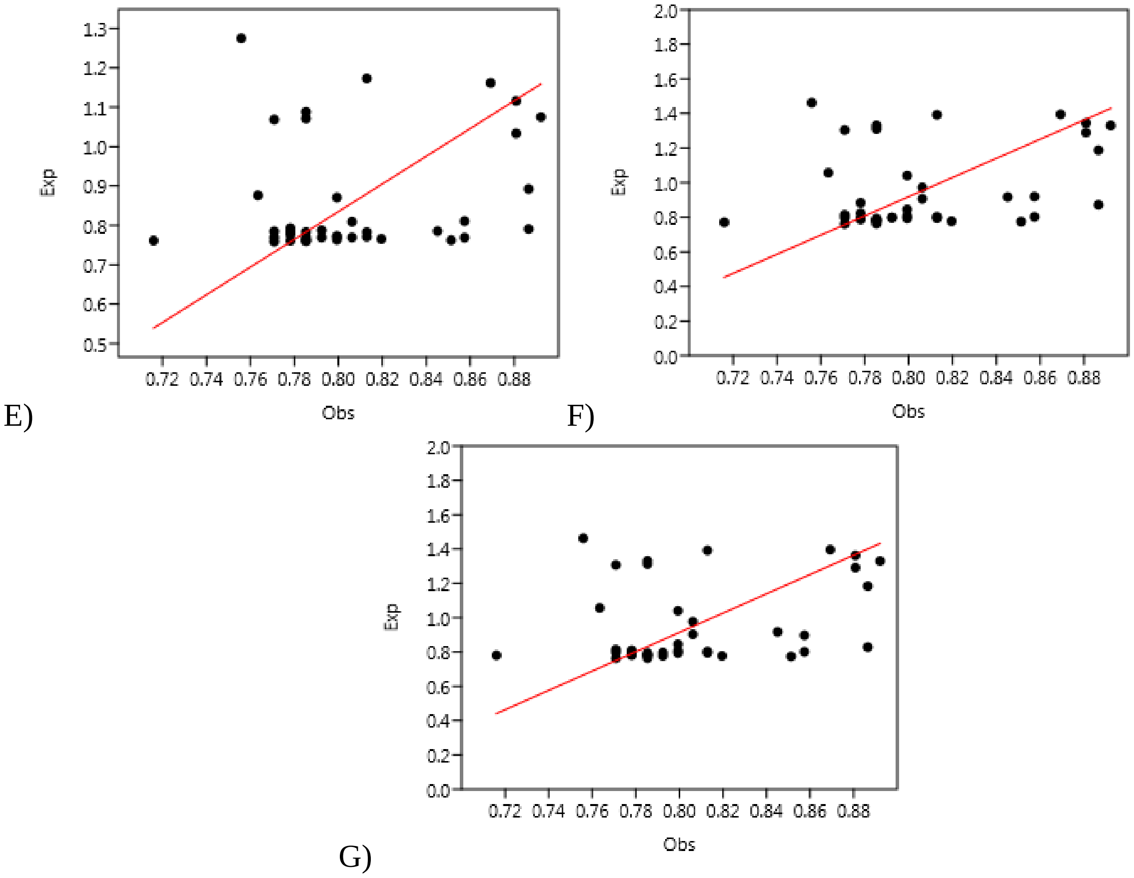


Fig. 2. Observed vs. expected pH in the different data sets based on the data set with no species deletions (A: all species), relative abundances (B:≥1%, C:≥2%, D:≥5%) and occurrence frequencies (E: ≥1%, F: ≥2%, G: ≥5%).

Discussion

Gradient analytical weighted averaging (WA) regression and calibration modelling (and related techniques) have recently been developed for and successfully applied to historical monitoring of lakes, or used to infer past environmental conditions from the remains of different organisms (Hämäläinen, 2000). In this regard, an usual data treatment is the exclusion of species that occur in only a small number of samples assuming that the model will be inadequately able to characterize the optima and tolerances of species that occur only a few times, and that overall model performance may be improved by eliminating them (Payne et al., 2005).

Singular observations (singletons, taxa occuring in only one sample) often occur in ecological series. In nature, singletons are the result of random fluctuations, migrations, or local changes in external forcing. In an aquatic system studied at a fixed location... such changes may be due to temporary movements of water masses. Singletons may also result from improper sampling or inadequate preservation of specimens (Legendre and Legendre, 1998).

It has been observed that taxa deletion in chironomid-based inference models substantially improved the predictive ability of inference models (measured as RMSEP, Martens and Naes, 1989). The common practice of including taxa with only ≥ 2% abundance in at least two lakes was one of the deletion criteria that much improved inference models. Similar deletion criteria, such as ≥ 2% in at least 3 lakes and ≥ 3% in at least 1 lake, produced comparable improvements (up to 18% reduction in RMSEP) (Quinlan and Smol, 2001).

In the same way, Payne et al. (2006) developed transfer-function models based on different techniques, including weighted averaging, to investigate testate amoebae ecology for southern Alaska. Results showed that model performance was improved by selective taxa exclusion. The relatively poor model performance compared with previous studies was explained by the limitations of one-off water-table measurements, the very large environmental gradients covered and by recent climate change in the study area.

Our findings partially disagree the above-mentioned studies: Our observed "all taxa" dataset *p* value is lower than in other cut-offs, suggesting that this model could have the best performance. In this way, removing rare taxa was counterproductive and the developed transfer function models by removing rare taxa actually reduced the model performances. This result corroborate those of Wilson et al. (1996) and Birks (1994), in which diatom and pollen pH calibration datasets, as well as in other data sets, the lowest prediction (measured in terms of $RMSE_{boot}$,) always occurs in WA regression and calibration prior to deletion of taxa on the basis of their effective number of occurrences. Birks (1994) also emphasizes that the largest prediction errors occur when only the commonest and numerically most abundant taxa are included in the WA regression and calibration. According to Wilson et al. (1994), the effects of species deletions and dataset size on the predictive ability of the models emphasizes the value of training sets with a large number of taxa to develop transfer functions with robust and reliable estimates of species optima and tolerances.

The ability of diatom-based metrics to infer nutrients increasing through extension of the dataset was also demonstrated by Philibert et al. (2006). Presumably, even the estimated WA optima of very rare taxa (their absences are ignored in WA regression) are contributing to some ecological "signal" to the calibration, rather than, as might be expected, having no effect or even having deleterious effects by introducing "noise" into the calibration (Birks, 1994).

On the other hand, in a transfer function training set there is a tendency of nearby sampling locations to resemble floristically one another more than randomly selected sites with similar species assemblages and environmental conditions, this possibly resulting in inappropriate model choice, and misleading and over-optimistic estimates of a transfer function’s performance (Telford and Birks 2009). Nevertheless, the WA-based models have demonstrated to be more robust to spatial autocorrelation than other model types in applied foraminifera-based studies based on a unimodal species-environment response (Amesbury et al., 2013; Telford and Birks, 2005).

Our results differed considerably between abundance-based and occurrence -based cutoff criteria. Despite the latter models led to enhanced inference performance, the removal of taxa occuring in <1% of sampling stations did not improve the model performance. The opposite was observed for the <1% model on relative abundances. In this cutoff, the rare taxa are probably still restricted to very few samples, and their optimal may be uncertain, with little impact on the predictive ability of the model. On the other hand, the cutoffs based on relative abundances (≥2% and ≥5%) may have selected only widespread taxa, considered to have wide tolerances and, thus, considered bad indicators, leading to unreliable environmental inferences. The incorporation of abundance data in biotic indices can bias accuracy and reduce precision in two ways, e.g. numerically dominant taxa can skew the result in the direction of their indicator scores, and presence/absence data or strongly transformed abundances can skew the result in favor of rare taxa by assigning them equal weighting as abundant taxa (Monaghan, 2016).

Finally, it is important to note a possible not evaluated season’s influence on our data. As demonstrated in Winter and Duthie (2000), the inference models constructed during a study on epilithic diatoms as indicators of stream nutrients concentration were better when seasonal variation was removed from the dataset by using mean summer values, than those using the full data set.

Conclusion

The results revealed that bioassessment by means of WA modelling is a powerful modeling technique to evaluate precisely the species response to a single as well as multiple environmental descriptors. However, manipulation of the different datasets significantly influenced the model performances. In this sense, removing rare taxa was counterproductive, and the developed transfer function models by removing rare taxa actually reduced the model performances. Although our results agree with those of Wilson et al. (1996), with a strong evidence that sample size has a great influence on results, the influence of rare taxa in bioassessment is an ongoing topic of discussion and study, and choosing a cut off to avoid rare taxa noise may be subjective. However, our work contributes to a better understanding of diatom ecology especially those of tropical reservoirs, supporting the development of more accurate diatom biological monitoring protocols for the study area.

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Capítulo 6

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Type analysis of *Achnanthidium minutissimum* and *A. catenatum* and description of A. tropicocatenatum sp. nov. (Bacillariophyta), a common species in Brazilian reservoirs

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Abstract

Background and aims -- A commonly occurring *Achnanthidium* Kützing species has been observed in Brazilian reservoirs. The morphological features were described based on light and scanning electron microscope observations and it is described here as *A. tropicocatenatum* sp. nov.

Methods -- The new species was compared with the type material of morphologically similar taxa: *Achnanthidium catenatum* (J.Bílý & Marvan) Lange-Bertalot and *A. minutissimum* (Kützing) Czarnecki. Comparisons with the most related species based on literature were also provided. Traditional weighted-averaging approaches (WA) were applied to model ecological optima.

Key results -- Morphometric analysis of the valve shape supported the taxa separation; they can be easily differentiated by the morphological and ultrastructural features, including subcapitate to capitate apices, strongly radiate striae and a prominent axial area. This new species has been found in alkaline waters with low electrolyte content.

Conclusions -- Records of *A. catenatum* in Brazil are considered questionable because the species may have been confused with *A. tropicocatenatum* sp. nov.

Key words -- Distribution, ecological optimum, ecology, geometric morphometry, morphology, new species, taxonomy

INTRODUCTION

Achnanthidium Kützing is a taxonomically difficult group of monoraphid diatoms (Round & Bukhtiyarova 1996). Despite its representatives are commonly recorded in worldwide freshwaters, the determination and delimitation of many taxa are still a challenge, because of both the small size of its valves, which often requires scanning electron microscopy (SEM), and the lack of comprehensive descriptions of nomenclatural types and undescribed variability of diagnostic features that often overlap (Hlúbiková et al. 2011).

Because of the high diversity in this group and its importance as indicators of water quality (Van de Vijver et al. 2011a, b), *Achnanthidium* has received considerable attention and, during the last decade, the genus has been the subject of intense revisions (e.g. Hlúbiková et al. 2011, Van de Vijver et al. 2011a, Wojtal et al. 2011, Novais et al. 2015), especially taxa belonging to the *A. minutissimum* (Kützing) Czarnecki complex (Ector 2011). These revisions have also contributed with more detailed information of the geographical distributions and biogeography of the genus in general.

Nowadays, *Achnanthidium* comprises more than 200 taxa (Fourtanier & Kociolek 2011) including many species described from various parts of the world in the last decade (e.g. Potapova & Ponader 2004, Cantonati & Lange-Bertalot 2006, Potapova 2006, Zidarova et al. 2009) and new species have been regularly described (e.g. Wojtal et al. 2010, Jüttner & Cox 2011, Van de Vijver et al. 2011b, Witkowski et al. 2012, Peres et al. 2014, Novais et al. 2015, Pinseel et al. 2015, Liu et al. 2016). However, the genus has received relatively little attention in Brazil, where *A. minutissimum* and *A. catenatum* (J.Bílý & Marvan) Lange-Bertalot are among the most commonly recorded species (e.g. Bertolli et al. 2010, Faria et al. 2010, Faustino et al. 2016). Although they are widely used names, some misidentifications were observed and they are discussed in this work. All these findings contributed to the generally accepted idea that *A. minutissimum* and *A. catenatum* species are globally distributed. Besides, it is important to note that a number of mentions to the species above are ecological contributions (e.g. Santos & Ferragut 2013, Burliga et al. 2014, Fonseca et al. 2014) or diatoms are used as indicators of environmental change (e.g. Lobo et al. 2004a, b, Hermany et al. 2006, Molisani et al. 2010, Bere & Tundisi 2011, Elias et al. 2017), emphasizing the importance of accurately defining the identity of the most common taxa in order to improve the accuracy of the diagnostic tools that rely on diatom taxonomy and ecology (Wetzel & Ector 2014). Other records about these species are in unpublished doctoral dissertations (e.g. Carneiro 2003, Nascimento 2012) or do not present illustrations and/or descriptions (Souza & Oliveira 2007). *Achnanthidium catenatum* is considered to be invasive in Europe (Bílý & Marvan 1959, Druart &

Straub 1992, Coste & Ector 2000, Straub 2002, Hlúbiková et al. 2011) and more recently in East China (Ma et al. 2013).

Freshwater diatom flora of Brazil has been shown to contain many unique species (e.g. Tremarin et al. 2011, Wengrat et al. 2015, Marquardt et al. 2016). However, no new *Achnanthidium* species has been published so far. During a diatom survey in reservoirs of São Paulo State, an *Achnanthidium* species morphologically similar to *A. catenatum* was found, and it is proposed as a new species for science: *A. tropicocatecatum* sp. nov. The morphology is presented by light (LM) and scanning electron microscopy (SEM). Furthermore, we compared it with the type materials of morphologically similar species, *A. minutissimum* and *A. catenatum*. Examining type material has become a valuable tool to solve taxonomic issues (e.g. Cejudo-Figueiras et al. 2011). We also presented information based on ecology optima. In this way, besides solving the taxonomic issues about the species it is as well characterized from the autecological standpoint (e.g. Trobajo et al. 2013, Morales et al. 2015, Bicudo et al. 2016).

MATERIALS AND METHODS

Study area

The Jurupará and Cachoeira do França reservoirs (CBH-RB 2009) are located in the catchment area of the Ribeira de Iguape/Litoral Sul basin which presents high availability of water in relation to the demand, due to a rich and extensive drainage network. The area is considered as a Conservation Unit (PEJU, Portuguese for Jurupará State Park) and widely covered with native forests.

The Jurupará reservoir is part of a hydropower facility located in Piedade township at an elevation of 781 m along the Peixes River, tributary to the Juquiá River (SMA 2012). Built in 1947, the reservoir accumulation capacity is up to $42x10^6$ m^3. This environment is predominantly mesotrophic, according to the Trophic Status Index (TSI) (Lamparelli 2004) calculated presently. The reservoir is also used for fish farming (fig. 1). The TSI results are in accordance with those of the PEJU Management Plan report (SMA 2012) in which observations carried out through ichthyofauna studies indicated a mesotrophic site in the network tanks region, probably a result of the psychulture activities.

The Cachoeira do França reservoir is located in the region of Juquitiba at an elevation of 685 m. Its construction began in 1954 and was completed in 1957 and, operation started in the following year. It was the first hydroelectric plant built by Companhia Brasileira de Alumínio (CBA) for power generation which corresponds to the first of the cascading projects along the Juquiá River

(SMA 2012). This reservoir is currently classified as oligotrophic (TSI) and presents an accumulation capacity of up to 135.2×10^6 m^3 (fig. 1).

Information about the study area is summarized in table 1.

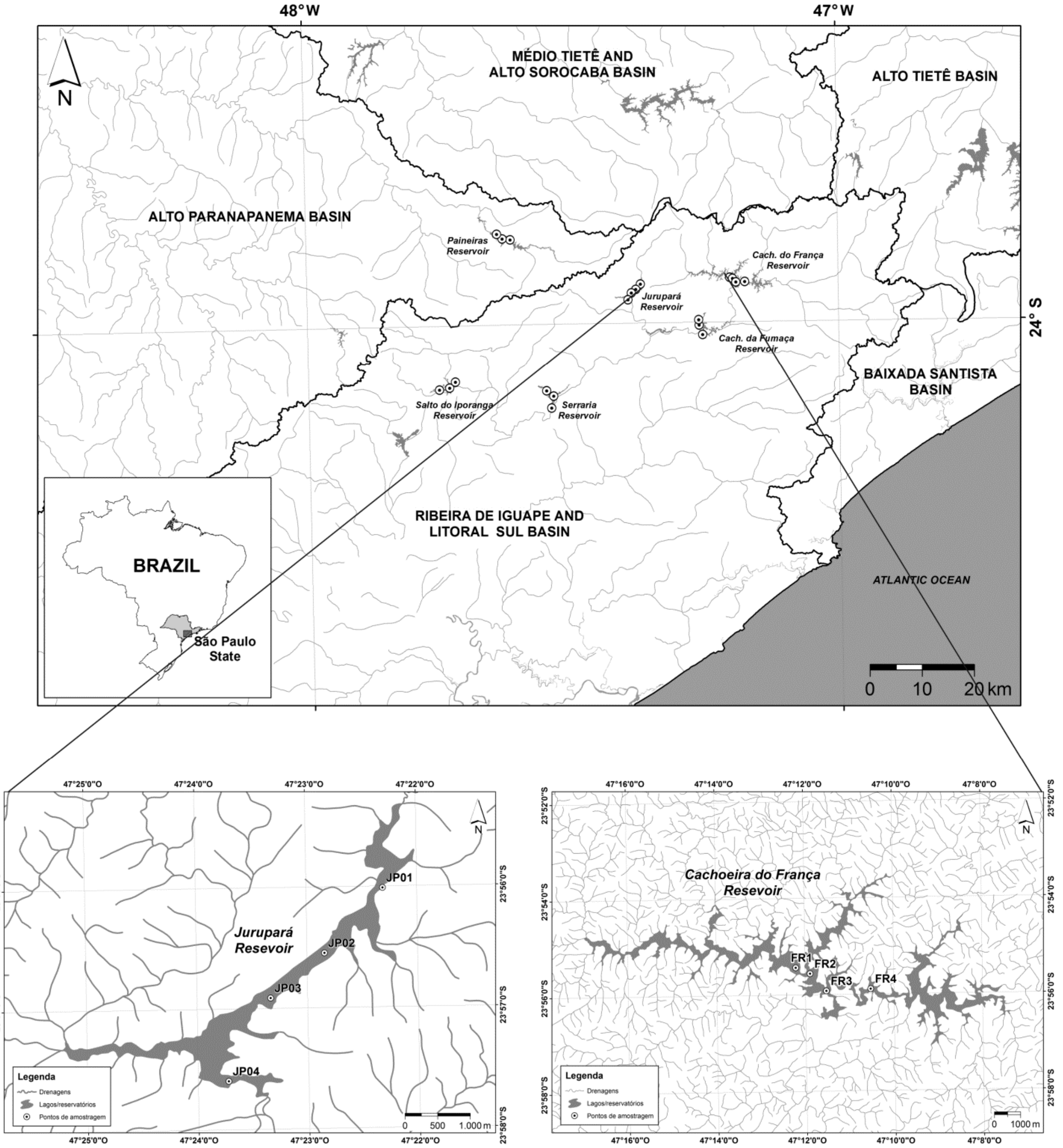

Figure 1 -- Study area showing the *Achnanthidium tropicocatenatum* type locality (Cachoeira do França reservoir) and Jurupará reservoir.

Table 1 -- Data from sampling sites of *Achnanthidium tropicocatenatum*, state of São Paulo, Brazil, habitat and the material number at the Herbarium of the Institute of Botany (SP).

Reservoir	Herbarium number	Habitat	Municipality	Main river	Sampling date	GPS coordinates
Cachoeira do França (type material)	SP469444	periphyton (stones)	Juquitiba	Juquiá	05/08/2014	23°55'58.8"S 47°11'31.4"W
Jurupará	SP469208	surface sediment	Piedade	Peixes	25/06/2014	23°56'00"S 47°22'18.0"W

Environmental variables

Water samples for chemical analysis were collected during austral summer and winter campaigns in 2014 with a Van Dorn sampler along a vertical profile. Temperature (°C), pH and specific conductance (µS cm^{-1}) were measured in the field using standard electrodes (Horiba U-53). The analytical procedure for dissolved oxygen (mg L^{-1}), ammonium (µg L^{-1}), alkalinity (mEq L^{-1}), nitrate (µg L^{-1}), soluble reactive silica (mg L^{-1}), total nitrogen and total phosphorus (µg L^{-1}), free carbon dioxide (mg L^{-1}) and bicarbonate ions (mg L^{-1}) followed Standard Methods (APHA 2005). Chlorophyll-*a* (µg L^{-1}), corrected for phaeophytin, was measured using 90% ethanol (Sartory & Grobbelaar 1984). Details of the environmental conditions at the sampling sites are summarized in table 2.

Table 2 -- Means (and standard deviation) of abiotic variables in the studied samples in which *A. tropicocatenatum* occurred and its weighted average optima and tolerances. Abbreviations: Sec (Secchi depth; m), Temp (Temperature; °C), Cond (Conductivity; µS cm^{-1}), Alk (Alkalinity; mEq L^{-1}), DO (Dissolved oxygen; mg L^{-1}), TN (Total nitrogen; µg L^{-1}), TP (Total phosphorus; µg L^{-1}), SRS (Soluble reactive silica; mg L^{-1}), Chlo (Chlorophyll-a; µg L^{-1}).

	Abiotic variables values	Ecological optimum	Ecological tolerance
Sec (m)	2±0.86	1.59	0.45
Temp (°C)	23.9±3.04	24.22	3.11
Ph	7.21±0.72	7.54	0.92
Cond (µS cm^{-1})	30.3±9.46	28.94	7.45
Alk (mEq L^{-1})	0.22±0.44	0.23	0.04
DO (mg L^{-1})	7.2±0.65	7.39	0.83
TN (µg L^{-1})	288.0±114.0	292.32	118.8
TP (µg L^{-1})	13.3±7.64	12.60	5.73
SRS (mg L^{-1})	3.6±1.07	3.38	1.03

Sampling and diatom preparation

The following type materials were examined under LM and SEM microscopy, respectively:

1. *Achnanthidium minutissimum*: type material, Kützing's Algarum Aquae Dulcis Germanicarum, Decade VIII, no. 75, Kützing 301 (Van Heurck Collection, Botanic Garden Meise, Belgium). Type locality: Near Aschersleben, Germany; epiphytic on filamentous algae (Lange-Bertalot & Ruppel 1980, Potapova & Hamilton 2007, Novais et al. 2015).

2. *Achnanthidium catenatum*: lectotype material, sample n° E9877 (Hustedt Collection, Alfred Wegener Institute in Bremerhaven, Germany). Type locality: Sedlice reservoir on Želivka River, Czech Republic (Bílý & Marvan 1959, Hlúbiková et al. 2011).

For the new species we selected two samples from two different habitats (see table 1). Periphytic material scrapped from stones during the summer and winter 2014 and surface sediment (2 cm deep samples) collected using a gravity core (UWITEC) only during the winter. Diatoms were cleaned from organic matter with hot hydrogen peroxide (35%) and hydrochloric acid (37%) (Battarbee et al. 2001). For LM observations, cleaned diatoms were mounted with Naphrax®. LM and morphometric measurements were performed with a Leica® DMRX brightfield microscope with 100x oil immersion objective, and light micrographs were taken with a Leica® DC500 camera. For scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered with additional deionized water through a 3-μm Isopore™ polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminum stubs and coated with platinum using a Modular High Vacuum Coating System BAL-TEC MED 020 (BAL-TEC AG, Balzers, Liechtenstein). An ultra-high-resolution analytical field emission (FE) scanning electron microscope Hitachi SU-70 (Hitachi High-Technologies Corporation, Japan) operated at 5 kV and 10 mm distance was used for the analysis. SEM images were taken using the lower (SE-L) detector signal and up to 28° tilted. Plates were assembled by CorelDraw Graphics Suite X7®. Relative abundances were estimated following Batarbee et al. (2001) with a minimum of 400 valves counted per slide at 1000× magnification on up to six random transects and until reaching an efficiency of at least 90% (Pappas & Stoermer 1996).

Holotype permanent slides, as well as the raw and cleaned samples were deposited at Herbário Científico do Estado “Maria Eneyda P. Kauffmann Fidalgo” (SP), São Paulo State Department of Environment, Brazil. Isotypes were deposited at BR, the Botanic Garden Meise, Belgium.

Morphological terminology followed Round et al. (1990) and Krammer & Lange-Bertalot (1991).

Diatom distribution

We estimated the new species ecological optima and tolerances with respect to pH, conductivity and total phosphorus based on weighted averaging (ter Braak & van Dam 1989), and tested its ecological profiles for differences in the ecological optima with respect to the similar species *A. catenatum*, by using a weighted comparison of means (Bland & Kerry 1998).The weighted average estimates of a species optimum is the mean of a measured environmental variable (such as total phosphorus concentration or pH) weighted by the abundance of the species in a sample data set, whereas species tolerance is the weighted standard deviation (Porter et al. 2008).

Calculations were based on data from the Acquased project called, “Base line diagnosis and reconstruction of anthropogenic impacts in the Guarapiranga Reservoir, focused on sustainable water supply and quality management in the reservoirs of the Alto Tietê and surrounding basins”, in which this study is included. This dataset involves 33 tropical reservoirs and 227 samples, covering a wide range of trophic states. In this sense, the chosen abiotic variables were considered relevant for water quality assessment.

Statistical analysis

Geometric morphometric techniques were applied to different *Achnanthidium* groups to evaluate whether these morphotypes present differences in shape compared with *A. tropicocatenatum*. For this analysis, a total of 68 LM photographs were taken from *A. minutissimum* type material, 32 from *A. catenatum* type material, 100 from *A. tropicocatenatum* (population from JP reservoir) and 83 from *A. tropicocatenatum* (type material from FR reservoir) (fig. 2). The pseudolandmarks of the outlines to further identification and characterization were performed with CLIC (Collection of Pseudolandmarks for Identification and Characterization) software (Dujardin et al. 2010).

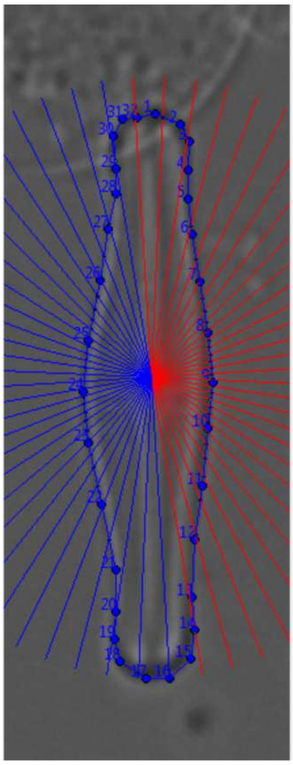

Figure 2 -- Position of the pseudolandmarks on the valve outline used to perform the geometric morphometric analysis. Scale bar = 10 μm.

Matrices, consisting of pseudolandmarks defining outlines, were built based on the populations and shape variables were obtained through the Generalized Procrustes Analysis (GPA) superimposition algorithm (Rohlf 1990). After superimposition, shape differences can be described by the differences in coordinates of corresponding pseudolandmarks between objects (Adams et al. 2004). Principal component analysis (PCA) was carried out for the resulting normalized coordinates by means of the Past software version 1.78 (Hammer et al. 2001). Finally, analysis of similarity (NPMANOVA) was performed between the Cartesian coordinates of resulting groups in the PCA with the Chord distance measure.

RESULTS

Taxonomical description

Achnanthidium tropicocatenatum Marquardt, C.E.Wetzel & Ector, **sp. nov.** (figs 3--5).

Frustules usually solitary (fig. 3A--BH). Girdle view rectangular, slightly arched, with apices recurved to the rapheless valve (fig. 3BI--BL). Valves linear-lanceolate, slightly inflated in the central portion of the valve, with protracted, subcapitate to capitate apices (fig. 3A--BH). Length 10.5--30 µm, width 2.5--3.5 µm in the middle. Axial area narrow linear and silicified, widening towards the central area (figs 3A--BH, 4A & B). Raphe valve: concave with small rounded central area, bordered by one more widely spaced stria on one or both sides (fig. 4A & B). Externally raphe straight, filiform, with slightly expanded proximal and distal raphe endings (fig. 4A & B). Internally proximal raphe endings slightly deflected in opposite directions and distal endings terminating in small helictoglossae (fig. 5D). Transapical striae radiate throughout the valve (36--40 in 10 µm), becoming denser towards the apices (up to 45 in 10 µm), cannot be resolved in LM. Striae mainly composed of four, sometimes five or six, rounded transapical areolae (fig. 4A & B), often slit-like near the valve margin and covered by a delicate silica membrane (hymen). Rapheless valve: convex with central area indistinct or narrow lanceolate, bordered by 1-2 more widely spaced striae on one or both sides (fig. 4C). Transapical striae radiate throughout the valve (38--40 in 10 µm), becoming denser towards the apices (up to 55 in 10 µm), cannot be resolved in LM. Striae mainly composed of 5-6, sometimes 4, elliptical transapical areolae (fig. 5A--C), often slit-like near the valve margin and covered by a delicate silica membrane (hymen) (fig. 5A). On both valves, a single row of elongated areolae on the mantle, separated from the striae on the valve face by a hyaline area (fig. 4A & C).

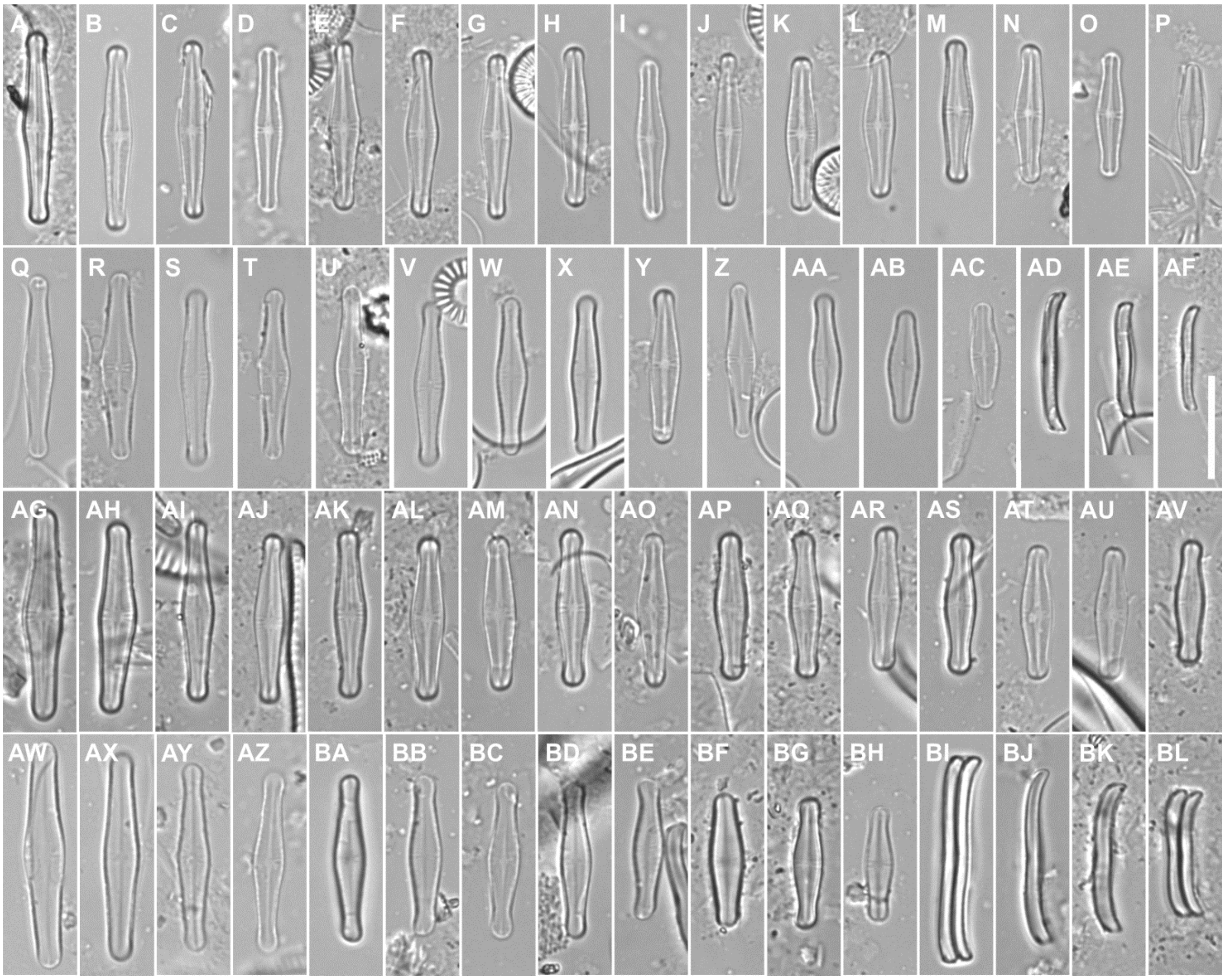

Figure 3 -- *Achnanthidium tropicocatenatum* sp. nov. LM: A--AF, specimens from type locality (Cachoeira do França reservoir, São Paulo, Brazil); A--P, raphe valves; Q--AC, rapheless valves; AD--AF, girdle view. AG--BL, specimens from Jurupará reservoir, São Paulo, Brazil; AG--AV, raphe valves; AW--BH, rapheless valves; BI--BL, girdle view. Scale bar = 10 µm.

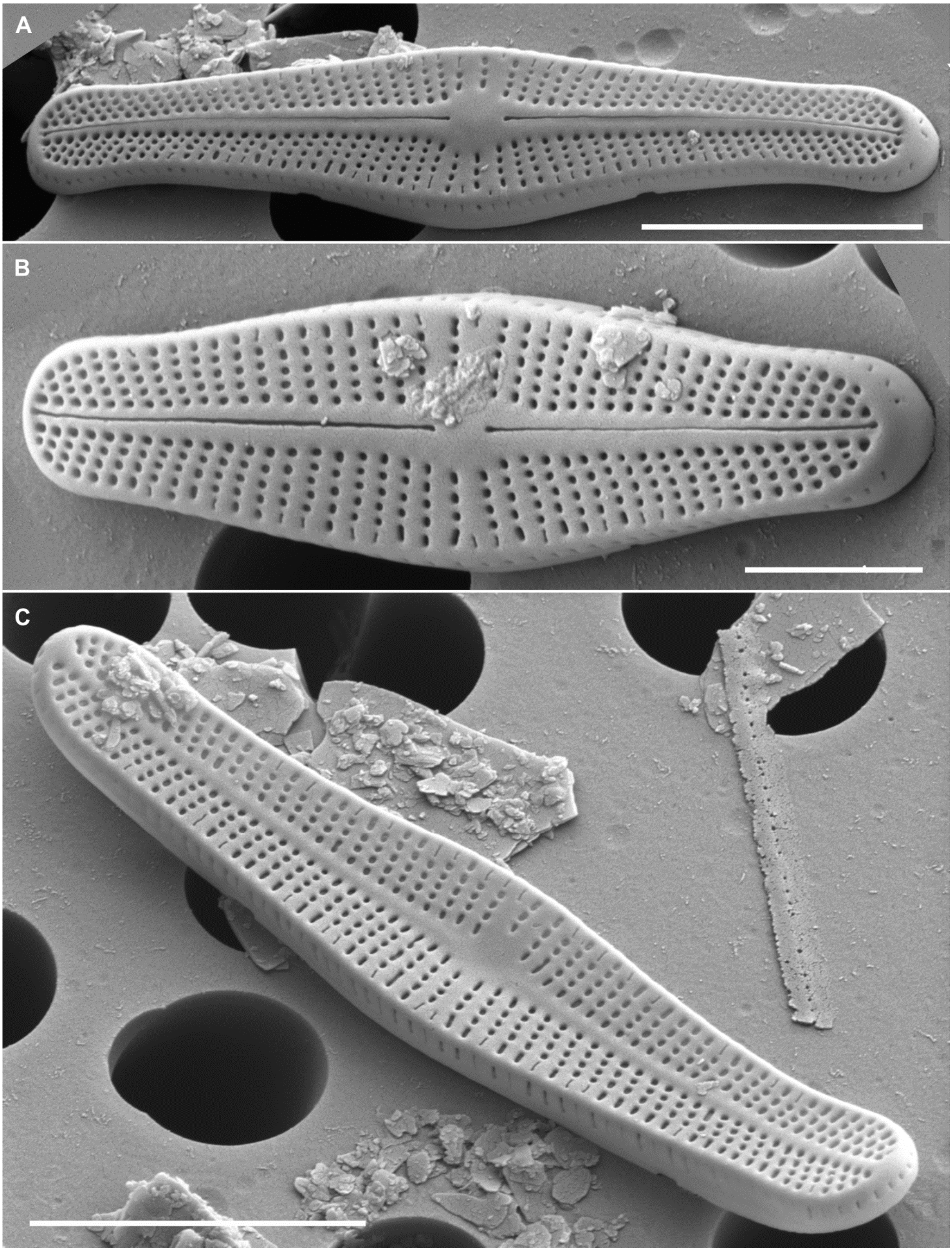

Figure 4 -- *Achnanthidium tropicocatenatum* sp. nov. SEM: A--C, external views; A & B, raphe valves; C, rapheless valve. Population from type locality (Cachoeira do França reservoir, São Paulo, Brazil). Scale bars: A & C = 5 µm, B = 2 µm.

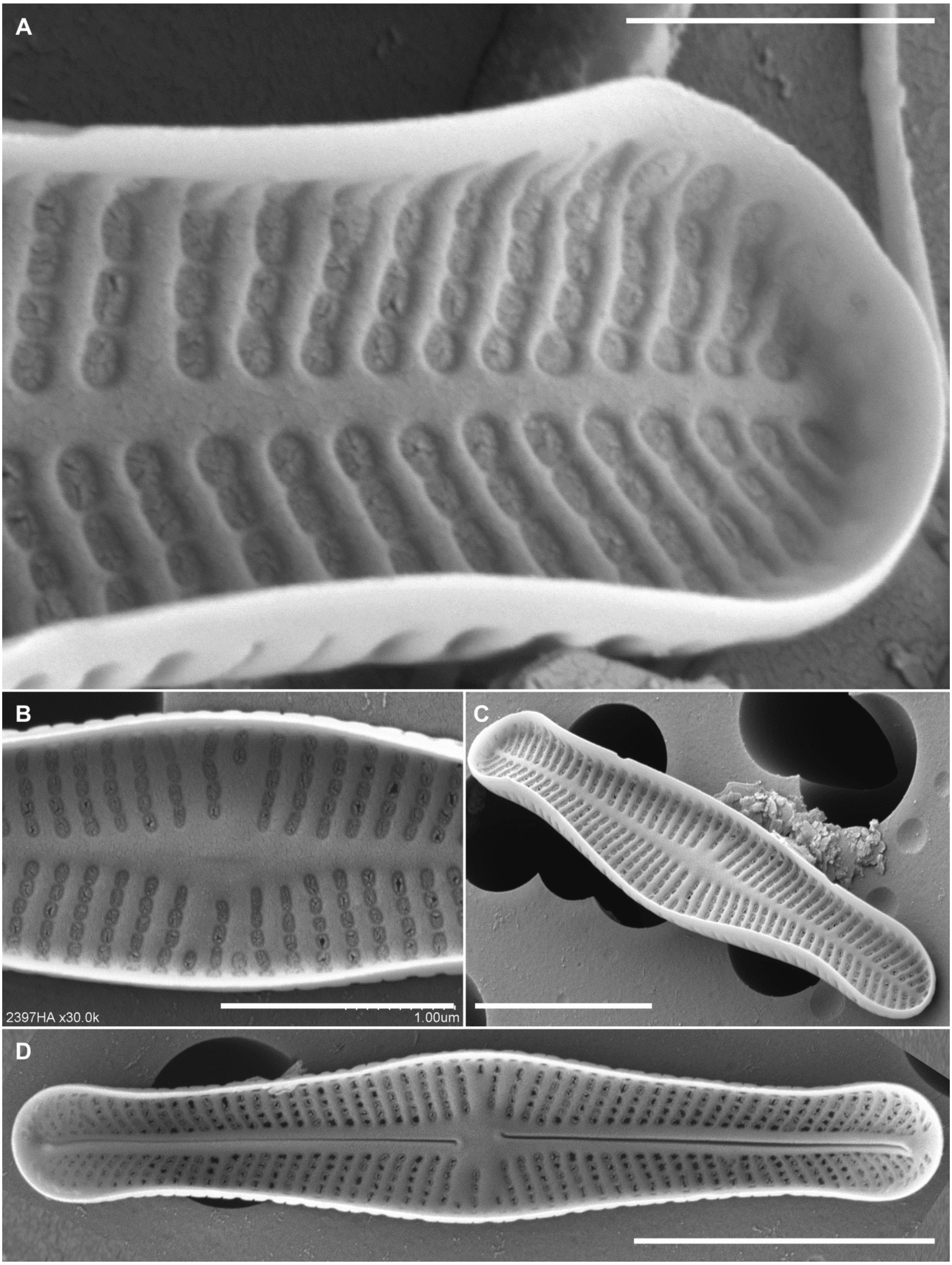

Figure 5 -- *Achnanthidium tropicocatenatum* sp. nov. SEM: A--D, internal views; A--B, rapheless valves; D, raphe valve; A, detail of apical striae and areolae; B, detail of central area and transapical striae. Population from type locality (Cachoeira do França reservoir, São Paulo, Brazil). Scale bars: A & C = 2 µm, B & D = 5 µm.

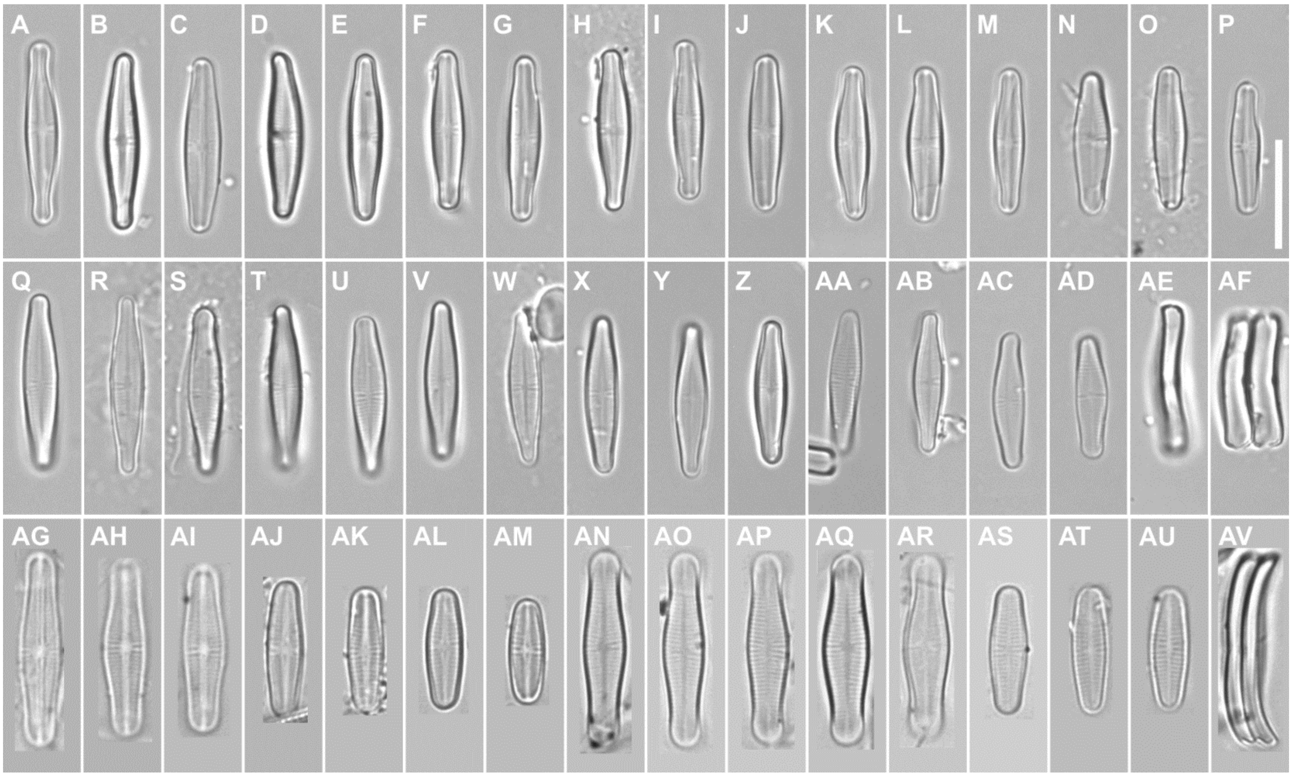

Figure 6 -- *Achnanthidium minutissimum* LM: A--AF, specimens from type material (Type locality: Near Aschersleben, Germany); A--P, raphe valves; Q--AD, rapheless valves; AE & AF, girdle view. *Achnanthidium catenatum* LM: AG--AV, specimens from lectotype material (Type locality: Sedlice reservoir on Želivka River, Czech Republic); AG--AM, raphe valves; AN--AU, rapheless valves; AV, girdle view. Scale bar = 10 µm.

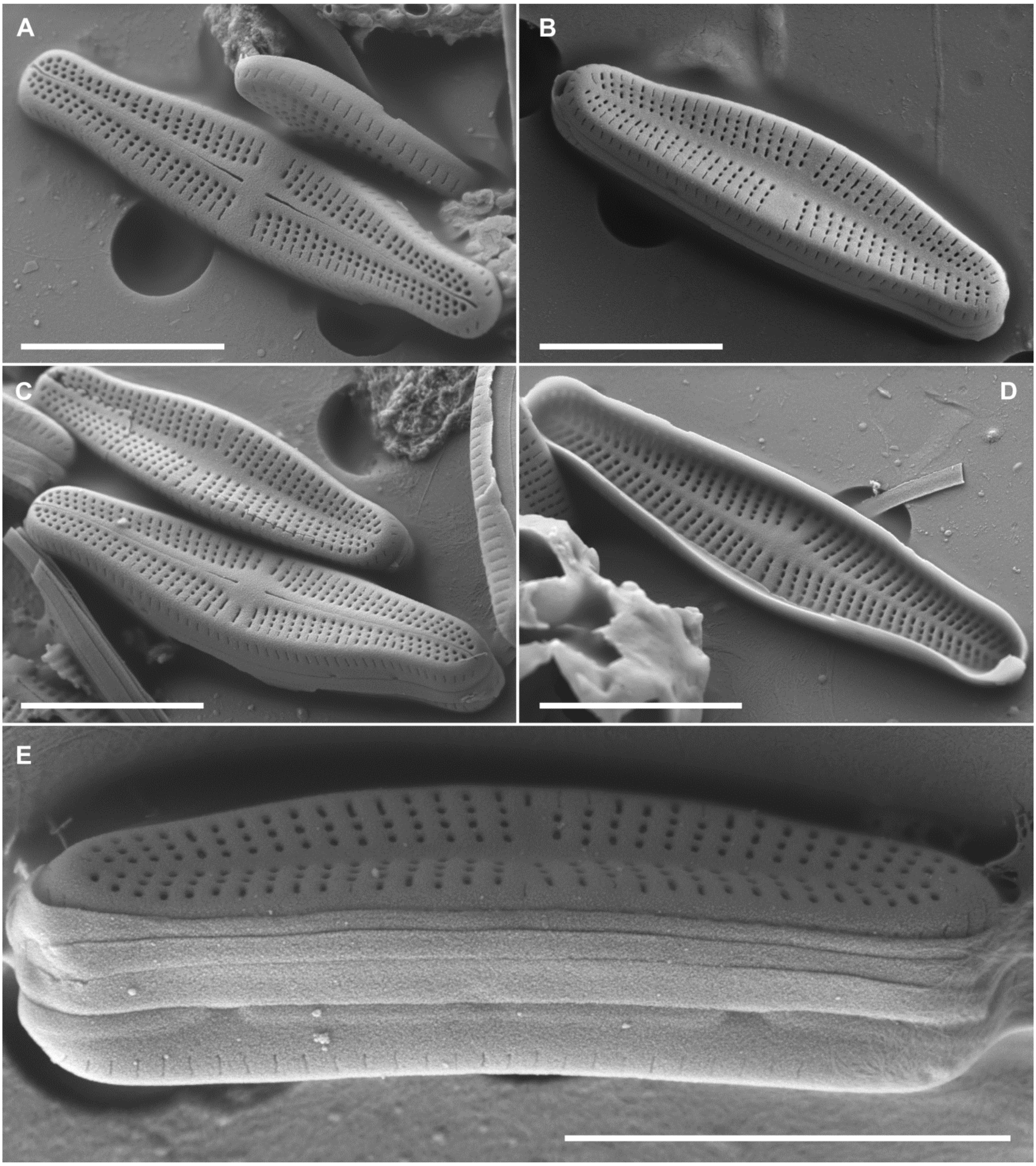

Figure 7 -- *Achnanthidium minutissimum* SEM: A--C, external views; D, internal view; E, girdle view; A, raphe valve; B & D, rapheless valve; C, raphe and rapheless valves. Scale bars = 5 µm.

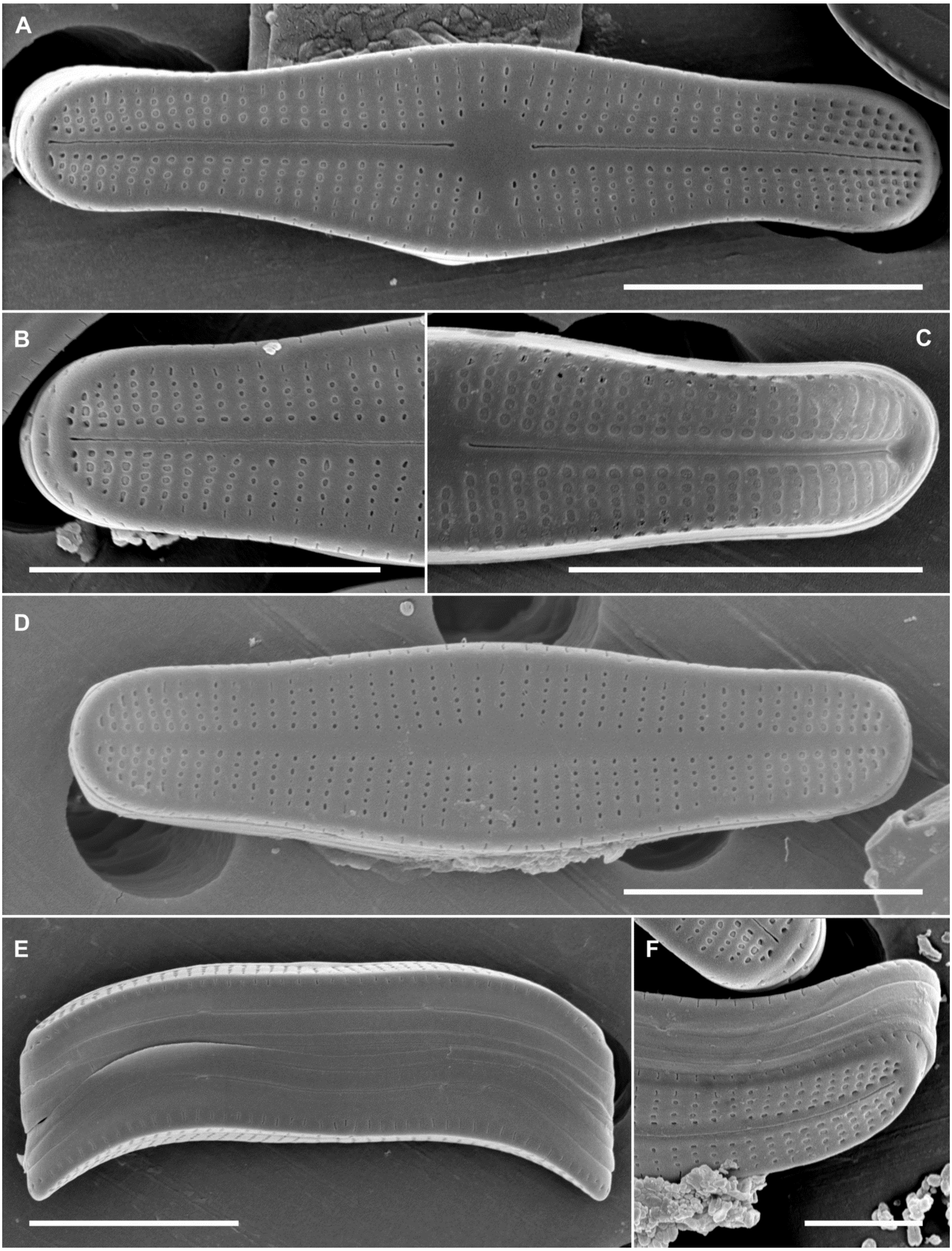

Figure 8 -- *Achnanthidium catenatum* SEM: A & B, D, F, external views; C, internal view; E & F, girdle view, B & C, detail of apices with its distal raphe endings; F, detail of apice. Specimens from lectotype material (Type locality: Sedlice reservoir on Želivka River, Czech Republic). Scale bars: A--E = 5 µm, F = 2 µm.

Holotype -- BRAZIL. São Paulo: Cachoeira do França Reservoir, 23°55'58.8"S, 47°11'31.4"W, G. Marquardt & D. Bicudo, 5 Aug. 2014 (holotype SP!, slide 469444, Herbário Científico do Estado Maria Eneyda P. Kauffmann Fidalgo, Juquitiba, Brazil, depicted in fig. 3A. Isotype BR!, slide XX, Botanic Garden, Meise, Belgium).

Etymology -- The epithet refers to the similarity of the new species, observed in a tropical region, to *A. catenatum*.

Morphological examination -- Although the morphology of *A. tropicocatenatum* under LM could resemble *A. catenatum* at first sight, important features as the ends, protracted, subcapitate to capitate in the new species and not protracted, broadly rounded in *A. catenatum* as well as the shape of the frustule in girdle view can easily separate them. Also, valves are slender in *A. catenatum,* with widened central portion resulting in a more undulated valve margin whereas valves are only slightly inflated in *A. tropicocatenatum.* Thus, the valve appears more elongated in the latter. Besides, the axial area in *A. tropicocatenatum* is silicified and preeminent (SEM) (fig. 5C & D).

The girdle view in *A. catenatum* resembles a “C” with the ends strongly curved and sharply pointed to the araphid valve, corresponding to one fifth of the frustule length (Hlúbiková et al. 2011). Also, it is commonly observed in more than one frustule together, forming short chains of 2 (Straub 2002), 3 (Hlúbiková et al. 2011) and up until 8 cells in the planktonic type material of Bohemia (Bílý & Marvan 1959). Druart & Straub (1992) also observed in Chaumeçon Lake (France) that the *A. catenatum* cells are joined together in long, recognizable chains, a very rare character in *Achnanthidium* species. On the other hand, valves in girdle view are not so strongly curved in *A. tropicocatenatum*, ends are not so sharply pointed and frustules are commonly observed as solitary (figs 4BI--BL). Besides striae are more strongly radiate in the new species (fig. 5C).

Apices are also clearly different in *A. minutissimum*, ranging from subrostrate to protracted (figs 7A--AD). Moreover, *A. minutissimum* differs in its valve dimensions (table 3) and in its not inflated central region.

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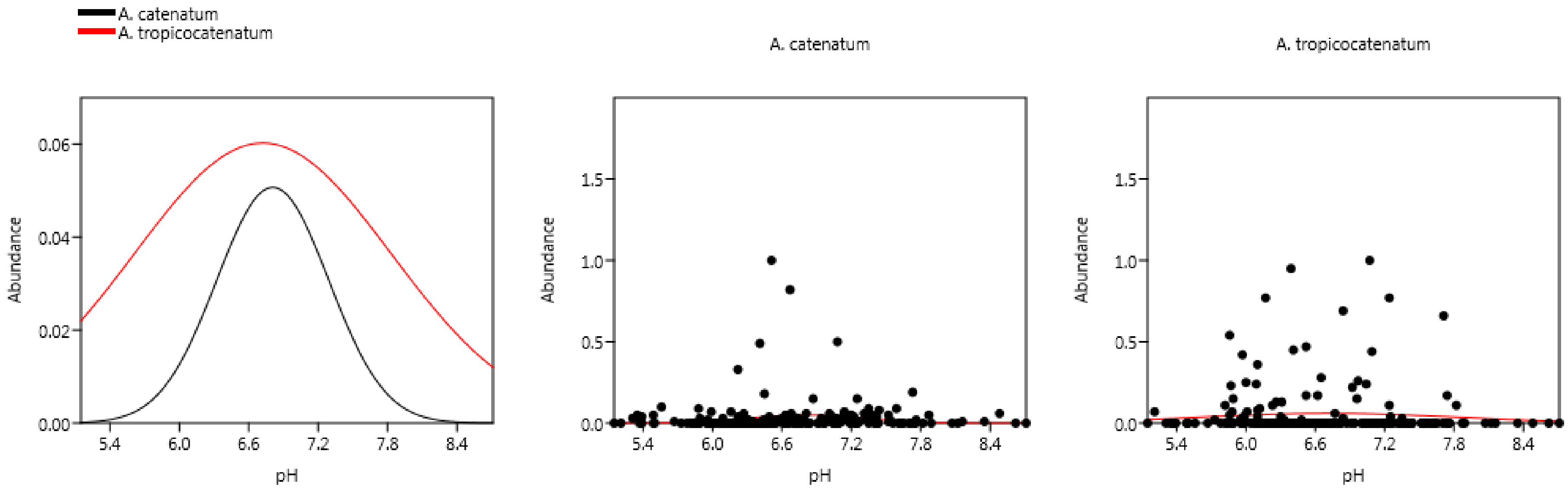
The species is also similar to *A. lusitanicum* Novais & M.Morais. Nevertheless, the new species is longer (10.5--30 µm) compared with *A. lusitanicum* (5.3--13 µm). Also, apices in the latter are rostrate and broadly rounded contrasting to the subcapitate to capitate in *A. tropicocatenatum* (table 3).

Achnanthidium lusitanicum is described from two different sites: 1) Janeiro de Baixo, Zêzere River (Tejo basin) as the type material and 2) Boeiro, Sertã Stream (Tejo basin), which can have much larger valve dimensions (length: 8.3--19.3 and width: 2.3--4.3 µm). *Achnanthidium* *tropicocatenatum* is more similar to the second illustrated population, especially in girdle view. However, *A. lusitanicum* population 2 is wider in the middle of the valve than *A. tropicocatenatum* (2.8--3.2 µm). The species is also more rhomboidal compared with *A. tropicocatenatum*. Thereby, it is important to note the confusion regarding the circumscription of *A. lusitanicum,* as the second population seems to represent a distinct species.

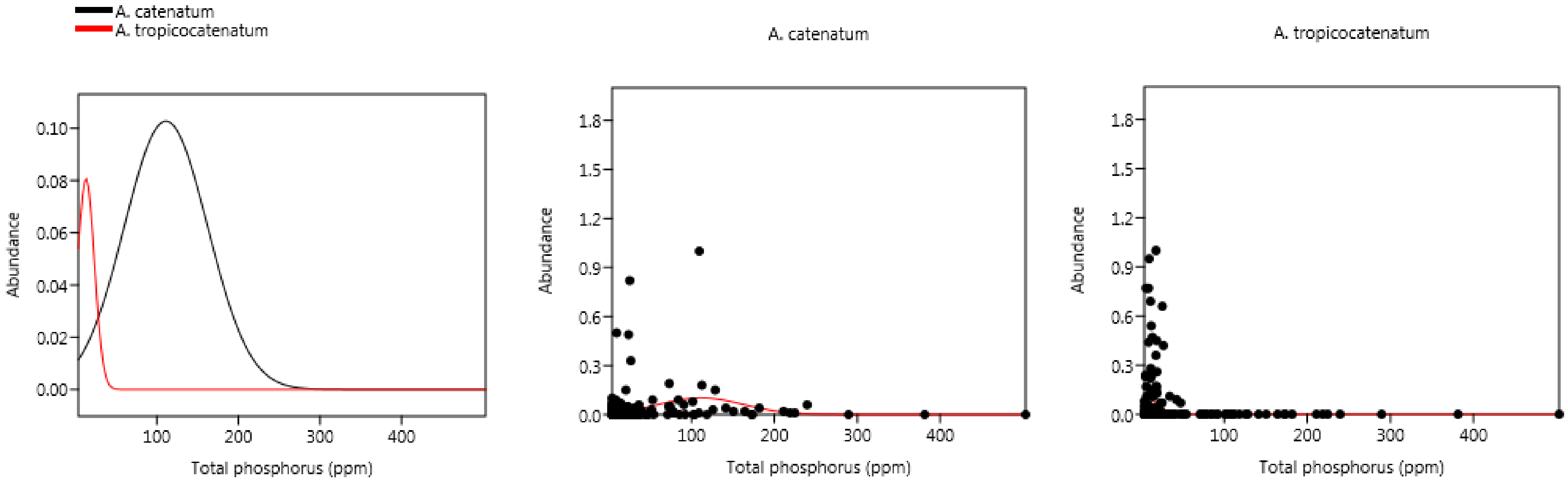
Ecology, distribution and associated diatom flora -- The type population of *A. tropicocatenatum* was observed in the Cachoeira do França reservoir in alkaline waters with low conductivity (27 µS cm^{-1}), low levels of nitrate (57.7 µg L^{-1}), total nitrogen (323.1 µg L^{-1}), total phosphorus (6.2 µg L^{-1}) and phosphate (4.3 µg L^{-1}) concentrations and pH of 7.2 (annual mean). Regarding its autoecology, the species reached its optimum when the temperature was around 24.2 ºC, values of pH were around 7.5, conductivity around 29 µS cm^{-1}, NT around 292.3 µ L^{-1} and TP around 12.6 µg L^{-1}. Based on phosphate measurements, this species presented an optimum at oligotrophic conditions (table 2). The new species has a different autecology when compared with *A. catenatum,* although both taxa shared similar preferences with respect to pH (fig. 9A, t=0.22, p=0.83). *A. tropicocatenatum* is an oligotraphentic species (fig. 9B, t=10.5, p<0.01) that prefers waters with lower electrolyte content opposite to *A. catenatum,* which prefer eutrophic waters (fig. 9C, t=9.2, p<0.01).

Achnanthidium tropicocatenatum, dominant in the sample of Cachoeira do França (86.6% relative abundance), was associated with *Brachysira neoexilis* Lange-Bertalot (3.8%) and *Discostella stelligera* (Cleve & Grunow) Houk & Klee (2%). *Navicula notha* J.H.Wallace, *Achnanthidium hoffmannii* Van de Vijver, Ector, A.Mertens & Jarlman, *Eunotia* sp. and *Aulacoseira tenella* (Nygaard) Simonsen also occurred but with low relative abundance (< 2%). The diatom flora is mainly composed of taxa indicating waters of low nutrient content.

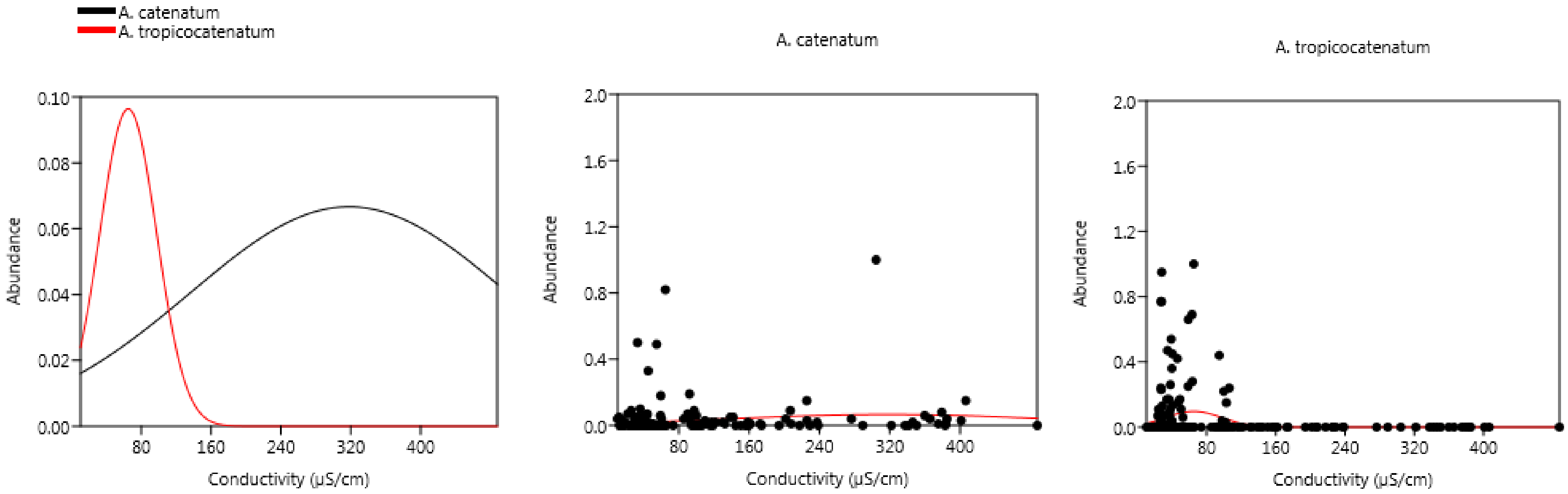
A. tropicocatenatum problably corresponds to *A.* cf. *catenatum* in Morales et al. (2011, figs 128--134) from Maylanco (Bolivia), a small stream about 2 m wide and 30 cm deep (17°23.73´ S, 66°3.16´W 2685 m a.s.l.) with alkaline waters, with relatively high conductivity (400 µS cm^{-1}), pH of 8.50 and temperature of 17.3 °C (Mesophytic, Province/Southern Puna, Mesophytic Sector).


A. catenatum
A. tropicocatenatum
Abundance
0.00
0.02
0.04
0.06
5.4
6.0
6.6
7.2
7.8
8.4
pH
A. catenatum
Abundance
0.0
0.5
1.0
1.5
pH
A. tropicocatenatum
Abundance
pH

A)


A. catenatum
A. tropicocatenatum
Abundance
0.00
0.02
0.04
0.06
0.08
0.10
100
200
300
400
Total phosphorus (ppm)
A. catenatum
Abundance
0.0
0.3
0.6
0.9
1.2
1.5
1.8
Total phosphorus (ppm)
A. tropicocatenatum
Abundance
Total phosphorus (ppm)

B)



C)

Figure 9 -- Relative abundance (%) of *Achnanthidium catenatum* (black lines) *Achnanthidium tropicocatenatum* (red lines) in São Paulo reservoirs, Brazil and relationships between pH (A), total phosphorus (B; µg L^{-1}) and conductivity (C; µS cm^{-1}). Percentages based in counting of at least 400 valves per sample. Lines indicates weighted average optima.

Table 3 -- Comparison between *Achnanthidium tropicocatenatum* and morphologically similar species.

	***Achnanthidium* sp. nov.**	***A. lusitanicum* Novais & M.Morais**	***A. catenatum* (J.Bílý & Marvan) Lange-Bertalot**	***A. minutissimum* (Kützing) Czarnecki**
Reference	This study	Novais et al. (2015)	Hlúbiková et al. (2011)	This study
Valve length (µm)	10.3--23.9	5.3--13	10--17.5	11.7–16.9
Valve width (µm)	2.7--3.5	2.3--3.0	2.8--3.6	2.6--3.3
Valve outline	Linear-lanceolate, slightly inflated in the central portion	Elliptic, linear-elliptic to linear-lanceolate, slightly inflated in the central portion	Slender with widened central portion resulting in an undulated valve margin	Linear-elliptic to linear-lanceolate
Valve ápices	Protracted, subcapitate to capitate	Protracted, rostrate and broadly rounded	Broadly capitate to subcapitate	Protracted, rostrate to subcapitate
Striation pattern	Radiate throughout the valve, denser towards the apices	Slightly radiate in the central portion, more radiate and denser towards the apices	Radiate in the central portion and weakly radiate or almost parallel, denser towards the apices	Radiate, denser towards the apices
Raphe valve				
Central área	Small rounded, bordered by one more widely spaced stria on one or both sides	Small rounded	Rounded, almost absent	Almost absent, slightly rounded or as a rectangular fascia
Striae (in 10 µm)	36--40 (up to 45 near the apices)	35 (up to 40 near the apices)	30--32	30 (in the middle)
Number of areolae (per striae)	3--6	3--5	4--6	-
Rapheless valve				
Central area	Indistinct or narrow lanceolate	Absent or small, elliptical	Small, lanceolate to rhombic	Indistinct or narrow lanceolate
Striae (in 10 µm)	38--40 (up to 55 near the apices)	30--35	30--34	30--34 (in the middle)
Number of areolae (per striae)	3--6	3--4 (5--6)	4--6	-

Geometric morphometry -- Morphological differences among the four studied populations were evidenced by their valve shape analysis (fig. 10). Similarity tests (NPMANOVA), performed on the Cartesian coordinates of resulting groups in the PCA, revealed statistically significant differences (*p* values: <0.001, <0.01, <0.05) between *A. minutissimum* and *A. catenatum* compared with the *A. tropicocatenatum* type and Jurupará populations, both of which belong to the same species (table 4).

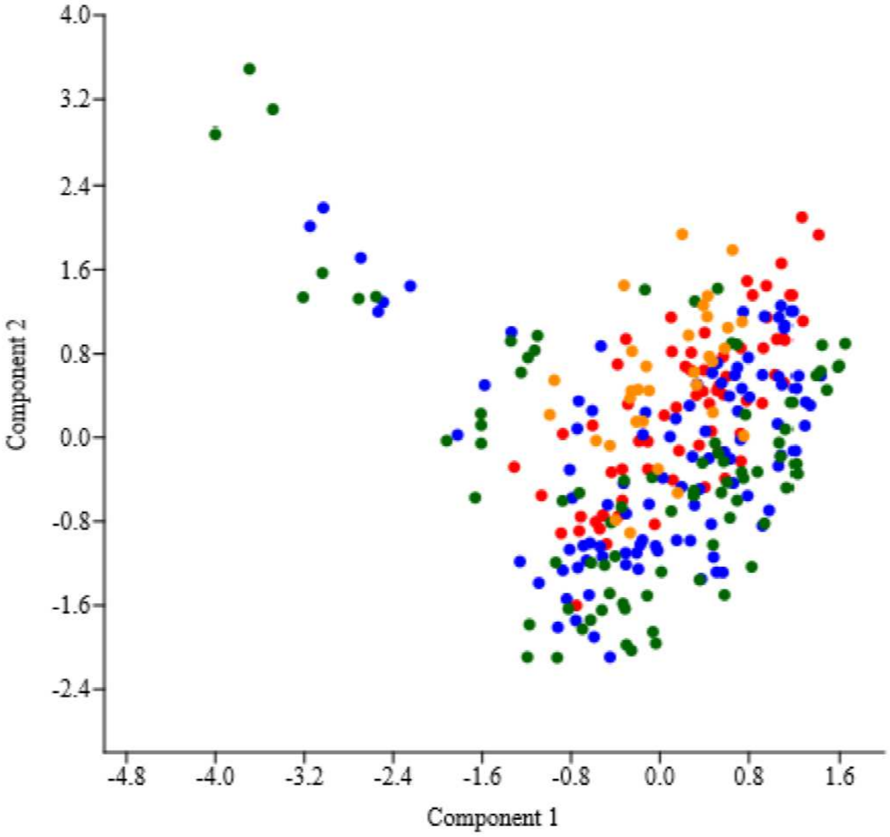


Figure 10 -- PCA plot of Procrustes-transformed valve outline pseudolandmark coordinates among *A. catenatum* (32 valve measurements) and *A. minutissimum* (68 valve measurements) lectotype materials, *A. tropicocatenatum* (population from JP reservoir, 100 valve measuments), and from *A. tropicocatenatum* (type material from FR reservoir, 83 valve measuments). Legend symbols: orange: *A. catenatum*, red: *A. minutissimum*; blue: *A. tropicocatenatum* (JP) and green: *A. tropicocatenatum* (type material).

Table 4 -- NPMANOVA test performed on morphological differences resulting groups in the PCA using the Chord distance measure. *P* values shown (<0.001, <0.01, <0.05).

	A. tropicocatenatum (Jurupará)	*A. tropicocatenatum* (type material)	*A. catenatum* (type material)
A. minutissimum (type material)	<0.05	<0.01	<0.05
A. tropicocatenatum (Jurupará)	---	0.4218	<0.01
A. tropicocatenatum (type material)	---	---	<0.01

DISCUSSION

Achnanthidium tropicocatenatum clearly belongs to the complex of species around *A. minutissimum* based on the simple and straight distal raphe endings, contrary to the species in the *A. pyrenaicum* group that have clearly deflected to even hooked distal raphe fissures (Kobayasi 1997, Potapova & Hamilton 2007, Van de Vijver & Kopalová 2014). This, widely distributed species in Brazilian reservoirs is characterized by a set of distinct morphological and ecological features that clearly separate it from all other similar *Achnanthidium* species.

Landmark-based geometric morphometrics was a powerful tool in quantifying the shape variation in the studied species. Comparison and examination of *A. tropicocatenatum* and related species (type materials) showed significant overlapping of valve outlines (fig. 10) making the identification based only on LM difficult. However, NPMANOVA statistically demonstrated differences in valve outline among them, supporting the placement of *A. tropicocatenatum* as a new taxon. The geometric morphometric approach has been increasingly applied in diatoms as a complementary tool for taxonomy, being very useful in the separation of morphologically similar taxa, either with *Achnanthidium* (e.g. Potapova & Hamilton 2007) or other genera (e.g. Falasco et al. 2009, Cejudo-Figueiras et al. 2011, Peng et al. 2014, Wengrat et al. 2015, Urbánková et al. 2016).

Currently, *A. minutissimum* s.l. is regarded as a very generalized indicator species with a seemingly broad ecological tolerance and different North American *Achnanthidium* morphotypes exhibit different ecological preferences that could be useful in biomonitoring (Potapova & Hamilton 2007, Pinseel et al. 2017). The correct identity and biogeographical distribution of this taxon have long been obscured by force-fitting and taxonomic drift (Van de Vijver & Kopalová 2014). A recent study of Pinseel et al. (2017) has argued that the implementation of molecular data in the taxonomy of *Achnanthidium* will be essential to solve the taxonomic problems associated with this group may result in better understanding of the biogeography and niche differentiation of different species belonging to the *A. minutissimum* complex. In Brazil, the species have been reported especially for the South (e.g. Bertolli et al. 2010, Faria et al. 2010, Marra et al. 2016, and Nardelli et al. 2016; all of them in Paraná State) and the Southeast region (e.g. Faustino et al. 2016; São Paulo State).

However, the specimens represented as *A. minutissimum* by Ferrari & Ludwig (2007) for the Ivaí basin (Paraná State) seem to represent two distinct taxa and probably the diatom illustrated in the fig. 30 is closer to *A. catenatum*. Likewise, the images 120--122 in Silva et al. (2010) from the Iraí reservoir, Paraná state (eutrophic system) are not related to *A. minutissimum*.

Similarly, *Achnanthidium catenatum* is also an indicator of organic pollution (Berthon et al. 2011). The species is widely reported from around the world and it is the only planktonic species of the genus, so records of blooms in which *A. catenatum* is dominant are not uncommon (e.g. Straub 2002, Ma et al. 2013).

Nevertheless, its distribution in Brazil is uncertain and *A. catenatum* have been recorded by diatomists especially in samples from the South and the Southwest regions of the country. However, some of the cells illustrated bear little resemblance to *A. catenatum.* Mostly cited to São Paulo localities (e.g. Faustino et al. 2016), this species has been reported as an indicator of an environmental shift particularly associated with the eutrophication process in paleolimnological reconstruction studies (Costa-Böddeker et al. 2012, Fontana et al. 2014). However, no illustration of the taxon was available. Also, *A. catenatum* was reported by Fontana & Bicudo (2012) from surface sediments in the cascading reservoirs of Paranapanema River (São Paulo and Paraná States, Brazil). Nevertheless, the species is described to possess smaller dimensions for length (9.6--9.8 µm) and valve width (1.4--1.7 µm), not compatible with the *A. catenatum* lectotype material measurements (table 3). Finally, the species reported by Marra et al. (2016) (figs 110--112) in a mesotrophic reservoir in Paraná State is probably conspecific with *A. tropicocatenatum*.

Originally applied to paleolimnological research, weighted-averaging regression and calibration methods (Birks et al. 1990) have been increasingly used to quantify relations between species and various environmental variables (Kelly & Whitton 1995, Pan & Stevenson 1996, Leland & Porter 2000, Winter & Duthie 2000, Leland et al. 2001, Potapova et al. 2004). *A. tropicocatenatum* not only differs morphologically but it is also ecologically distinct compared with *A. catenatum* and *A. minutissimum*. Thereafter, the presence of a taxon can be used to indicate the probable availability of its preferred conditions at the time of collection. However, if subsequently this taxon is confused with, or wrongly assumed to include another taxon with different and unknown ecological preferences (perhaps because fine morphological differences have not been noted), records of these algae will not indicate the prevalence of any particular conditions, and the bioindicator system breaks down (Cox 1987).

Achnanthidium minutissimum and *A. catenatum* are considered two of the most commonly reported taxa in floristic and ecological works worldwide. With increased use of diatoms as indicators of ecosystem health, establishing the identity of at least the most common taxa could improve the accuracy of ecological diagnostic tools relying heavily on the most inclusive taxonomic categories as the basic units expressing environmental change (Morales et al. 2013). The description of *A. tropicocatenatum* improves our knowledge about the biogeography of species in this complex and allows their use in more precise ecological and biogeographical studies.

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Capítulo 7

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Sellaphora tropicomadida sp. nov., a new freshwater diatom species (Bacillariophyta) from a tropical Brazilian reservoir

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Abstract

A new periphytic freshwater diatom species belonging to the genus *Sellaphora* Mereschkowsky has been recorded from a reservoir in São Paulo (Southeast Brazil) and is described as *S. tropicomadida* Marquardt & C.E.Wetzel, sp. nov. The new species most distinctive feature is the areolae internal ultrastructure, composed of large occlusions laid very close to the raphe, restricting an almost absent central area. *Sellaphora madida* (Kociolek) C.E.Wetzel in Wetzel et al. originally described from the U.S.A. (Oregon) has been recently collected from locations different from the type locality and presents a disjunct distribution, with confirmed records in subtropical regions of South America (southern Brazil) and Europe (Bulgaria). These specimens were compared with those of *S. tropicomadida* sp. nov. to highlight the differences between them. All populations are illustrated in detail with light (LM) and scanning electron (SEM) microscopes. Further comparisons of *S. tropicomadida* with similar species based on the literature and information about their ecology are also provided. We suggest careful examination of the small size dominant diatoms with SEM in routine monitoring, to get better results and increase taxonomic accuracy for ecological assessment, mainly concerning species with distinct ecological preferences, despite their morphological resemblance.

Keywords geographical distribution, new species, surface sediment, *Sellaphora*, taxonomy, ultrastructure

Introduction

Sellaphora Mereschkowsky has been a main focus of taxonomic investigations (e.g. Beszteri et al. 2001, Bruder & Medlin 2008, Evans et al. 2008, Mann et al. 2008, Poulíčková et al. 2008, Vanormelingen et al. 2013, 2015). Supported by several molecular studies (e.g. Novis et al. 2012, Zgrundo et al. 2013, Kulikovskiy et al. 2014), clades of related species previously placed in *Eolimna* Lange-Bertalot & W. Schiller in Schiller & Lange-Bertalot (1997) have been recently transferred to the genus *Sellaphora* (e.g. Wetzel et al. 2015). Its representatives are characterized by symmetrical, linear to elliptical valves, obtuse apices, distant terminal nodules, usually fine striae and a simple connecting zone (Beauger et al. 2016).

These small naviculoid diatoms belong to an ecologically important heterogeneous group with high abundance in freshwater benthic communities (Wetzel et al. 2015). Studies in Africa (e.g. Taylor & Lange-Bertalot 2006), Asia (e.g. Hustedt 1942), Europe (e.g. Coste & Ector 2000, Werum & Lange-Bertalot 2004, Blanco et al. 2009, Kulikovskiy et al. 2010, Beauger et al. 2016), North America (e.g. Kociolek et al. 2014) and Oceania (Moser et al. 1998, Novis et al. 2012) present the high richness of *Eolimna* with most of the described species, whereas tropical small naviculoid diatom reports are scarce and insufficiently documented.

In Brazil, despite the increasing number of taxonomic studies in a number of published (e.g. Ferrari et al. 2009, 2014, Garcia 2010, Wetzel et al. 2010, 2015, Tremarin et al. 2012, 2013, 2014, 2015, 2016, Silva et al. 2013, Marquardt & Bicudo 2014, Pereira et al. 2014, 2015, Talgatti 2014, Wetzel & Ector 2014, Almeida et al. 2015, 2016, Silva & Souza 2015, Wengrat et al. 2015, 2016, Bertolli et al. 2016, Marquardt et al. 2016) and unpublished (e.g. master thesis and doctoral dissertations) articles on diatoms, no records of new *Sellaphora* species have been published. According to Flora do Brasil (2020, under construction) and recent searches for available manuscripts, among the small naviculoid representatives, *S. nigri* (De Notaris) C.E. Wetzel & Ector in Wetzel et al. (2015) (= *E. minima* (Grunow) Lange-Bertalot in Moser et al. 1998) has been one of the most registered species (e.g. Leandrini et al. 2002, Ludwig et al. 2005, Moresco et al. 2011), although sometimes just in listed without illustration or/and description of the taxa (e.g. Rodrigues 1991, Souza & Oliveira 2007, Moresco & Rodrigues 2014). Other records include *S. neocaledonica* (Manguin ex Kociolek & Reviers) C.E. Wetzel, Ector, Van de Vijver, Compère & D.G. Mann [≡ *E. neocaledonica* (Manguin) Gerd Moser, Lange-Bertalot & Metzeltin in Ludwig et al. 2005] and *S. submuralis* (Hustedt) C.E. Wetzel, Ector, Van de Vijver, Compère & D.G. Mann [≡ *E. submuralis* (Hustedt) Lange-Bertalot & Kulikovskiy in Kulikovskiy et al. 2010 in Bartozek et al. 2013]. However, both species records lack SEM images.

During a diatom survey in reservoirs of São Paulo State, an unknown species morphologically similar to *Sellaphora madida* (Kociolek) C.E. Wetzel in Wetzel et al. (≡ *E. madida* Kociolek in Kociolek et al. 2014) is found and described here as *S. tropicomadida* sp. nov. Moreover, *S. madida*, which is a recently described species, first collected from Sparks Lake, Oregon, is recorded for the first time in Brazil (Rio Grande do Sul) and Europe (Bulgaria). In this paper we describe and compare size variability, frustule morphology and ultrastructural detail based on LM and SEM analyses of all these *Sellaphora* populations to better determine their differences and to justify the establishment of the new species *S. tropicomadida*. We also compare the morphological features of the *S. tropicomadida* with similar taxa based on the literature. Information about its ecology is also provided.

Methods

Study areas

Jurupará reservoir (23°56'01.8"S, 47°22'18.4"W) is located in the Ribeira do Iguape and Litoral Sul basin, in Piedade Municipality (781 m a.s.l.), southwestern São Paulo in a protected area in PEJU State Park (Parque Estadual do Jurupará). The main reservoir water is for power generation but it is also used for recreation and fish farming activities. The reservoir has an area of 3.9 km² and its accumulation capacity is up to 4.10^6 m³ (information from Votorantim Energia) (Fig. 1).

According to the Trophic State Index (TSI) for reservoirs (Lamparelli 2004) based on the abiotic and biotic analyses, this reservoir is currently classified mostly as mesotrophic.

A population of *S. madida* from Pardinho river (Pardo River hydrographical basin, Sinimbú, Rio Grande do Sul, Brazil, 52°31'00.69" W, 29°31'30.86" S) and from Bulgarian river (Maritsa̓s basin, Luda River, 41°24’14.7” N, 26°09’40.6” E, at 64 m a.s.l.) (Tsvetelina Isheva sample) was also studied (Fig. 2).

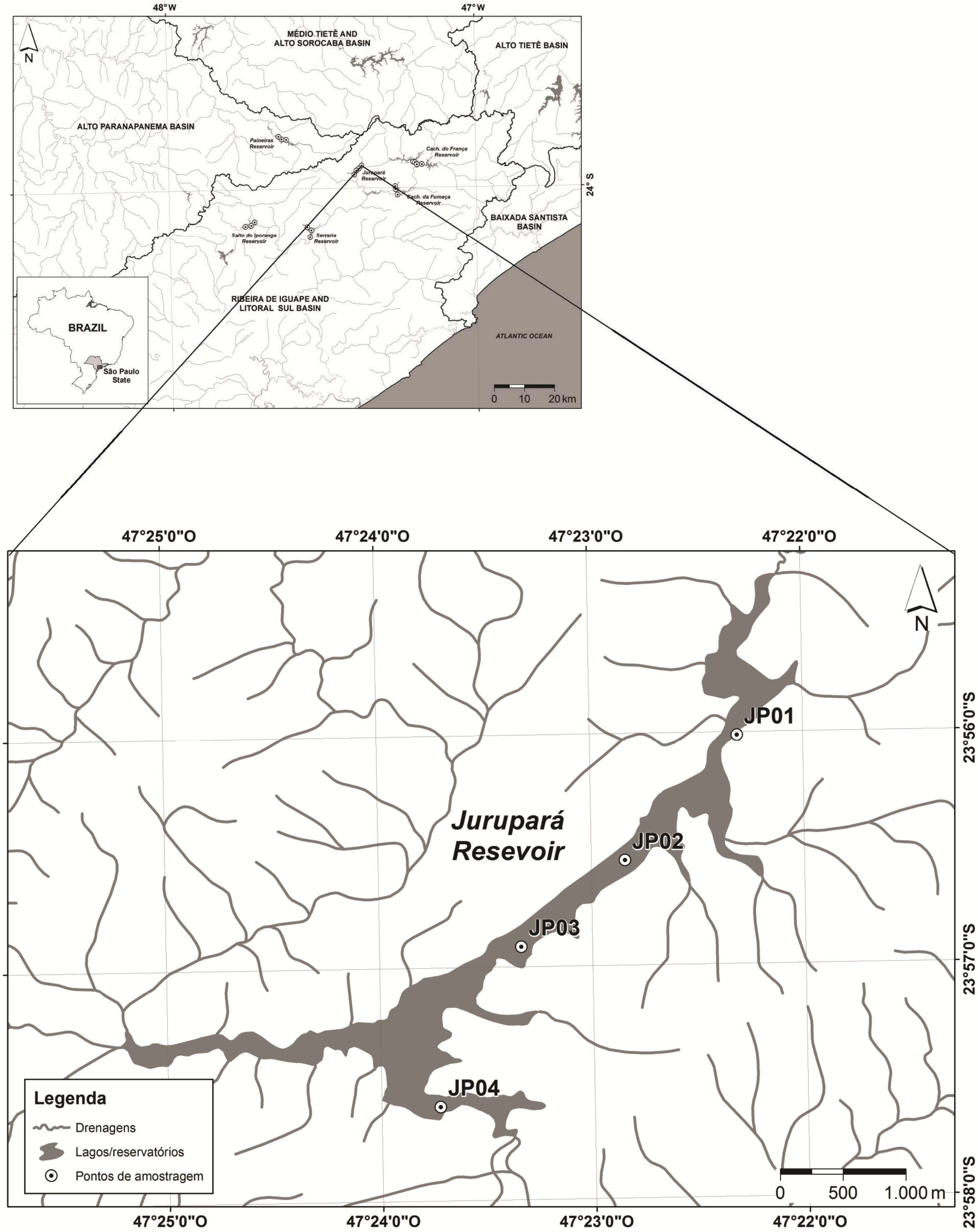


Fig. 1. “Ribeira do Iguape and Litoral Sul” basin and location of Jurupará reservoir.

Sampling design and slide preparation

Samples from the Jurupará reservoir were collected during the summer (January 2014). Environmental parameters were measured in the field with a multiparameter probe (Horiba U-53) and the analytical procedures followed Standard Methods (APHA 2005). Chlorophyll a (µg L^{-1}) corrected for phaeophytin was measured with 90% ethanol (Sartory & Grobbelaar 1984). Limnological characteristics are listed in Table 1.

Table 1. Limnological characteristics of the sampling sites located at the Jurupará Reservoir. Annual mean for four sampling sites during winter (June 2014) and summer (January 2014). Values are means ± standard deviation.

pH	6.4±1.3
Dissolved oxygen (mg O_2 L^{-1})	7.8±0.8
Conductivity (µS cm^{-1})	24.0±2.8
Soluble reactive silica (mg Si-SiH_2O_2)	3.9±0.3
Total phosphorus (µg L^{-1} TP)	19.5±4.2
Total nitrogen (µg L^{-1} TN)	464.3±89.4

Periphytic material was scraped off stones and processed by hot digestion with hydrogen peroxide (35%) and hydrochloric acid (37 %) (Battarbee et al. 2001). Samples from the Pardinho river were brushed off stones and the resulting suspension was collected in a 150 ml plastic sample bottle. A portion of the sample was oxidized in a boiling mixture of 2:1 nitric and sulphuric acid, and rinsed (Taylor & Lange-Bertalot 2006).

Cleaned and rinsed samples were mounted in permanent slides with Naphrax. LM observations and morphometric measurements were performed with an optical Leica DMRX microscope with a 100x oil immersion objective. Light micrographs were taken with a Leica DC500 camera. For SEM observations, the cleaned samples were filtered with additional deionized water through a 3-µm Isopore polycarbonate membrane filter (Merck Millipore). The filter was mounted on aluminium stubs and coated with platinum with a Modular High Vacuum Coating System BAL–TEC MED 020 (BAL–TEC AG, Balzers, Liechtenstein). An ultrahigh-resolution analytical field emission (FE) scanning electron microscope Hitachi SU–70 (Hitachi High-Technologies Corporation, Japan) operated at 5 kV and 10 mm distance was used for the analysis. SEM images were taken with the lower (SE-L) and upper (SE-U) detector signal. Micrographs were digitally manipulated and plates containing light and scanning electron microscopy images were created with CorelDraw X7.

Relative abundance of the new species was estimated according to Battarbee et al. (2001) with a minimum of 400 valves counted per slide at 1000× magnification on up to six random transects and until reaching efficiency of at least 90% (Pappas & Stoermer 1996). All slides used for diatom identification and enumeration were deposited at the "Herbário Científico do Estado Maria Eneyda P. Kauffmann Fidalgo" (SP), São Paulo State Department of Environment, Brazil.

The symbols "≡" and "=" before the names of specific and infraspecific taxa are used to represent homotypic or nomenclatural, and heterotypic or taxonomical synonyms, respectively.

Morphological terminology followed Round et al. (1990), Kociolek et al. (2014) and Wetzel et al. (2015).

Results and discussion

Sellaphora tropicomadida **Marquardt & C.E.Wetzel, sp. nov. (Figs 2–23)**

Valves small, elliptical to oval with broadly rounded apices (Figs 2–17). The valve face is flat and the mantle very shallow (Figs 18–20). Valve length 5.2–8.3 µm, width 3–3.7 µm. Striae uniseriate and radiate throughout the entire valve, not resolved in LM; 40 in 10 µm; striae bordering the central area slightly curved (Figs 18–20). Shorter striae (usually 3), often with uneven lengths, are present in the very middle of the valve. Axial area linear, narrow. Central area small, symmetrical but irregularly 'bow–tie' shaped (Figs 18–20). In external view, raphe filiform, slightly curved, with tear–drop central pores slightly curved to one side of the valve whereas terminal fissures are deflected to the opposite side (Figs 18–20). In internal view, raphe straight; proximal raphe fissures are slightly deflected to the same side and the distal raphe fissures ends as small helictoglossae (Figs 21–23). Areolae closed by hymenes, slightly angular, quadrate to nearly rounded, smaller near the margins; 70 in 10 µm (Figs 18–23). Internally, large areolae almost touching the raphe occlusions (Figs 21–23). Internally, raised axial area; almost absent central area, X-shaped (Figs 21–23). Striae extending down onto a relatively shallow mantle (Fig. 20). Girdle bands lacking areolae (Fig. 20).

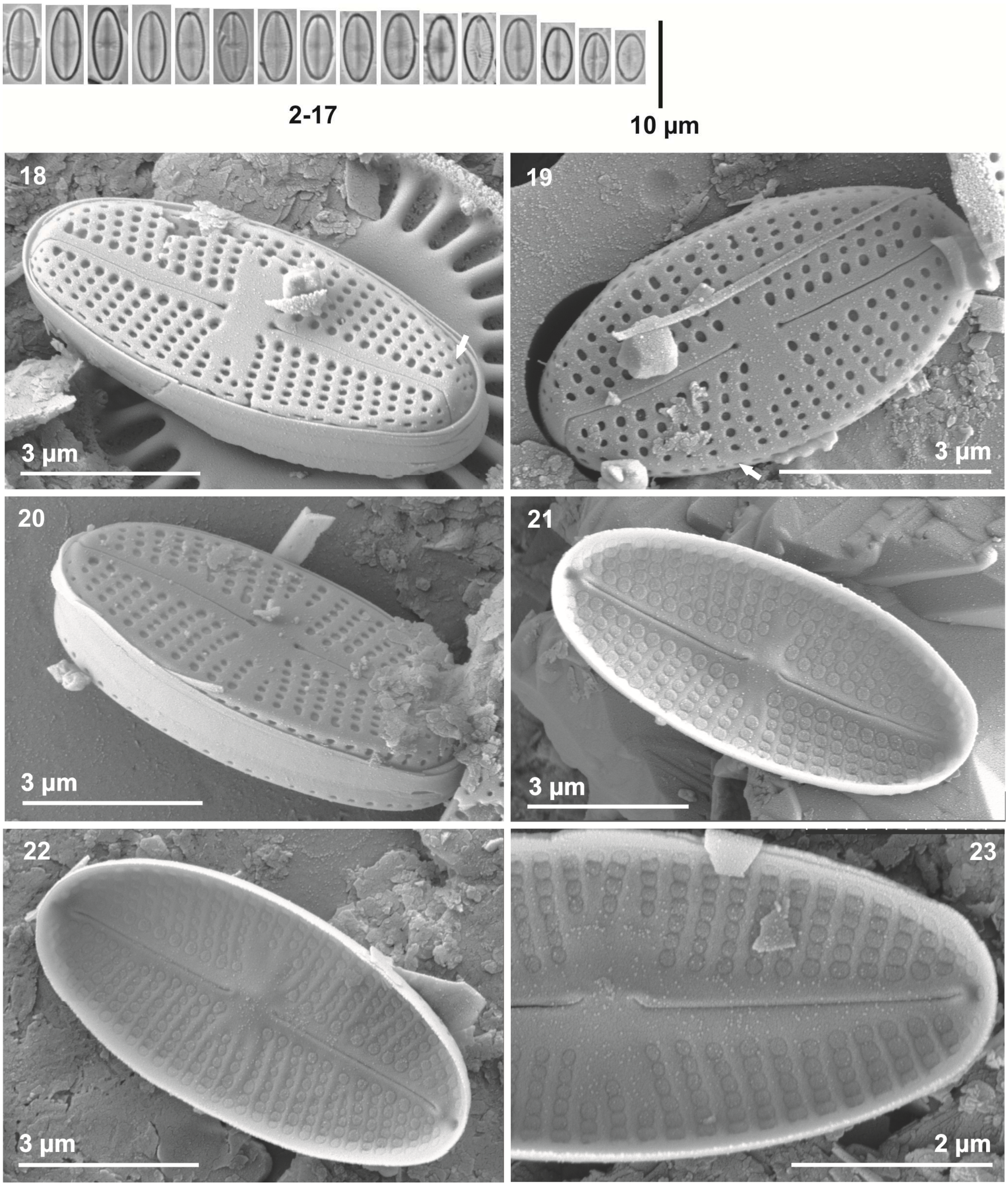
Type:–BRAZIL. São Paulo (SP), Piedade: Jurupará reservoir, periphyton, 23°56'01.8"S, 47°22'18.4"W, 781 m a.s.l. DC Bicudo and GC Marquardt, 28/I/2014 (holotype: SP!, slide 469435. Isotype: BR! slide BR-4471).

Etymology:–The epithet refers to the similarity with *S. madida*, but observed in a tropical region.

Ecology and associated taxa

Sellaphora tropicomadida presented a relative abundance of 4.67% in periphyton of Jurupará reservoir in alkaline waters with low levels of nutrient concentrations and high levels of dissolved oxygen (Table 1).

The co-occurring dominant diatom species (more than 2% relative abundance) in the sample were *Discostella stelligera* (Cleve & Grunow) Houk & Klee, *Aulacoseira tenella* (Nygaard) Simonsen and *Spicaticribra kingstonii* J.R. Johansen, Kociolek & R.L. Lowe.



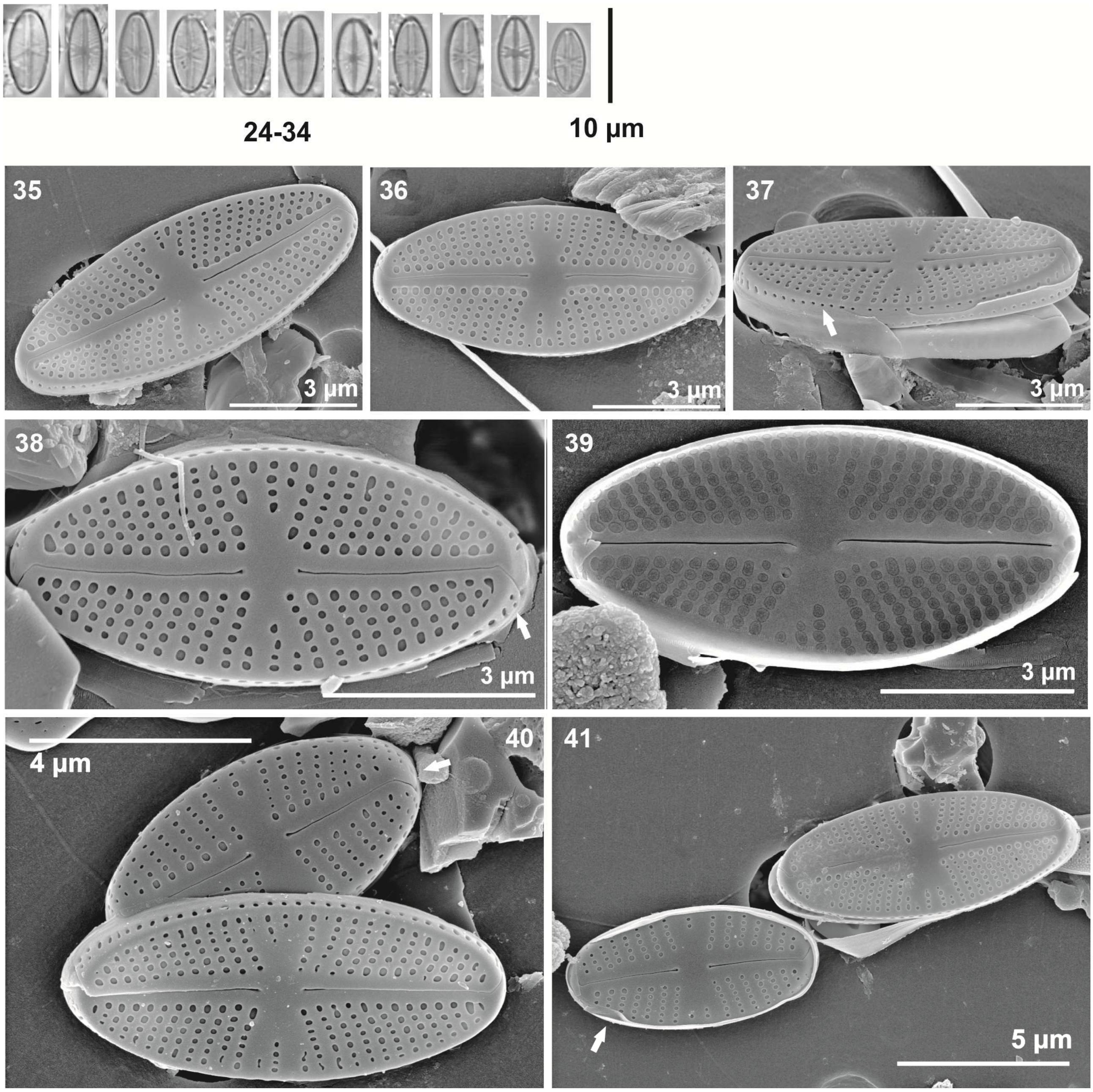
Figs 2–23. *Sellaphora tropicomadida* sp. nov. Light and scanning electron micrographs taken from the type material (SP469435). Figs 2–17. LM pictures showing the variation in size and valve outline. Scale bar = 10 µm. Figs 18–23. SEM pictures. Figs 18–20. External valve view of entire valve showing the raphe and areolae structure. Figs 18–19. Detail shows the areola which accompanies a stria near the valve face/mantle junction (arrows). Fig. 20. Detail of the valvar

mantle. Figs 21–23. Internal view showing the areolae occlusions, helictoglossae and the almost absent central area.

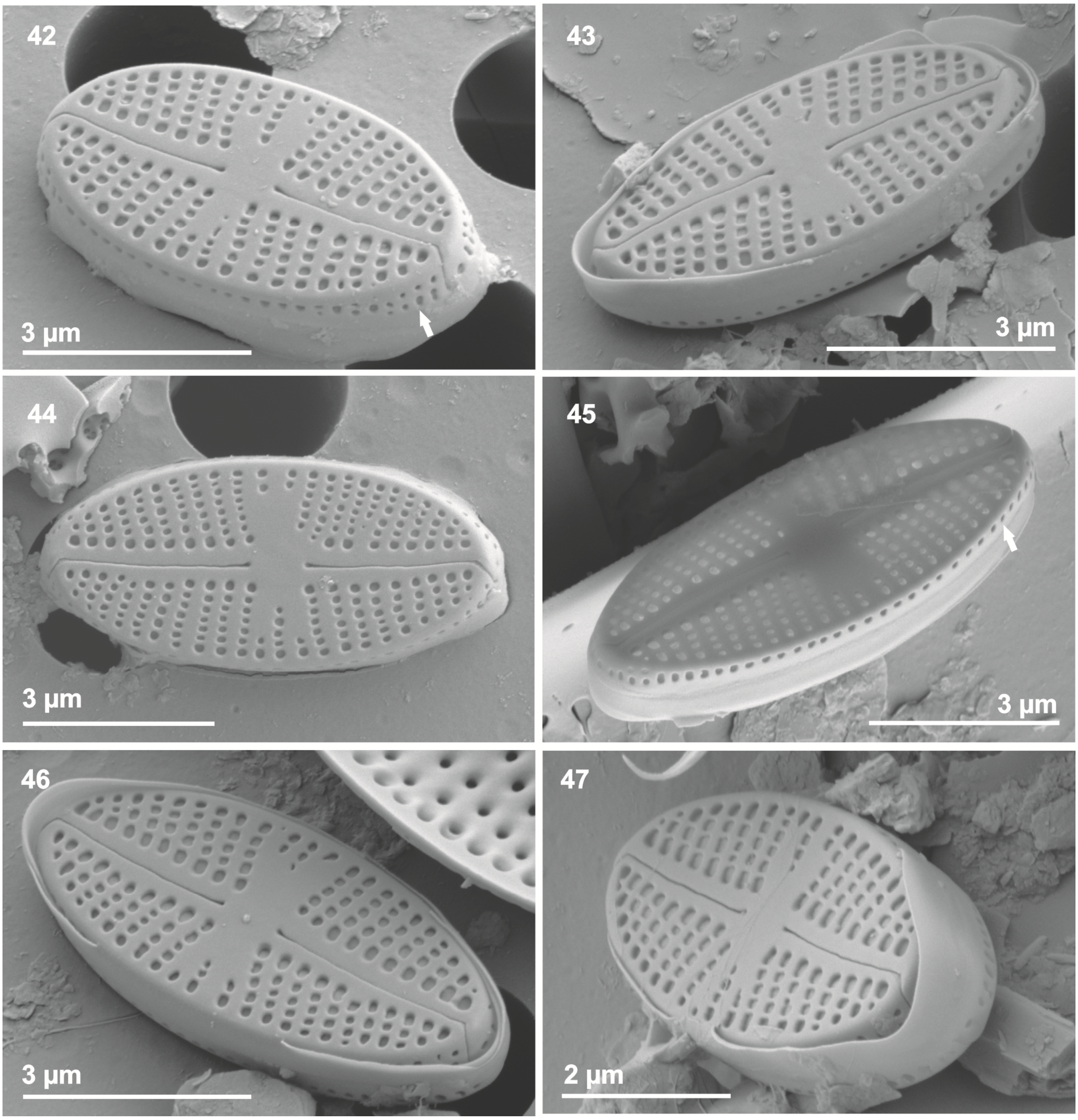
Taxonomical remarks and geographical distribution

Wetzel et al. (2015, p. 222–225) discuss at length the generic limits and concepts of *Eolimna* and *Sellaphora* (including the catch-all genus *Naviculadicta* Lange-Bertalot) and conclude that there seem to be no clear differentiation between extant species of the mentioned genera. Several molecular studies have shown that indeed some former *Eolimna* species belong to the genus *Sellaphora* (e.g. Novis et al. 2012, Zgrundo et al. 2013, Kulikovskiy et al. 2014).

The new *Sellaphora* found in the Brazilian sample is similar to *S. madida*. This species originally described from Oregon (Spars Lake) and also occurring in Wyoming (Fremont Lake) seems to have an interesting disjunct distribution. A population of *S. madida* from south Brazil was also observed. The sample was collected at the Pardinho River in southern Brazil (Figs 24–41) and it was also observed for the first time in Europe, from one river in the Maritsa's basin (Bulgaria) (Figs 42–47) where it was wrongly identified as *Psammothidium* cf. *rossii* (Hustedt) Bukhtiyarova & Round in Isheva & Ivanov (2016, figs 7–11). They also mixed *Achnanthidium* cf. *catenatum* in fig. 12. In this, the taxon was recorded with a relative abundance of 1.1% under low-flow conditions, occurring at 30°C water temperature, slightly alkaline pH (8.3), relative low specific conductivity (179 µS cm^{-1}), dissolved oxygen content 8.2 mg L^{-1} and 94% oxygen saturation, on a silicate substrate. The river is under very low anthropogenic impact (near reference conditions), but the environmental conditions change during low-flow periods due to significantly lower levels of surface water verging on an almost complete lack of flow, with the presence of stagnant pools. The associated taxa with the highest relative abundance were *Epithemia sorex* Kützing (21%), *Rhopalodia gibba* (Ehrenberg) O.Müller (8%), *Nitzschia inconspicua* Grunow (6%) and *N. elegantula* Grunow in Van Heurck (5%), common diatoms for lentic environments and/or such environments with moderate (high) trophic levels (Isheva & Ivanov 2016).



Figs 24–41. *Sellaphora madida*. Light and scanning electron micrographs taken from Pardinho River (Rio Grande do Sul, Brasil) material. Figs 24–34. LM pictures showing the variation in size and valve outline. Scale bar = 10 µm. Figs 35–41. SEM pictures. Figs 36–38, 40. External valve view of entire valve showing the raphe and areolae structure. Fig. 37. Detail of the valvar mantle showing the areola at the interstria (arrow). Fig. 39. Internal view showing the areolae occlusions, helictoglossae and the x-shaped central area. Fig. 38, 40. Detail of the valvar mantle showing a double row of areolae (arrow). Figs 40- 41. *Sellaphora nigri* (arrow), a different taxa that can easily be misidentified at first sight. Fig. 42. Detail of a double row of striae.



Figs 42–47. *Sellaphora madida*. Scanning electron micrographs taken from Luda River material. Figs 42–47. External valve view of entire valve showing the central area, raphe and areolae structure. Fig. 42. Detail of the valvar mantle showing a double row of areolae (arrow). Fig. 45. Detail of the valvar mantle showing the areola at the interstria (arrow).

Even though these are only few observations, the current distribution of *S. madida* is quite interesting (Fig. 48). Disjunct distribution of diatoms is not unusual and several recent publications report it (e.g. Szabó et al. 2004, Cremer et al. 2011, Lowe et al. 2013). One possible reason is the undersampling. The other is the confusion with other similar taxa or the transport by wind, animals (e.g. birds or fishes) or other human activities (e.g. aquacultures). The inherent difficulty in the identification in light microscopic studies is presumable and the use of SEM may reveal the presence of different taxa that can easily be misidentified at first sight (Figs 40–41). However, we cannot reject the possibility that alien diatom species might at times be transported outside their native range through human activities (Mann 2015). It is noticeable that subtropical freshwater diatom species can find their living conditions in natural surface waters in temperate zone as well, which might be connected with global warming (Coste & Ector 2000).

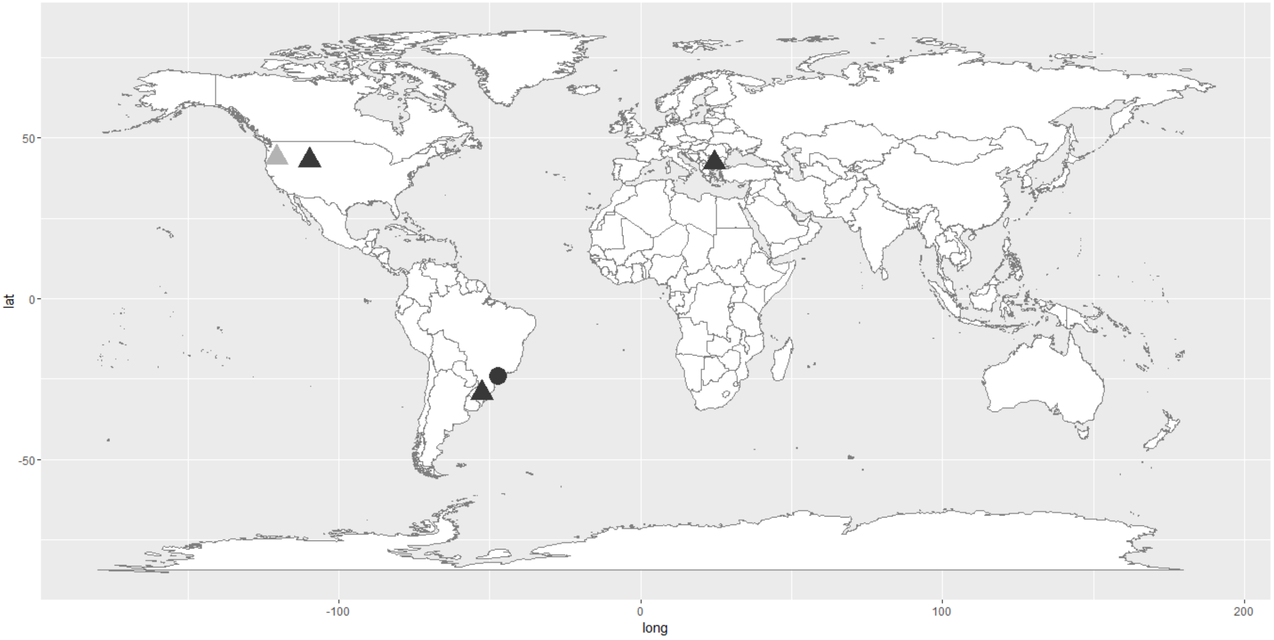


Fig. 48. Distribution records of *Sellaphora madida* (black triangle; type locality: grey triangle) and *S. tropicomadida* (type locality: black circle).

Despite the same valve width and length of both taxa, in general *S. tropicomadida* and *S. madida* can be easily differentiated by the valve outline, clearly elliptical and broadly rounded in the new species (Figs 2–17) and clearly tapered at the apices in *S. madida*, although this feature is less evident in the Pardinho River population than in the type material (Figs 24–41). Moreover, *S. tropicomadida* has a distinctly higher density of striae in 10 µm (Table 2), making it harder to be observed in LM. A higher number of areola in 10 µm (Table 2) is also observed. Additionally, in the new species *S. tropicomadida* each areola accompanies a stria near the valve face/mantle junction (Fig. 19) whereas in *S. madida* a supplementary areolae in each interstria (Fig. 44) can be observed. In addition, a double row of striae is commonly observed near the *S. madida* apices (Fig. 41). Another distinctive feature of the new species is the internal ultrastructure of the areolae, composed of large occlusions almost reaching the raphe and restricting an almost absent central area (Figs 18–23). This pattern was not observed in any other related species, usually characterized by a wider X-shaped central area (Table 2).

Sellaphora tropicomadida is also similar to *S. nigri*. However, the latter has larger valve dimensions and a small number of striae in 10 µm that seems clearly solved when observed in LM (Tab. 2). Internally, the areolae are quite smaller in *S. nigri*. Further, this taxon has a larger axial area, widening near the central portion of the valve (Tab. 2). Finally, *S. nigri* has a wider X-sapped central area almost reaching the border of the valve (Tab. 2). Another similar species, *S. atomoides* (Grunow) C.E.Wetzel & Van de Vijver in Wetzel et al. can be easily differentiated by a wider central and axial area and smaller number of striae in 10 µm (Tab. 2).

Apart from *Sellaphora* and especially due to its small size and similar valve outline, *S. tropicomadida* can be confused with some species in the genera *Mayamaea* Lange-Bertalot, such as *M. atomus* var. *alcimonica* (E. Reichardt) E. Reichardt in Lange-Bertalot (1997) and *M. agrestis* (Hustedt) Lange-Bertalot (2001). However, it is important to note that representatives of these genera have developed polar nodules that are visible as white dots under LM (Morales & Manoylov 2009) (Tab. 2). Besides, *S. tropicomadida* shows a higher striae density in 10 µm (Tab. 2).

Table 2. Morphological and metric variation of related *Sellaphora* species.

	***Sellaphora tropicomadida* sp. nov.**	***S. madida***	***S. nigri***	***S. atomoides***	***Mayamaea atomus* var. *alcimonica***	***M. agrestis***
Reference	This study	Kociolek et al. (2014)	Wetzel et al. (2015)	Wetzel et al. (2015)	Reichardt (1984)	Lange-Bertalot (2001)
Valve outline	elliptical to oval	rhombic-lanceolate	elliptical to oval	elliptical to oval	elliptical to oval	elliptical to oval
Apices	broadly rounded	Rounded	broadly rounded	broadly rounded	rounded	Rounded
Length	5.2–8.3	6–9	3.7–13.0	3.4–16.3		9–11
Width	3–3.7	3–3.5	2.7–4.8	2.6–3.7		(2.5) 3–3.8
Raphe	slightly undulate, proximal fissures: tear–drop; distal fissures: strongly bent to the same side of the valve	straight, proximal fissures very small expanded; distal fissures: strongly bent to the same side of the valve	straight, proximal fissures: tear–drop; distal fissures: strongly bent to the same side of the valve	straight, proximal fissures: tear–drop; distal fissures: strongly bent to the same side of the valve	straight, proximal fissures: tear–drop; distal fissures: strongly bent to the same side of the valve	Straight proximal fissures: tear–drop; distal fissures: strongly bent to the same side of the valve
Axial area	narrow, straight	narrow, straight	linear, wider at the central area	linear, wider at the central area	narrow, linear, thickened	narrow, linear, thickened
Central area	small x-shaped	wide x-shaped	wide x-shaped	wide x-shaped	small, rounded and limited by shorter striae	rounded and limited by shorter striae
Striae (10 µm)	32–40	28	25–32	30–36	28	24–28
	uniseriate and radiate, curved at the central area	uniseriate and radiate, curved at the central area	uniseriate, radiate, curved at the central area	uniseriate and radiate, curved at the central area	uniseriate and radiate	uniseriate and radiate
Aerolae (10 µm)	70	50	---	---	---	---
Areolae pattern	larger closest to the raphe; slightly angular, quadrate to nearly rounded; smaller near the margins; closed by vela; large and rounded internally	nearly rounded; closed by vela; large and rounded internally	larger closest to the raphe; slightly angular, quadrate to nearly rounded; smaller and circular near the margins; closed by vela	usually larger closest to the raphe; rounded and smaller near the margins	rounded	Rounded
Areolae mantle	one per striae	one per striae and one at each interstriae; sometimes double row of areolae	one per striae	one per striae	---	---

The analyses of the samples collected from Brazilian reservoirs have revealed a very diverse flora, with unknown and unique diatom species highlighting the importance of evaluating the identity and the distribution of tropical diatoms. Also, our studies have shown the importance of the routine use of SEM in the structure of small diatoms. Deeper knowledge of the flora inhabiting this insufficiently investigated region and its colonization pattern will play a key role for the preparation of specific management guidelines and devices.

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Capítulo 8

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Morphology and distribution of *Encyonema angustecapitatum* Krammer species complex (Bacillariophyceae) with description of four new species from São Paulo, southeast Brazil

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MARQUARDT et al.: ***Encyonema angustecapitatum* species complex**

Abstract

Type material of *Encyonema angustecapitatum* KRAMMER was investigated to establish the identity of several Brazilian *Encyonema* populations. In order to elucidate the differences and similarities of that species complex, morphological features under light and scanning electron microscopy were detailed. Notes on their distribution were also discussed. MDS analysis distinguished four *Encyonema* species presently described as new to science: *Encyonema acquapurae* sp. nov., *Encyonema sparsistriatum* sp. nov., *Encyonema tenue* sp. nov. and *Encyonema paradisiacum* sp. nov. The four new species can be separated on the basis of a combination of the following morphological features: valve outline, axial area, valve length, valve width, length to width ratio, number of striae in 10 μm and number of areolae in 10 μm. Regarding ecological preferences, all four species were mainly found in oligotrophic and oligo-mesotrophic environments.

Key words: morphology, multidimensional scaling procedure (MDS), new species, taxonomy, type material, ultrastructure

Introduction

The biraphid diatom genus *Encyonema* KÜTZING (Cymbellales) has over 400 described taxa (FOURTANIER & KOCIOLEK 2011); 17 species and 5 varieties were recorded for Brazil (ESKINAZI-LEÇA et al. 2015), and five new taxa were recently described (TREMARIN et al. 2011; SILVA et al. 2013a, b; SILVA & SOUZA 2015; MARQUARDT et al. 2016). *Encyonema* species are very common in freshwater benthic communities with low electrolyte content (ROUND et al. 1990; KRAMMER 1997b).

Encyonema was separated from *Cymbella* C. AGARDH by KRAMMER (1997a, b) after an extensive revision of the cymbelloid taxa, including 50 new species and some material from Brazil (Tapajós River, Brazilian Amazon). The genus was proposed to designate dorsiventral individual specimens with distal raphe ends curved to the ventral margin, opposed to all *Cymbella* species. *Encyonema angustecapitatum* KRAMMER and *E. ponteanum* KRAMMER were described in the above contribution, both after the study of Venezuelan material. In general, this 'species complex' shows a more or less similar morphology characterized by having (i) strongly dorsiventral asymmetrical valve outline; (ii) small capitate ends; and (iii) narrow ventral axial area with central area absent (KRAMMER 1997b).

Several populations of the *E. angustecapitatum* complex were found in samples from São Paulo state (southeast Brazil) during a project that aimed at identifying the São Paulo state algal flora (BIOTA-FAPESP Program) and also during a paleolimnological investigation that aimed at contributing towards the diatom biodiversity and autecology for the establishment of environmental scenarios and policy maker's information (AcquaSed Project).

Analysis of *E. angustecapitatum* and *E. ponteanum* type material revealed the existence of four distinct *Encyonema* taxa that were misidentified at first sight. Such inadequate identifications improved the uncertainty of those species distribution besides affecting the accuracy of the diagnostic tools relying on diatom taxonomy and ecology. Such species are presently described as new to science using both light (LM) and scanning electron microscopy (SEM). Notes on their ecological preferences and distribution are also included. The description of the four new species contributes to the knowledge of their diversity, geographic distribution and morphology.

Material and Methods

The original gathering from Rio Caroni (Venezuela) containing both *Encyonema angustecapitatum* and *E. ponteanum*, i.e. sample 1114B in KRAMMER collection kept at the Hustedt Diatom Study Centre of the Alfred Wegner Institute, Bremerhaven (BRM), corresponding to the slide 1099C, was observed. The material was prepared only for SEM analysis since there was not enough raw material available. Morphological measurements of *E. ponteanum* were based on KRAMMER's (1997b) light microscopy photos.

Altogether, four sample units containing *E. angustecapitatum* complex representatives collected from semi-lentic and lentic environments and plankton, periphyton and surface sediments from different localities in the São Paulo state (southeast Brazil) were analyzed under LM and SEM.

Plankton samples were obtained with a 20 µm mesh nylon plankton net and a VAN DORN water sampler (VAN DORN 1956). Periphytic material was scrapped from stones and macrophytes (floating and/or submerged). Surface sediments (first superficial 2 cm) were collected using a gravity core (UWITEC). A list of all samples studied and their characteristics is given in Table 1. Also, their water chemical and physical data are compiled in Table 2.

Table 1. Data from sampling sites of *Encyonema angustecapitatum* complex, state of São Paulo, Brazil, habitat and the material number at the Herbarium of the Institute of Botany (SP).

Sample	Coordinate	Year of collection	Municipality	Site description	Habitat
SP188327	---	1989	Casa Branca	Marsh	Plankton
SP401589	223°47'1.62"S, 46°26'11.28"W	2009	São Paulo, Diadema, Ribeirão Pires, Santo André, SBC, RGS	Billings Reservoir, Rio Pequeno branch, upstream region	Benthos
SP468841	23°39'31.8"S, 45°49'23.22"W	2010	Salesópolis	Ribeirão do Campo Reservoir	Benthos

Table 2. Available ecological data from sampling sites of *Encyonema angustecapitatum* complex (mean values from the water column), State of São Paulo, Brazil. Cond. (conductivity). N-NH_4 (ammonium). TN (total nitrogen). TP (total phosphorus).

Sample	Temp. (°C)	pH	Cond. (µS cm^{-1})	N-NH_4 (µg L^{-1})	TN (µg L^{-1})	TP (µg L^{-1})
SP401589	17.8	5.1	31.9	137.8	632.9	<4.0
SP468841	16.8	5.5	---	24.1	362.0	<4.0

Permanent slides were prepared using BATTARBEE et al. (2001) technique, with heated peroxide hydrogen (H_2O_2 37%) to remove the organic matter. The reaction was further completed by addition of hydrochloric acid (HCl 37%) and following centrifugation cycles (1500 rpm) to rinse the acid excess. A permanent slide from the organic-free material was mounted using Naphrax (R.I. = 1.74). The diatom community was investigated using a Zeiss Axio Imager A2 light microscope (LM) at 1000× magnification, equipped with Differential Interference Contrast (DIC) and an AxioCamMR5 digital camera. For the scanning electron microscopy (SEM) analysis, a subsample of the cleaned material was dried out on filters mounted on aluminium stubs and coated with platinum using a BAL–TEC MED 020 Modular High Vacuum Coating System for 30 s at 100 mA. An ultra-high-resolution analytical field emission (FE) scanning electron microscope Hitachi SU–70 (Hitachi High-Technologies Corporation, Tokyo, Japan) operated at 5 kV and 10 mm distance was used for the analysis. SEM images were taken using the lower (SE-L) detector signal. Photomicrographs were digitally manipulated and plates containing light and scanning electron microscopy images prepared using the CorelDRAW Graphics Suite X7.

Morphological terminology and comparisons between species were mostly based on KRAMMER (1997a, b) and ROUND et al. (1990).

The species complex morphological differences were valued using Bray-Curtis similarity multidimensional scaling procedure (MDS) performed with R version 3.1.2 (R DEVELOPMENT CORE TEAM 2015) using the package ‘vegan’ package (OKSANEN et al. 2016) and an analysis of similarity (NPMANOVA) was performed between the values of resulting groups in the MDS using the Euclidean distance measure. A scatterplot matrix (n = 63) was achieved using the package ‘Performance Analytics’ (PETERSON et al. 2014). The following features were taken into consideration: (1) valve length (apical axis) and width (transapical axis), (2) length to width ratio, (3) number of dorsal striae in 10 μm, and (4) number of ventral striae in 10 μm (Fig. 1). Differences in morphometric data were examined using Kruskal-Wallis non-parametric “Rcmdr” test and the package version 2.1-7 (FOX 2005, 2007).

Holotype permanent slides, as well as the raw and cleaned samples were deposited at Herbário Científico do Estado “Maria Eneyda P. Kauffmann Fidalgo” (SP), São Paulo State Department of Environment, Brazil. Isotypes were deposited at BR, the Botanic Garden Meise, Belgium.

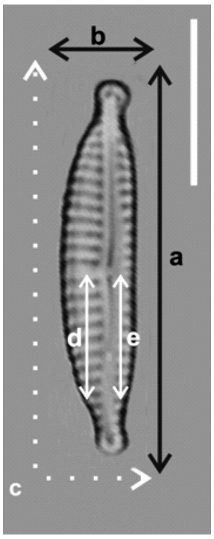


Fig. 1. Morphological differences considered in MDS procedure: a) valve length, b) valve width, c) length to width ratio, d) number of dorsal striae in 10 μm and e) number of ventral striae in 10 μm.

Results and Discussion

KRAMMER type material

***Encyonema angustecapitatum* KRAMMER**, Bibliotheca Diatomologica 37: 68, pl. 130, figs 8–15. 1997b (Figs 4–12).

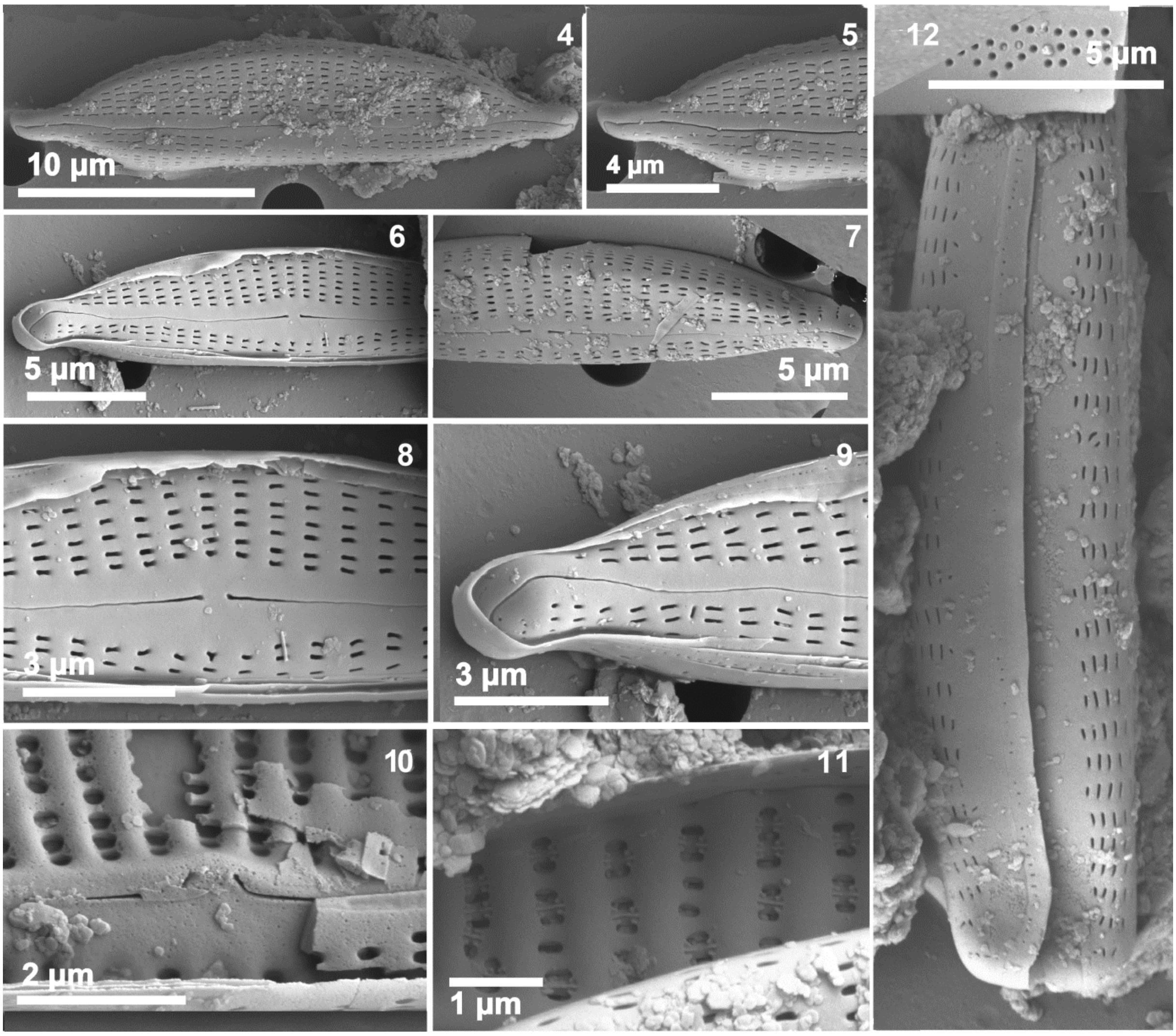
Type: Venezuela. Caroni river-Fährstellen. Leg. Rumrich, April 1, 1990. Holotype: 1099C IOK, housed at the Hustedt collection BRM(!). Corresponding to sample 1114B.

Scanning electron microscopy (SEM) (Figs 4–12): External raphe fissure slightly undulate (Figs 4, 6, 7). Proximal raphe end almost straight, the enlarged proximal endings slightly curved to the dorsal side (Figs 4, 6–8). Raphe distal ends are first dorsally bent, than strongly hooked to the ventral side ending onto the mantle (Figs 4–7, 9). Axial area is narrow, linear (Figs 4, 6, 7). Striae uniseriate composed by lineolae (Figs 4–12), numbering 36–38 in 10 μm. Internally, struts provide structural support to the foramen, whose opening bears three spines at each side (Figs 10, 11). Internally, the raphe fissure is interrupted by an intermissio (Fig. 10).

Distribution and ecology

Encyonema angustecapitatum and *E. ponteanum* are sporadically mentioned, suggestive of an underestimation of their real distribution. Register of *E. angustecapitatum* is in a specific webpage about diatoms, that includes illustration and information on its geographic distribution in the United States (Phycology Section, Patrick Center for Environmental Research, Academy of Natural Sciences of Drexel University. *Encyonema angustecapitatum* (NADED 110034). https://diatom.ansp.org/taxaservice/ShowTaxon1.ashx?naded_id=110034. Accessed 26 Oct 2016).

For South America, the species was reported by METZELTIN & LANGE-BERTALOT (1998) in a survey of material collected in the Tapajós River (Brazil). RUMRICH et al. (2000) documented *E. angustecapitatum* from the San Lucior pond (Colombia) and VOUILLOUD et al. (2010) from the Colombian Amazon material (Porvenir River, Amazonas). The latter was collected in a phytoplankton sample of a river with pH 6.3, conductivity of 10 μS cm^{-1}, Secchi depth of 62 cm and a temperature of 27.5 ºC. Then, MONTOYA-MORENO et al. (2013) through a bibliographical revision to recognize freshwater diatom species present in Colombia, registered the species for the sites already mentioned previously (Porvenir River, San Lucior pond) and also for Frontino moorland. However, no illustration nor description were provided.



Figs 4–12. Type material of *Encyonema angustecapitatum*. Scanning electron micrographs. All pictures taken from the holotype population (1114B). 4–9. SEM external views of entire valve showing the raphe and striae structure. 10, 11. SEM internal detail of areolae and raphe. Note the areolae with struts providing structural support to the foramen. 11. SEM internal detail of central area with intermissio. 12. SEM external view of girdle bands. Note the line of small pores.

Precisely for Brazil, only three *E. angustecapitatum* published records were found. Souza & Oliveira (2007) reported the species from an epilithic diatom floristic survey of the Paraná River Basin (Goiás State), but no illustrations or descriptions were provided. Marquardt et al. (2010) registered the species in a study of periphytic diatoms in the Rio das Pedras, located in Guarapuava (Paraná State). Nevertheless, this taxon differs from the type material for absence of shoulders especially on its ventral margin. Finally, Marquardt & Bicudo (2014) reported *E. angustecapitatum* during the floristic survey of the Cymbellales (Bacillariophyceae) from the Parque Estadual das Fontes do Ipiranga (PEFI), São Paulo city, southeast Brazil. Although a brief description and illustration of the above specimens were available, clear differences can be noticed when it is compared to the type material, such as the presence of a lanceolate, ventral axial area and the absence of well-defined shoulders.

The only register about *E. ponteanum* is, however, its original description in KRAMMER (1997b).

Formal descriptions of the new species

Encyonema acquapurae WENGRAT, MARQUARDT & C.E. WETZEL **sp. nov.** (Figs 13–26).

Light microscopy (LM) (Figs 13–21): Valves strongly dorsiventral. Dorsal margin broadly arched. Ventral margin linear. Apices narrow, subcapitate to capitate, sometimes slightly deflected to the ventral margins. Shoulders clearly developed. Length 20.8–27.4 µm. Width 5.0–6.0 µm. Length to width ratio 4.0–4.7. Axial area narrow, linear, wider on the ventral margin. Central area absent. Raphe filiform, lateral. Proximal fissures weakly expanded, curved to dorsal margin. Raphe distal ends strongly deflected to the ventral margin. Striae parallel to slightly radiate at the ends. Dorsal striae 13–14 in 10 µm, ventral striae 13–16 in 10 µm. Indistinct areolae. Stigmoid absent.

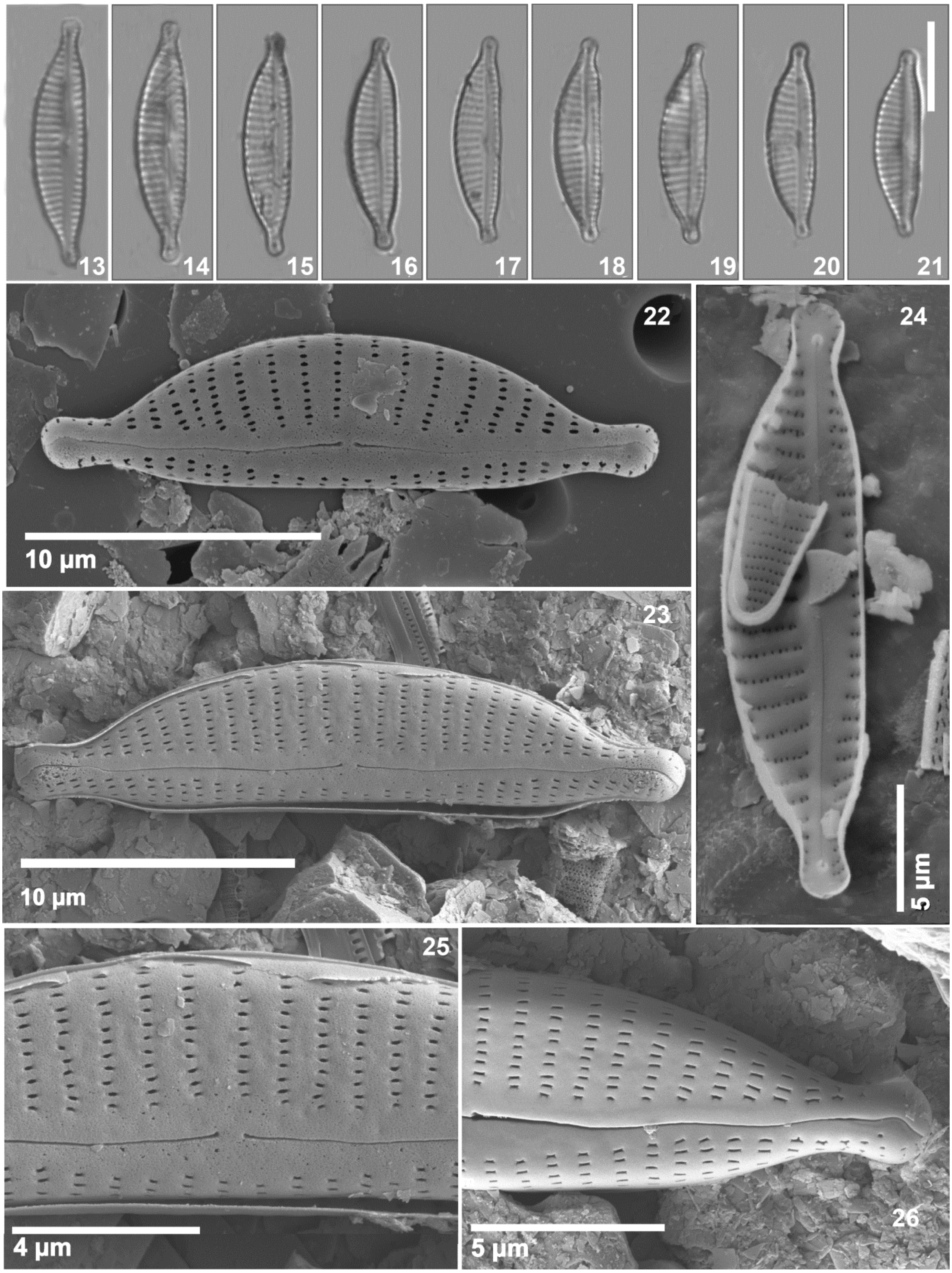
Scanning electron microscopy (SEM) (Figs 22–26): External raphe fissure slightly undulate (Figs 22, 23). Proximal end of raphe almost straight, the enlarged proximal endings slightly curved to the dorsal side (Figs 22, 23, 25). Raphe distal ends are first dorsally bent, then strongly hooked to the ventral side ending onto the mantle (Figs 22, 23, 26). Axial area is narrow, linear, and wider on the ventral margin (Figs 22, 23). Striae uniseriate composed by apically-elongate areolae (Figs 22, 23, 25, 26), numbering ca. 36 in 10 µm. Internally, raphe fissure is interrupted by an intermissio (Fig. 24). Raphe distal endings terminating on well-developed helictoglossae (Fig. 24).

Etymology: From Latin ‘acquapura’, meaning pure water, in reference to the very clear waters of the Rio Pequeno branch, in which the species was collected.

Type locality: Brazil. São Paulo, Billings Reservoir, Rio Pequeno branch, sample SP401589 (23°47’1.62”S, 46°26’11.28”W), leg. S. WENGRAT & D. BICUDO, coll. date 06/08/2009.

Holotype: SP401589 (Herbário Científico do Estado “Maria Eneyda P. Kauffmann Fidalgo”, São Paulo, Brazil, depicted in Figs 13-21).

Isotype (here designated): BR-4422 (Botanic Garden, Meise, Belgium).



Figs 13–26. *Encyonema acquapurae* sp. nov. Light and scanning electron micrographs. All pictures taken from the holotype population (SP401589). 13–21. LM views showing variation in size and valve outline. 22, 23, 25, 26. SEM external views of entire valve showing the raphe and striae structure. 24. SEM internal view of entire valve showing the raphe and striae structure, central area with intermissio. LM scale bar = 10 μm (in Figs 13–21).

Taxonomic remarks: This species is very similar to *E. angustecapitatum* and they can be misidentified at first sight, especially under light microscopy (LM). The type material here investigated (Figs 4–12) and which was illustrated by KRAMMER (1997b, pl. 130: figs 8-15) clearly show the differences between the two species. Thus, *E. acquapurae* can be discriminated by having (i) more radiate dorsal striae, (ii) wider axial area on ventral side, (iii) greater measurements (length: 20.8–27.4 µm and width: 5.0–6.0 µm), and (iv) smaller length to width ratio (4.0–4.7).

This species is to be compared to *E. pankowii* LANGE-BERTALOT & KRAMMER in KRAMMER. Although their measurements overlap, the latter has less striae and areolae in 10 µm (Table 3), so that they appear somewhat coarse when they are seen in LM. *Encyonema acquapurae* is distinguished from *E. ponteanum* by its largest dimensions and smaller length to width ratio (Table 3). Another similar species, *E. gaeumannii* (F. MEISTER) KRAMMER is smaller (4–5 µm) and has comparatively more striae (15–18) and more areolae in 10 µm (38–42), as well as more protracted ends. Furthermore, it shows very narrow axial area and larger apices (Table 3). In addition, all these species show a parallel striae pattern whereas *E. acquapurae* has a radiated one.

Encyonema acquapurae is also similar to *E. kabaniense* RODIONOVA & POMAZKINA in POMAZKINA & RODIONOVA (2014). Although no LM images of the latter species are available, differences regarding its areolae pattern (rounded and internally supported by struts) may be useful in separating both taxa.

Table 3. Main morphological characters of the new *Encyonema* species populations.

	Encyonema acquapurae	*E. tenue*	*E. sparsistriatum*	*E. paradisiacum*
Valve outline	dorsal side convex, ventral side moderately convex to straight	dorsal side convex, ventral side slightly convex to straight	dorsal side convex, ventral side slightly convex to straight	dorsal side convex, ventral side straight to slightly convex
Valve ends	subcapitate to capitate, sometimes deflected to the ventral side	capitate, sometimes slightly deflected to the ventral side	rostrate to capitate, sometimes deflected to the ventral side	rostrate to subcapitate
Length (µm)	20.8–27.4	20.4–25.3	15.5–21.5	18.3–26.8
Width (µm)	5.0–6.0	4.0–4.5	4.0–7	3.2–3.8
Striae arrangement	parallel to slightly radiate	parallel to slightly radiate	parallel to slightly radiate	parallel to slightly radiate
Maximum length to width ratio	4.0–4.7	4.6–6.1	4.0–4.8	5.3–7.2
Shoulder	clearly developed	clearly developed	poorly developed	absent
Central area	absent	absent	absent	absent
Axial area	narrow, ventral	narrow, ventral or sometimes dorsal	narrow, ventral	narrow, ventral or sometimes dorsal
Dorsal striae in 10 µm	13–14	11–14	11–12	13–15
Ventral striae in 10 µm	13–16	11-14	11–12	14–16
Areolae in 10 µm	36	36–40	40–50	35–40
Morphology of the areolae in external view (SEM)	lineolate	rounded, lineolate	lineolate, irregularly arranged along axial area	lineolate and Y-shaped

Ecology: *Encyonema acquapurae* material was common in samples collected from plankton and surface sediments of the Billings reservoir Rio Pequeno branch (relative abundance ≤ 2%). The water is oligo-mesotrophic with low nutrient concentrations (Table 2). The new species was collected associated with *Brachysira brebissonii* R. ROSS, *Encyonopsis sanctipaulensis* WENGRAT et al., *Eunotia veneris* KÜTZING and *Encyonema sparsistriatum* sp. nov.

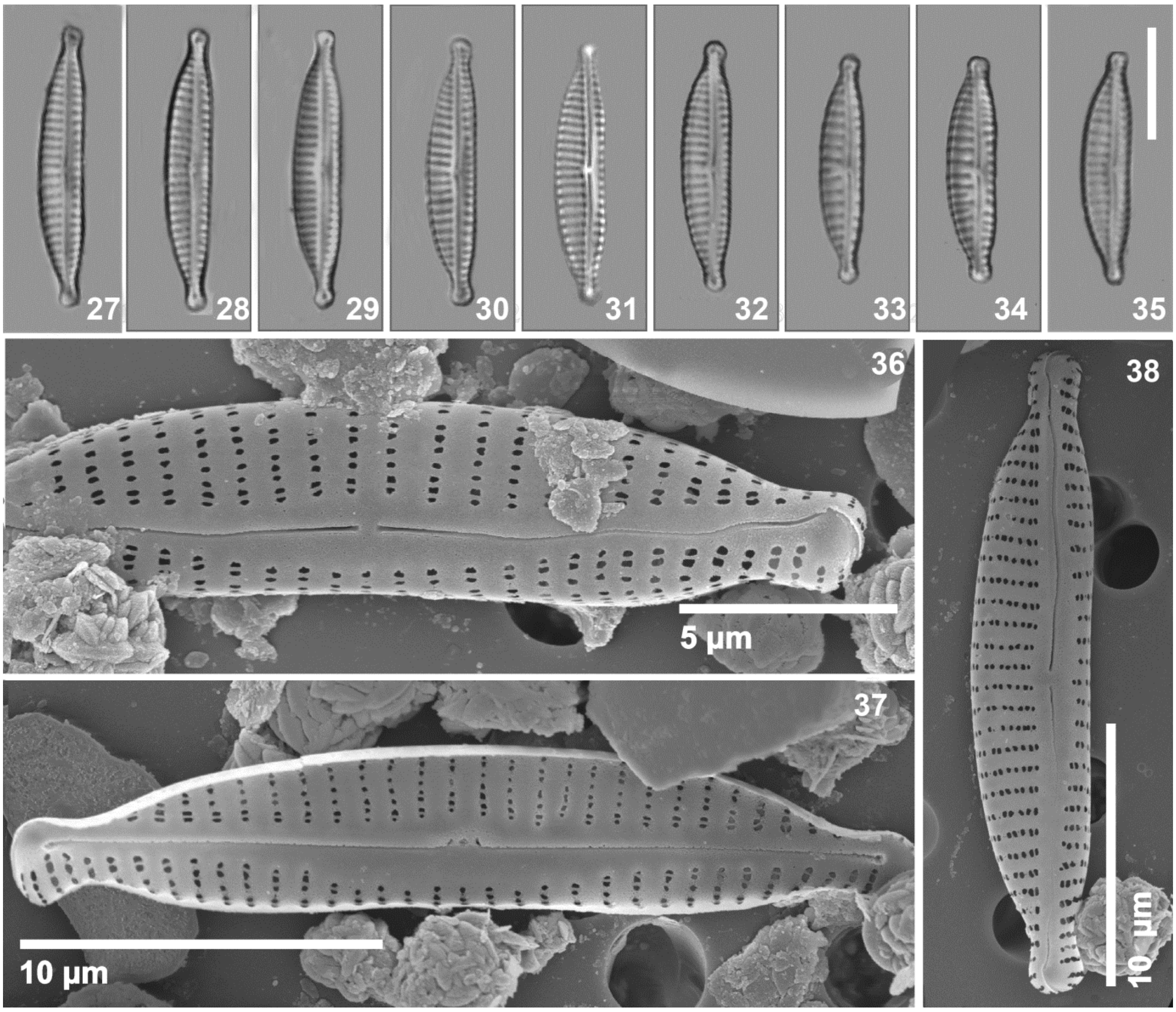
Encyonema tenue MARQUARDT, WENGRAT & C.E. WETZEL **sp. nov.** (Figs 27–38).

Light microscopy (LM) (Figs 27–35): Valves strongly dorsiventral. Dorsal margin arched. Ventral margin slightly arched to linear, with a slight indentation near the median area. Apices narrow, capitate, sometimes slightly deflected to the ventral margin. Shoulders clearly developed. Length 20.4–25.3 µm. Width 4.0–4.5 µm. Length to width ratio 4.6–6.1. Axial area narrow, linear, wider on the ventral margin. Central area absent, sometimes with shorter and spaced striae on dorsal side (Figs 32–34). Raphe filiform, lateral. Proximal fissures weakly expanded, slightly curved to dorsal margin. Raphe distal ends strongly deflected to the ventral margin. Striae parallel to slightly radiate at the ends. Dorsal striae 11–14 in 10 µm, ventral striae 11–14 in 10 µm. Indistinct areolae. Stigmoid absent.

Scanning electron microscopy (SEM) (Figs 36–38): External raphe fissure slightly undulate (Figs 36, 38). Proximal end of raphe almost straight, enlarged, slightly curved to the dorsal side (Figs 36, 38). Raphe distal ends are first dorsally bent, and then strongly hooked to the ventral side ending onto the mantle (Figs 36, 38). Axial area is narrow, linear (Figs 36, 38), slightly wider to the ventral side. Striae are composed of rounded to lineolate areolae (Figs 36, 38), numbering 35–40 in 10 µm. Internal striae are composed of rounded, lineolate and unequal areolae (Fig. 37). Internally, the raphe fissure is interrupted by an intermissio (Fig. 37). Raphe distal ends terminating in well-developed helictoglossae (Fig. 37).

Etymology: Specific epithet refers to the lesser valve width when compared to *E. acquapurae*.

Type locality: Brazil, São Paulo, Casa Branca, sample SP188327, leg. A.A.J. CASTRO & C.E.M. BICUDO, coll. date 17/10/1989.



Figs 27–38. *Encyonema tenue* sp. nov. Light and scanning electron micrographs. All pictures taken from the holotype population (SP188327). 27–35. LM views showing variation in size and valve outline. 36, 38. SEM external views of entire valve showing the raphe and striae structure. 37. SEM internal view of entire valve showing the raphe and striae structure, central area with intermissio. LM scale bar = 10 µm (in Figs 27–35).

Holotype: SP188327 (Herbário Científico do Estado "Maria Eneyda P. Kauffmann Fidalgo", São Paulo, Brazil, depicted in Figs 27–35).

Isotype (here designated): BR-4468 (Botanic Garden, Meise, Belgium).

Taxonomic remarks: The new species was recorded as *E. angustecapitatum* in MARQUARDT & BICUDO (2014). However, ultrastructural analysis showed that the Casa Branca specimens have rounded areolae, whereas in the type material are lineolate. Spaced dorsal striae at median region were not observed in *E. angustecapitatum.* All other features usually overlap.

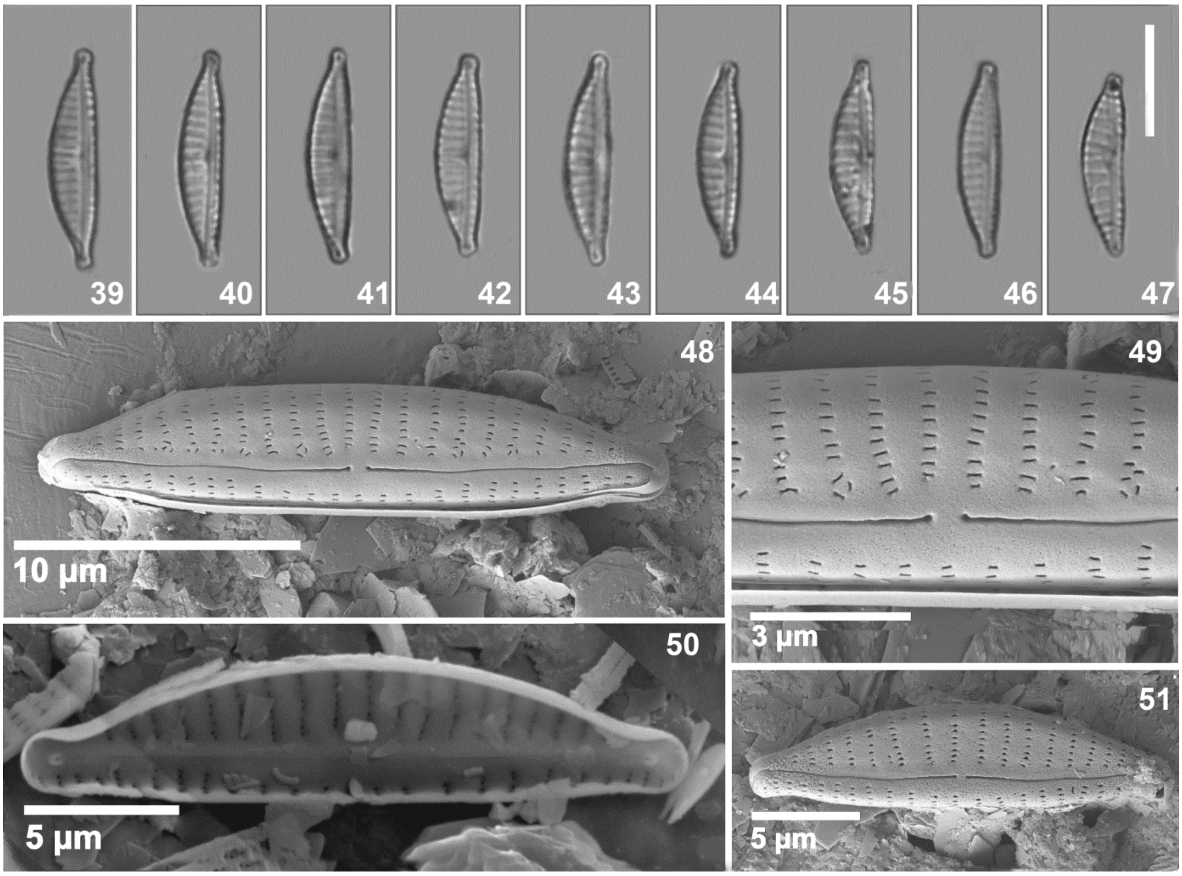
Encyonema pankowii has less striae (10–11) and areolae (18–20) in a 10 µm interval, wider valvar width (5.5–6.5 µm) and length to width ratio (4.4) (Table 3).

Ecology: *Encyonema tenue* was collected from a marsh (Casa Branca city, São Paulo State) with relative abundance ≤ 2%. The new collected species was associated with *Encyonopsis schubartii* (HUSTEDT) KRAMMER, *Kurtkrammeria frequentis* (KRAMMER) BAHLS and *Gomphonema* sp. There is no information about the local water nutrients.

Encyonema sparsistriatum MARQUARDT, WENGRAT & C.E. WETZEL **sp. nov.** (Figs 39–51)

Light microscopy (LM) (Figs39–47): Valves strongly dorsiventral. Dorsal margin arched. Ventral margin slightly convex to linear. Apices narrow, rostrate to capitate. Shoulders poorly developed. Length 15.5–21.5 µm. Width 4.0–4.7 µm. Length to width ratio 4.0-4.8. Axial area narrow, linear, wider on the ventral margin. Central area absent. Raphe filiform, lateral. Proximal fissures weakly expanded, curved to dorsal margin. Raphe distal ends strongly deflected to the ventral margin. Striae parallel to slightly radiate at the ends. Dorsal striae 11–12 in 10 µm, ventral striae 11–12 in 10 µm. Indistinct areolae. Stigmoid absent.

Scanning electron microscopy (SEM) (Figs 48–51): External raphe fissure slightly undulate (Figs 48, 51). Proximal raphe end almost straight, the proximal endings are slightly curved to the dorsal side (Figs 48, 49, 51). Raphe distal ends first dorsally bent, and then strongly hooked to the ventral side (Figs 48, 51). Axial area is narrow, linear, and wider to the ventral side (Figs 48, 51). Striae composed of lineolate areolae along the valve irregularly arranged along the axial area (Figs 48, 49) numbering 45–50 in 10 µm. Internally, the raphe fissure is interrupted by an intermissio (Fig. 50). Raphe distal endings terminating in well-developed helictoglossae (Fig. 50).



Figs 39–51. *Encyonema sparsistriatum* sp. nov. Light and scanning electron micrographs. All pictures taken from the holotype population (SP401589). 39–47. LM views showing variation in size and valve outline. 48, 49, 51. SEM external views of entire valve showing the raphe and striae structure. 50. SEM internal view of entire valve showing the raphe and striae structure. LM scale bar = 10 µm (in Figs 39–47).

Etymology: The specific epithet refers to its more spaced and smaller striae number when compared to other species of the *E. angustecapitatum* complex.

Type locality: Brazil. São Paulo, Billings Reservoir, Rio Pequeno branch, sample SP401589 (23°47’1.62”S, 46°26’11.28”W), leg. S. Wengrat & D. Bicudo, coll. date 06/08/2009.

Holotype: SP401589 (Herbário Científico do Estado “Maria Eneyda P. Kauffmann Fidalgo”, São Paulo, Brazil, depicted in Figs39–47).

Isotype (here designated): BR-4422 (Botanic Garden, Meise, Belgium).

Taxonomic remarks: *Encyonema sparsistriatum* differs from *E. acquapurae* in its smaller dimensions (15.5–19.8 µm length and 4.0–4.5 µm width) and smaller striae density (11–12 in 10 µm) composed by rounded areolae, that also are more numerous (40–50) in a 10 µm interval.

Encyonema sparsistriatum differs from *Encyonema menezesiae* W.J. SILVA & M.G.M. SOUZA mainly in its valve outline and areolae shape: *E. sparsistriatum* is strongly dorsiventral and has rounded areolae, whereas *E. menezesiae* is slightly dorsiventral and has areolae with linear foramina.

Ecology: This new species was rare in the samples collected from the surface sediments of Billings reservoir Rio Pequeno branch (relative abundance ≤ 2%). The water is oligo-mesotrophic with low nutrient contents (Table 2). The new species was associated with *Brachysira brebissonii*, *Encyonopsis sanctipaulensis*, *Encyonema angustecapitatum*, *Eunotia veneris* and *Encyonema acquapurae*.

Encyonema paradisiacum MARQUARDT, WENGRAT & C.E. WETZEL **sp. nov.** (Figs 52–66).

Light microscopy (LM) (Figs 52–60): Valves strongly dorsiventral. Dorsal side arched. Ventral margin slightly arched to linear. Apices narrow, rostrate, subcapitate to capitate. Length 18.3–26.8 µm. Width 3.2–3.8 µm. Length to width ratio 5.3–7.2. Axial area narrow, linear, broader on the ventral margin. Central area absent. Raphe filiform, lateral. Proximal fissures weakly expanded, slightly curved to dorsal margin. Raphe distal ends strongly deflected to the ventral margin. Dorsal striae parallel to slightly radiate at the ends, dorsal striae 13–15 in 10 µm, ventral striae 14–16 in 10 µm, very short in the median area. Indistinct areolae. Stigmoid absent.

Scanning electron microscopy (SEM) (Figs 61–66): External raphe fissure slightly undulate (Fig. 61). Proximal end of raphe almost straight, slightly curved to the dorsal side (Figs 61, 62). Raphe distal ends are first dorsally bent, than strongly hooked to the ventral side (Figs 61, 62, 64). Axial area is narrow, linear, and wider to the ventral side (Figs 61, 62). Striae are composed of lineolate areolae, sometimes Y-shaped (Figs 61, 62, 64), numbering 35–40 in 10 μm. Internally, the raphe fissure is interrupted by an intermissio (Figs 63, 65). Raphe distal endings terminating in well-developed helictoglossae (Fig. 66).

Etymology: From Latin 'paradisus' or Greek 'paradeisos': paradise) in reference to the place where it was collected (Ribeirão do Campo reservoir).

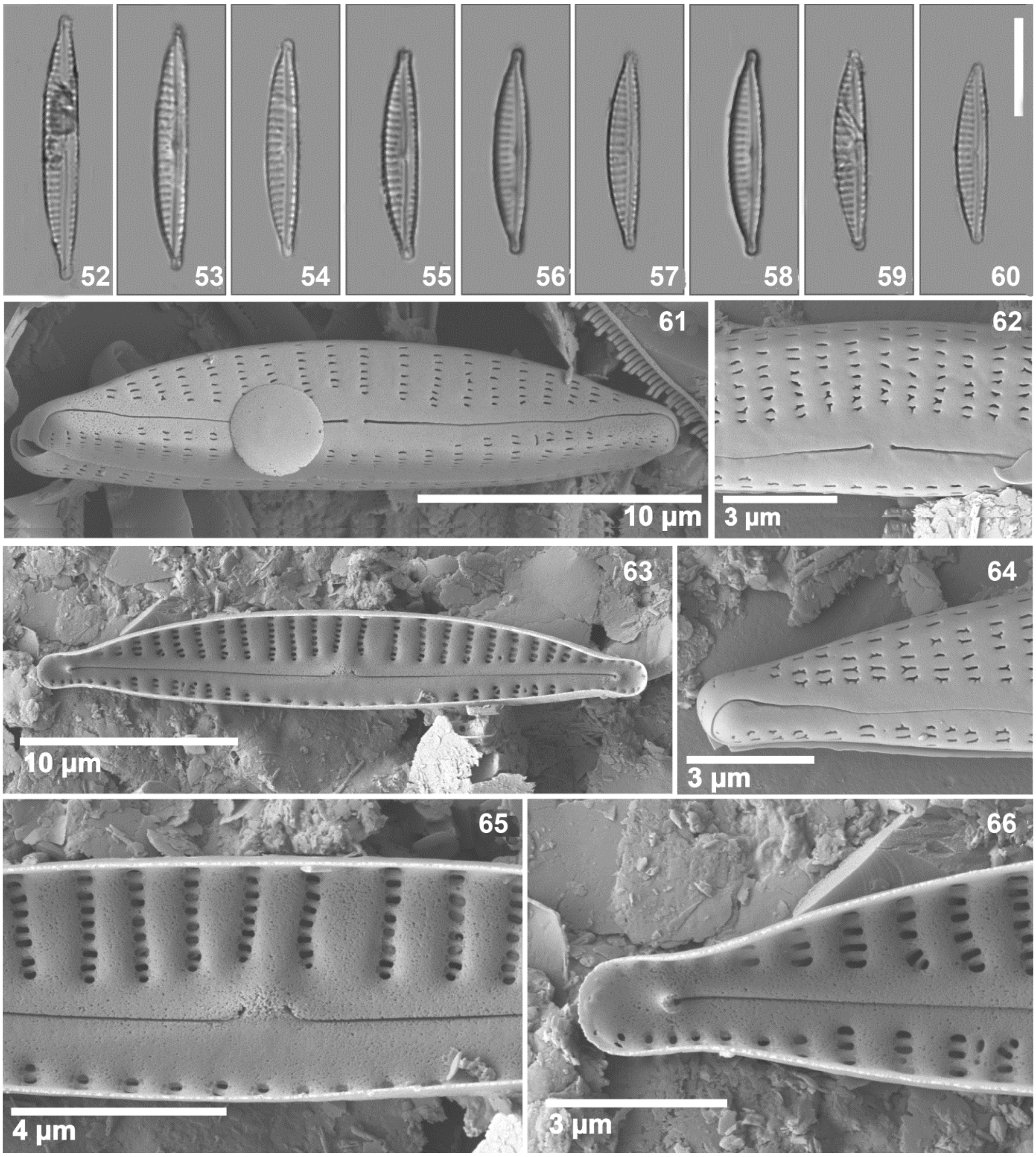
Type locality: Brazil, São Paulo, Ribeirão do Campo Reservoir, sample SP427990 (23°39'31.8"S, 45°49'23.22"W), A.C.R. ROCHA & D.C. BICUDO, coll. date 13/08/2010.

Holotype: SP468841 (Herbário Científico do Estado "Maria Eneyda P. Kauffmann Fidalgo", São Paulo, Brazil, depicted in Figs 52–60).

Isotype (here designated): BR-4430 (Botanic Garden, Meise, Belgium).

Taxonomic remarks: *Encyonema paradisiacum* has strongly curved raphe to a very narrow ventral margin, with short striae placed only marginally or entirely absent. These features are similar to those of some *Cymbellopsis* KRAMMER species. However, according to KRAMMER (1997b) *Encyonema* structure significantly differs from *Cymbellopsis*. In the latter genus, the areolae are quite irregular and some of them often form clusters that appear as rough points at LM. Also, the foramina are either delicate apical elongated slots or irregularly x-shaped openings. Regarding the areolae ultrastructure, SEM observations of *E. paradisiacum* showed that they are always lineolate or Y-shaped (Figs 62, 63, 65) and their striae are not interrupted.

Ecology: The new species was somewhat rare in the surface sediments samples collected from Ribeirão do Campo reservoir (Salesópolis city, São Paulo state) (relative abundance ≤ 2%). The water is oligotrophic with low nutrient contents (Table 2). The species was associated with *Eunotia botuliformis* F. WILD, NÖRPEL & LANGE-BERTALOT, *E. bilunaris* (EHRENBERG) SCHAARSCHMIDT and *Brachysira serians* (BRÉBISSON) ROUND & D.G. MANN.



Figs 52–66. *Encyonema paradisiacum* sp. nov. Light and scanning electron micrographs. All pictures taken from the holotype population (SP427990). 52–60. LM views showing variation in size and valve outline. 61, 62, 64. SEM external views of entire valve showing the raphe and striae structure. 63, 65, 66. SEM internal view of entire valve showing the

raphe and striae structure. Fig. 65. Detail of the intermissio. LM scale bar = 10 μm (in Figs 52–60).

Morphological examination

Differences between the studied groups were evidenced by using their morphological measurements (Fig. 1). Similarity tests (NPMANOVA) performed on the resulting groups in the MDS, revealed statistically significant differences (p values: <0.001, <0.01, <0.05) between the four studied taxa (Table 4) but not for *E. angustecapitatum* and for *E. ponteanum.* The small number of *E. ponteanum* representatives may have contributed to this result. However, no representative of the species was found during the re-examination of the type material, and the measures used in this analysis were based only on KRAMMER (1997b).

The procedure distinguished four groups of specimens as follows: *E. acquapurae* (N = 15 specimens), *E. tenue* (N = 11 specimens), *E. sparsistriatum* (N = 12 specimens) and *E. paradisiacum* (N = 15 specimens), besides *E. angustecapitatum* (N = 7) and *E. ponteanum* (N = 3) type materials (Fig. 4). These groups are evident during the ordination analysis, with a 'stress' of 0.07596, indicating that graphical distances among species were close to the original similarities. All species were easily distinguished by the morphometric analysis approach (Fig. 2).

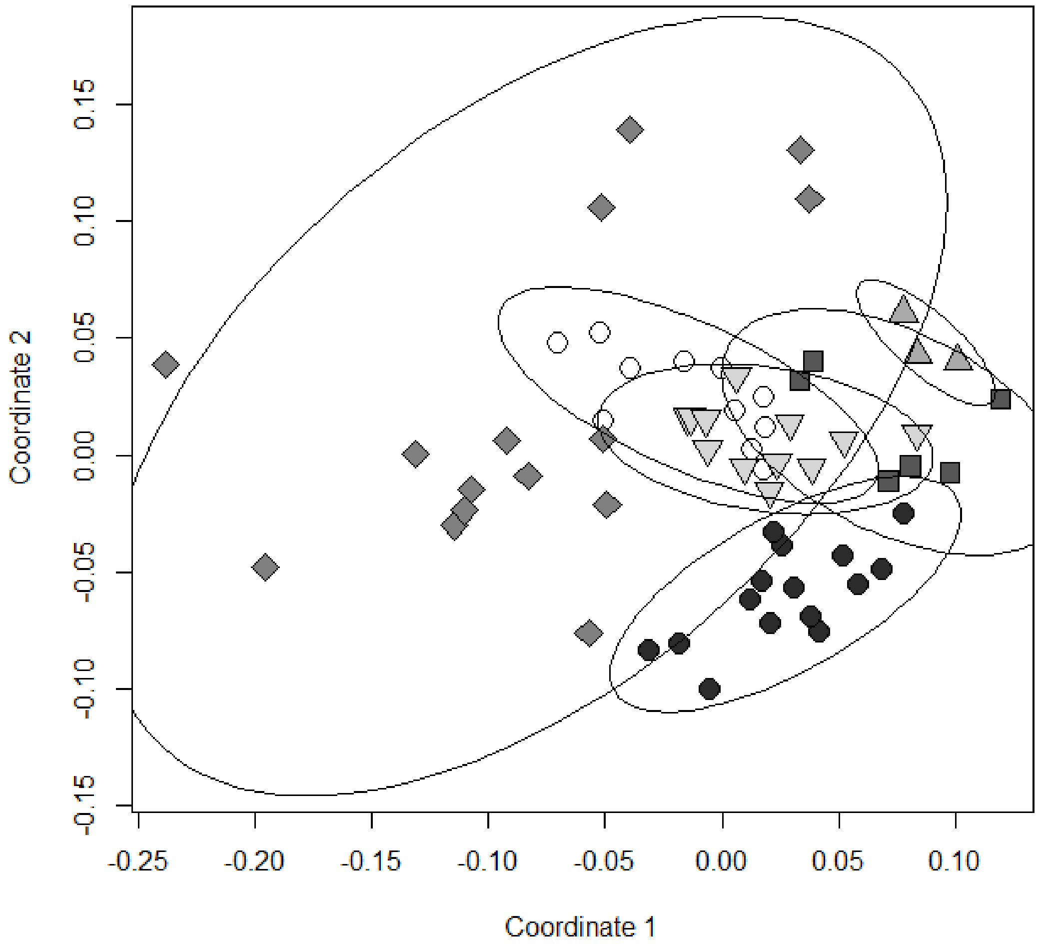


Fig. 2. Multidimensional scaling procedure (MDS) plot based on Bray-Curtis similarities between *E. angustecapitatum* species complex in this study and 4 species distinguished by cluster analysis (symbols). Legend symbols: filledl circle: *E. acquapurae*; white circle: *E. tenue*; inverted triangle: *E. sparsistriatum*; diamond: *E. paradisiacum*; square: *E. angustecapitatum* and triangle: *E. ponteanum.*

For the scatterplot matrix, correlation between length and width (0.45), length and length to width ratio (0.57), width and length to width ratio (-0.38), width and ventral striae (0.27) and dorsal and ventral striae (0.87) were significant and very useful features for species differentiation (Fig. 3).

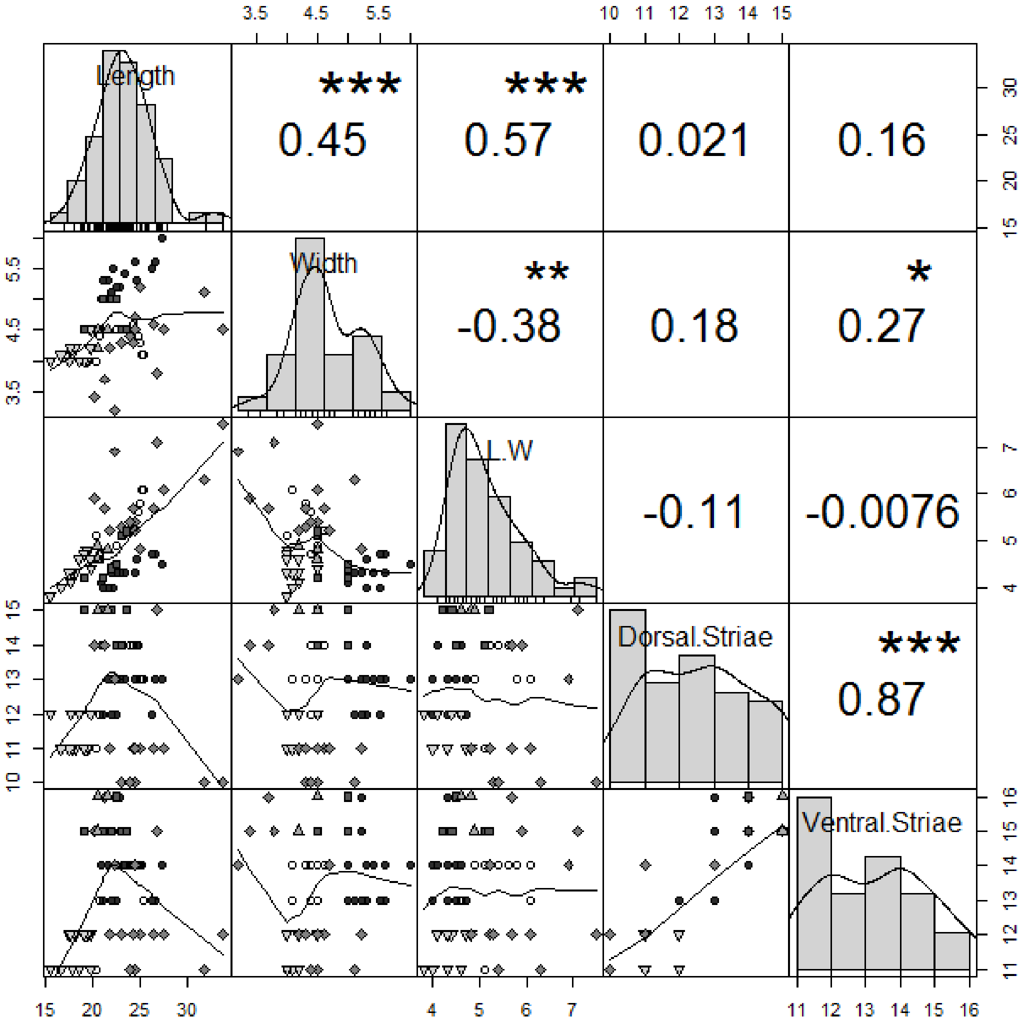


Fig. 3. Scatterplot matrix of measurements of new *Encyonema* species. Variation in the valve length, width, striae in 10 μm and length to width ratio (L:W). Distribution of each variable is shown on the diagonal. Legend => filled circle: *E. acquapurae*; square: *E. tenue*; diamond: *E. sparsistriatum*; triangle: *E. paradisiacum*; inverted triangle: *E. angustecapitatum* and white circle: *E. ponteanum*. Lower panel: the bivariate scatter plots with a fitted line are displayed. Upper panel: the value of the correlation plus the significance level as stars. Each significance level is associated to a symbol: *p*-values (0, 0.001, 0.01, 0.05, 0.1, 1) <=> symbols ("***", "**", "*", ".", " ").

Table 4. NPMANOVA test performed on morphological differences resulting groups in the MDS using the Euclidean distance measure. *P* values shown (<0.001, <0.01, <0.05).

	E. tenue	*E. sparsistriatum*	*E. paradisiacum*	*E. angustecapitatum*	*E. ponteanum*
E. acquapurae	<0.01	< 0.001	0.002	< 0.001	< 0.001
E. tenue	---	0.0012	<0.05	< 0.001	<0.01
E. sparsistriatum		---	< 0.001	<0.05	<0.01
E. paradisiacum			---	0.0004	< 0.001
E. angustecapitatum				---	0.1593

Table 4. Main morphological characters of *Encyonema angustecapitatum* type materials and related species. nd: no data.

	Encyonema angustecapitatum KRAMMER	*E. ponteanum* KRAMMER	*E. gaeumannii* (F. MEISTER) KRAMMER	*E. pankowii* LANGE-BERTALOT & KRAMMER in KRAMMER
Valve outline	dorsal side convex, ventral side moderately convex to straight	strongly dorsiventral, elliptic lanceolate, dorsal side convex, ventral side moderately convex, straight or slightly concave	strongly dorsiventral, elliptic lanceolate	strongly dorsiventral, elliptic lanceolate, dorsal side convex, ventral side moderately convex, straight or slightly concave
Valve ends	capitate	capitate, narrow and rounded	capitate, broad and rounded	capitate, narrow and rounded
Length (µm)	17–24	18–20	14–22	24–28
Width (µm)	4.1–5.1	3.8–4.2	4–5	5.5–6.5
Striae arrangement	Parallel	parallel to slightly radiate	parallel to slightly radiate	parallel to slightly radiate
Maximum length to width ratio	5.3	4.4	4.4	4.4
Shoulder	rounded and clearly developed	clearly developed on the ventral side	sometimes absent on the dorsal side	clearly developed on the ventral side
Central area	narrow, slightly ventral	absent	absent or slightly dorsal	Absent
Axial area	narrow, linear	narrow, ventral	narrow, linear	narrow, ventral
Dorsal striae in 10 µm	12–15	12–14	15–18	10–11
Ventral striae in 10 µm	14–15	15–16	18–19	17–18
Areolae in 10 µm	36–38	28–32	38–42	18–20
Morphology of the areolae in external view (SEM)	Lineolate	nd	lineolate	Nd

Final remarks and conclusion

Present observations of the type material of *E. angustecapitatum* culminated with a better understanding of the complex identity, and helped to identify four new taxa that were formerly misidentified. The species in the *E. angustecapitatum* complex can be separated mainly by differences of their morphological features such as valve outline, axial area, valve length, valve width, length to width ratio, number of striae in 10 µm and number and type of areolae in 10 µm. Although most morphological characteristics of the species examined are similar and overlap among themselves, analyses based on the combination of morphological data were useful for the identification of the *E. angustecapitatum* species complex. Morphometric and statistical analysis has been shown to be a useful and widely used tool for the separation of diatom complexes as well as to delimit important features in the species (e.g. WENGRAT et al. 2015; WETZEL & ECTOR 2015; ŻELAZNA–WIECZOREK & OLSZYŃSKI 2016).

All species were quite rare in all samples currently studied making LM and SEM observations quite difficult. Their biogeography is still unclear, but just American's distribution were found until now (Fig. 67). We cannot excluded that the new species are more widespread than just in the São Paulo state. This observation concerns the significant amount of Cymbellales representatives recently described (e.g. BAHLS 2015; LE COHU et al. 2015; YANA & MAYAMA 2015; HEUDRE et al. 2016; MARQUARDT et al. 2016). Moreover, the new taxa might be characteristic for oligotrophic habitats in tropical areas.

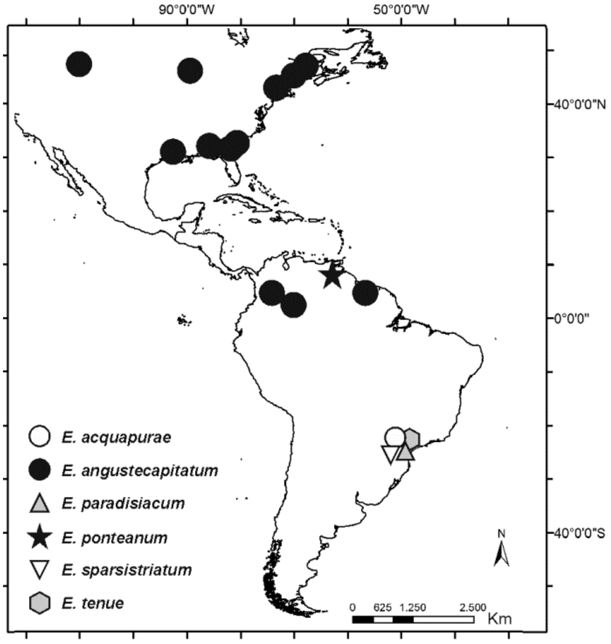


Fig. 67. Distribution of *E. angustecapitatum* complex reported from the literature: (black star) type locality of *E. angustecapitatum* and *E. ponteanum* in Venezuela; (white symbols) represent *E. Acquapurae* distribution; (black symbol) represent *E.*

angustecapitatum; (triangle) represent *E. paradisiacum*; (inverted triangle) represent *E. sparsistriatum* and (diamond) represent *E. tenue*.

This study contributes to the need of documenting and illustrating diversity to facilitate research on diatom biogeography, ecology and paleoecology in Brazil. Further research on the diatom diversity of Brazilian environments may yet lead to the discovery of other new species due to the few taxonomic studies already performed and the little attention given to rare taxa.

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Conclusões e considerações gerais

O presente estudo permite as seguintes conclusões e recomendações:

O **capítulo 1** fornece novas informações sobre a ecologia e a distribuição de diatomáceas tropicais. Foram delineados três grupos potenciais de diatomáceos indicadores da qualidade da água, que indicaram 1) condições oligotróficas, 2) de mistura de água com condições de baixa luminosidade e 3) espécies com maiores requerimentos de nutrientes, ocorrendo em águas de maior condutividade e pH, de acordo com os relatados na literatura. Reforçamos ainda a importância do PEJU para a manutenção da qualidade ecológica dos reservatórios e condições de referência para as fontes de água da Região Metropolitana de São Paulo.

No **capítulo 2**, utilizamos comunidades de diatomáceas fitoplanctônicas e de sedimentos superficial para avaliar diferentes cinjuntos de dados (separados por categorias de raridade) na análise de importância do ambiente local e do espaço na variação da comunidade. As diatomáceas são consideradas ubíquas e sua composição comunitária é determinada predominantemente pela classificação de espécies pelo ambiente. No entanto, há um contraste claro entre esta avaliação e a literatura recente diatomáceas, que contém muitas alegações de endemismo. Os resultados mostram que apesar de seguirem uma mesma tendência, a resposta das diatomáceas foi dependente da estação climática e do habitat. Nesta escala espacial, a distribuição de diatomáceas em ambos os compartimentos (fitoplâncton e sedimento superficial) foi organizada principalmente pelo componente espacial durante o verão, ao contrário de estudos recentes que suportam os fatores ambientais como principal preditor. Durante o inverno, o componente ambiental foi preponderante apenas para as comunidades de diatomáceas de fitoplâncton. No entanto, a conectividade hidrológica proporcionou maior influência dos processos de dispersão sobre a estrutura da comunidade, desempenhando um papel importante na explicação da dinâmica das metacomunidades de diatomáceas. Assim, esta variável não deve ser negligenciada nos modelos que predizem o funcionamento do ecossistema.

O **capítulo 3** tratou de dois grandes objetivos. Em primeiro lugar, utilizamos a partição aditiva da diversidade para examinar se os reservatórios em cascata no mesmo rio diferem na divisão da diversidade em diferentes escalas de reservatórios localizados em diferentes rios. Em segundo lugar, determinamos se os reservatórios com ligação direta ao curso de água possuem menor diversidade beta do que os reservatórios sem conexão. Descentramos as contribuições do turnover espacial e aninhamento aos padrões de diversidade beta e suas mudanças através da perda e ganho de espécies ao longo do período de estudo com o quadro quantitativo recentemente proposto por Baselga (2010). O estudo revelou que as conexões hidrológicas foram importantes na estruturação da comunidade, sugerindo maiores taxas de dispersão entre localidades em ambientes conectados. Para fins de conservação, nossos resultados sugerem uma proteção em grande escala, assegurando a diversidade beta entre os locais e conservando a diversidade total.

O **capítulo 4**, utilizamos a distância de decaimento como descritor da variação composicional da similaridade da comunidade de diatomáceas entre os diferentes hábitats (fitoplâncton e sedimento superficial) e estações (verão e inverno, apenas para fitoplâncton). Nosso estudo apóia a visão da teoria neutra, que prevê que o decaimento da similaridade da comunidade deve ser particularmente forte em distâncias espaciais curtas, determinando padrões em metacomunidades de diatomáceas dos reservatórios estudados. As diatomáceas nos sedimentos de superfície responderam de forma semelhante aos resultados observados na coluna de água, mas os resultados não foram estatisticamente significativos. Isto é provavelmente devido ao caráter integrativo do sedimento, que acumula 1 a 2 anos de eventos do reservatório ao invés de uma única estação. Além disso, os processos na natureza são dependentes da escala e o limite da dispersão pode ser mais importante em pequenas escalas (<100 km). Além disso, a área de estudo não impactada pode ser considerada um fator importante na determinação das contribuições relativas de componentes geográficos para a estrutura da comunidade de diatomáceas.

O capítulo 5 compara conjuntos de dados de diatomáceas em diferentes categorias de raridade, baseado nas abundâncias relativas e freqüências de ocorrência, para verificar suas respostas na abordagem WA e calibração das condições limnológicas que inferem. Os valores de correlação (r^2) do ajuste gaussiano (curvas de resposta) dos diferentes modelos foram utilizados para avaliar a precisão dos indicadores WA. O pressuposto subjacente é que o modelo é inadequadamente capaz de caracterizar os ótimos e tolerâncias de espécies que ocorrem apenas algumas vezes, e que seu desempenho geral pode ser melhorado eliminando-as. Os resultados revelaram que a manipulação dos diferentes conjuntos de dados influencia significativamente os desempenhos dos modelos. Nesse sentido, a remoção de táxons raros foi contraproducente e os modelos de função de transferência desenvolvidos, removendo táxons raros, reduziram os desempenhos do modelo. Embora nossos resultados corroborem com os de Wilson et al. (1996), com uma forte evidência de que o tamanho da amostra tem uma grande influência sobre os resultados, a análise de táxons raros no biomonitoramento é um tema em discussão e, escolher um corte para evitar “ruídos” causados por táxons raros nos conjuntos de dados pode ser subjetivo. No entanto, o trabalho contribui para uma melhor compreensão da ecologia das diatomáceas, especialmente as de reservatórios tropicais, apoiando o desenvolvimento de um protocolo de monitoramento biológico de diatomáceas para a área de estudo.

O **capítulo 6** possui um enfoque taxonômico com análise de material tipo de *Achnanthidium minutissimum* e *A. catenatum,* considerados dois dos táxons mais comumente relatados em trabalhos florísticos e ecológicos de todo o mundo e a descrição de uma nova espécie: *A. tropicocatenatum*. O capítulo ressalta que registros de *A. catenatum* no Brasil são considerados questionáveis porque podem ter sido confundidos com *A. tropicocatenatum* sp. Nov. Com o aumento do uso de diatomáceas como indicadores da saúde do ecossistema, o estabelecimento da identidade de pelo menos os táxons mais comuns poderia melhorar a precisão das ferramentas de diagnóstico ecológico que dependem fortemente das categorias taxonômicas mais inclusivas como unidades básicas que expressam a mudança ambiental. A descrição de *A. tropicocatenatum* melhora nosso conhecimento sobre a biogeografia de espécies neste complexo e permite seu uso em estudos ecológicos e biogeográficos mais precisos.

No **capítulo 7**, uma nova espécie de diatomáceas de água doce perifítica pertencente ao gênero *Sellaphora* registrada de um reservatório em São Paulo (sudeste do Brasil) é descrita como *S. tropicomadida* Marquardt & C.E.Wetzel, sp. nov. São também apresentadas implicações ecológicas e critérios diagnósticos diferenciais em relação a espécies similares do Sul do Brasil. Comparação com espécies semelhantes com base na literatura e informações sobre sua ecologia também são fornecidos. As análises das amostras coletadas nos reservatórios brasileiros revelaram uma flora muito diversa, com espécies desconhecidas e únicas de diatomáceas, que esclarecem a importância de avaliar a identidade ea distribuição de diatomáceas tropicais. Além disso, nossos estudos mostraram a importância do uso rotineiro de MEV na estrutura de pequenas diatomáceas. Um conhecimento mais profundo da flora que habita nesta região insuficientemente investigada e seu padrão de colonização desempenhará um papel chave na elaboração de diretrizes e dispositivos específicos de manejo.

No **capítulo 8**, o material de tipo *Encyonema angustecapitatum* foi investigado para estabelecer a identidade de várias populações brasileiras de *Encyonema*. Também foram discutidas notas sobre sua distribuição. A análise MDS distinguiu quatro espécies de *Encyonema* atualmente descritas como novas para a ciência: *Encyonema acquapurae* sp. nov., *Encyonema sparsistriatum* sp. nov., *Encyonema tenue* sp. nov. e *Encyonema paradisiacum* sp. Nov. As quatro novas espécies podem ser separadas com base numa combinação das seguintes características morfológicas: contorno da válvula, área axial, comprimento da válvula, largura da válvula, relação comprimento / largura, número de estrias em 10 μm e número de areolas em 10 μm. Quanto às preferências ecológicas, as quatro espécies foram encontradas principalmente em ambientes oligotróficos e oligo-mesotróficos. O estudo contribui para a necessidade de documentar e ilustrar a diversidade para facilitar a pesquisa sobre biogeografia de diatomáceas, ecologia e paleoecologia no Brasil. Pesquisas adicionais sobre a diversidade de diatomáceas dos ambientes brasileiros podem ainda levar à descoberta de outras novas espécies devido aos poucos estudos taxonômicos já realizados e à pouca atenção dada aos taxa raros.