CAMILA BARBOSA DE ARAÚJO

Estudo polifásico em *Micrasterias arcuata* (Desmidiaceae, Zygnematophyceae) e suas implicações taxonômicas e nomenclaturais

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

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ORIENTADOR: DR. CARLOS E. DE M. BICUDO

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RESUMO

Micrasterias arcuata Bailey (Desmidiaceae, Zygnematophyceae) apresenta ampla variabilidade morfométrica e um elevado número de categorias infraspecíficas muitas vezes mal circunscritas, o que a torna uma espécie de taxonomia e nomenclatura extremamente problemáticas. Informação em literatura sobre a influência das variáveis ambientais no polimorfismo e na autecologia dessa espécie é bastante incipiente. Além disso, seu posicionamento filogenético em relação ao gênero *Micrasterias*, assim como a outros gêneros e às categorias taxonômicas infragenéricas próximas permanece desconhecido. A presente tese visa a ampliar o conhecimento da caracterização morfológica de *M. arcuata* e de suas categorias infraspecíficas (morfotipos) *in situ* e em condições de cultivo, além de realizar o primeiro estudo filogenético sobre a espécie. No capítulo 1, foram realizadas análises morfológicas com base na morfometria tradicional, morfometria geométrica e MEV em populações naturais coletadas em um açude situado na Estação Experimental de Itirapina, Estado de São Paulo, Brasil. O principal objetivo desse capítulo foi avaliar os morfotipos de *M. arcuata* documentados para a área estudada e identificar possíveis características diagnósticas para diferenciá-los. No capítulo 2, foi realizado o primeiro estudo filogenético para a espécie em questão. Para isso, morfotipos de *M. arcuata* previamente identificados e coletados na área de estudo foram isolados e mantidos em condições ideais de cultivo. Foram usadas três cepas de morfotipos e/ou categorias infraspecíficas de *M. arcuata*. Para a extração do material genético foram utilizados três marcadores moleculares, o nuclear SSU rDNA e dois plastidiais, *psa*A e *rbc*L. Os resultados obtidos neste capítulo mostraram grande diversidade genética em *M. arcuata*, com a proposição de um novo gênero e três espécies pertencentes à família Desmidiaceae.

Palavras-chave: cultivo, desmídia, filogenia, polimorfismo, taxonomia polifásica.

ABSTRACT

Micrasterias arcuata Bailey (Desmidiaceae, Zygnematophyceae) includes a wide morphometrical variability and a high number of often poorly circumscribed infraspecific categories that makes it a species with an extremely problematic taxonomy and nomenclature. Data availability on the influence of environmental variables on the polymorphism and autoecology of this species is yet incipient. In addition, its phylogenetic position in relation to the genus *Micrasterias* and other close related taxonomic categories remains unknown. Present Doctoral Dissertation aims at expanding the knowledge of the morphological characterization of *M. arcuata* and some infraspecific taxa both in observational and experimental conditions. In addition, to carry out the first phylogenetic study on the species. Chapter 1 includes traditional, geometric morphometric analyses and SEM performed on natural populations collected from a reservoir at the Itirapina Experimental Station, São Paulo State, Brazil. The main objective of this chapter is to evaluate the *M. arcuata* morphotypes, and to identify possible diagnostic characteristics capable of differentiated them. In chapter 2, the first phylogenetic study carried out for the species in question. Therefore, morphotypes of *M. arcuata* were collected, isolated and kept under ideal culturing conditions. Three strains of previous identified morphotypes were studied. For extraction of genetic material, three molecular markers were used: nuclear SSU rDNA and two chloplastid ones, *psa*A and *rbc*L. Results obtained show a great genetic diversity in *M. arcuata* species, as well as the proposition of a new genus and three new species belonging Desmidiaceae.

Key words: culturing, desmid, phylogeny, polyphasic taxonomy, polymorphism.

ORGANIZAÇÃO E APRESENTAÇÃO DA TESE

A presente tese foi elaborada para conter os seguintes itens: introdução geral, justificativa, perguntas, hipóteses, objetivos, capítulos e considerações finais.

Com o objetivo de facilitar a publicação dos resultados obtidos no presente estudo, cada capítulo já está apresentado no formato de artigo científico, com seus respectivos itens: resumo, introdução, material e métodos, resultados e discussão, referências bibliográficas e material suplementar.

No capítulo 1 são apresentados os resultados referentes ao uso da morfologia tradicional e da morfometria geométrica para a caracterização e diferenciação de populações naturais de *Micrasterias arcuata* obtidas de coletas mensais realizadas em uma lagoa rasa situada na Estação Experimental de Itirapina, Município de Itirapina, São Paulo, Brasil.

No Capítulo 2 são apresentados os resultados de uma análise multigênica que utilizou os marcadores moleculares 18S rDNA *rbc*L e *psa*A para inferir o posicionamento filogenético de cepas de *Micrasterias arcuata* em relação ao gênero *Micrasterias* e outros gêneros pertecentes à família Desmidiaceae. Além disso, são apresentados os resultados referentes à caracterização morfológica das cepas estudadas baseada nas análises de morfometria tradicional, morfometria geométrica e análises em microscópio eletrônico de varredura (MEV).

Por fim, são apresentadas as considerações finais, com uma breve discussão geral dos resultados obtidos nos dois capítulos, assim como a conclusão geral da tese.

SUMÁRIO

Introdução geral

1. Zygnematophyceae

A classe Zygnematophyceae (nome automaticamente tipificado) ou Conjugatophyceae (nome descritivo) é a maior e mais diversa entre todas as seis classes de algas verdes classificadas na linhagem Streptophyta. Em conjunto com as algas verdes pertecentes ao clado Chlorophyta e as plantas terrestres (Embryophyta), as Zygnematophyceae constituem a linhagem Viridiplantae (Leliaert *et al.* 2012).

Do ponto vista morfológico e ultrastrutural, a classe Zygnematophyceae está dividida em duas ordens, Zygnematales ou Zygnemales (desmídias sacodermes) e Desmidiales (desmídias placodermes) (Brook 1981, Felisberto & Rodrigues 2010), ambas restritas a ambientes dulcícolas de regiões temperadas e tropicais (van-den-Hoek *et al*. 1997, Felisberto & Rodrigues 2011, Oliveira 2011).

A ordem Zygnematales é formada por organismos unicelulares de vida isolada que podem se reunir para formar colônias amorfas (Mesotaeniaceae) e individuos de aspecto filamentoso (Zygnemataceae), sendo polifilética do ponto de vista filogenético (McCourt *et al*. 2000). A ordem Desmidiales é monofilética e apresenta indivíduos unicelulares constituídos por duas semicélulas que se unem na região do istmo, além de possuírem a parede celular estruturalmente mais complexa e relativamente mais ornamentada com espinhos, poros e processos, entre outros. Atualmente, a ordem Desmidiales encontram-se composta por quatro famílias, quais sejam: Closteriaceae (2 gêneros), Gonatozygaceae (2 gêneros), Peniaceae (1 gênero) e Desmidiaceae (que apresenta a grande maioria dos gêneros descritos) (Mix 1972, McCourt *et al*. 2000, Gontcharov *et al*. 2003, 2004, Hall *et al*. 2008).

A família Desmidiaceae está formada por ao redor de 34 gêneros (Bicudo & Menezes 2017) e mais de 2.500 espécies validamente descritas (Gerrath 2003), porém, segundo alguns autores, o último número pode estar subestimado e ultrapassar até mais de 5.000 espécies (Škaloud *et al*. 2012). De qualquer modo, a família é considerada a mais especiosa e complexa das algas verdes incluídas na linhagem Streptophyta.

Desmídia é um termo genérico referente à palavra grega “desmós” que significa cadeia (Edward 1869). As desmídias são encontradas no perifíton e no metafíton de ambientes

oligotróficos a mesotróficos de água doce, que apresentem baixos valores de condutividade elétrica e sejam levemente ácidos (pH variando entre 3,5 e 5,5), contudo ressalte-se que algumas espécies são tolerantes a ambientes levemente alcalinos (Spijkerman 2004). Tais indivíduos são considerados K-estrategistas, pois necessitam de uma elevada demanda de recursos para seu desenvolvimento, além da estabilidade das variáveis físicas e químicas do ambiente, sendo muito sensíveis a quaisquer alterações ocorridas no meio em que habitam e, porisso, considerados excelentes indicadores de qualidade ecológica da água (Coesel 1991, Barbosa *et al*. 2013, Svoboda *et al*. 2014).

A taxonomia das desmídias encontra-se atualmente baseada, exclusivamente, nas características morfológicas de suas células, tais como forma, dimensões, planos de simetria, ornamentação da parede celular e configuração de seus cloroplastídios (Brook 1981, Šťastný 2013). Muitas das espécies classificadas nesse grupo apresentam ampla variação morfológica, mesmo em nível populacional, o que vem resultando em inúmeras confusões taxonômicas, com a descrição de inúmeros táxons infraespecíficos nos níveis variedade e forma taxonômica (Gontcharov & Melkonian 2010).

Ainda na década de 1970 do século passado, Bicudo (1972) já alertava para a necessidade de uma urgente revisão taxonômica de táxons classificados na família Desmidiaceae, afirmando que a mesma se encontrava entulhada de descrições de novas espécies, variedades e formas taxonômicas, muitas das quais haviam sido propostas de forma arbitrária, sem qualquer critério. Růžička (1981) considerou em sua importante revisão do material europeu do gênero *Micrasterias*, que muitos materiais constituíam variedades ou formas taxonômicas descritas a partir de pequenas diferenças morfológicas e seriam, portanto, representantes da mesma unidade taxonômica, isto é, sinônimos ou, até mesmo, só o resultado da elevada plasticidade fenotípica documentada para o grupo (Neustupa *et al*. 2010).

Com o advento das técnicas da biologia molecular e, portanto, com o surgimento dos primeiros resultados referentes ao posicionamento filogenético de táxons de Zygnematophyceae e, em especial, da ordem Desmidiales, certos gêneros foram identificados como polifiléticos (Gontcharov & Melkonian 2010) levantando-se, novamente, a questão da importância de revisões taxonômicas de desmídias, que incluam novas decisões e emendas a serem propostas, além de uma acurada revisão do conceito de espécie (não apenas com base na morfologia) para este grupo de microalgas. Além da criação de uma base taxonômica robusta, obtida a partir do uso da taxonomia polifásica de nível ômega, ou seja, com base em dados da taxonomia tradicional subsidiados pelas outras áreas do conhecimento como a morfologia, biologia molecular e filogenia, além de dados fisiológicos e ecológicos.

A delimitação de espécies é um requisito absolutamente indispensável para o entendimento da biodiversidade ecossistêmica. Entretanto, identificar espécies, consideradas unidades fundamentais no caso de muitos grupos de protistas e, em especial, os de microalgas é uma tarefa um tanto difícil, especialmente devido aos problemas relacionados com definir o que é uma espécie diante das várias tentativas constantes na literatura, que representam diferentes conceitos e definições (Škaloud *et al*. 2015).

A diversidade taxonômica das desmídias e os distintos conceitos de espécie foram discutidos por Coesel & Krienitz (2008), que destacaram a dificuldade na aplicação do conceito de espécie biológica frente à ausência e à indisponibilidade de dados referentes à reprodução sexuada no grupo e, atualmente, em 90-95% das espécies descritas. É extremamente difícil aplicar o conceito de espécie do ponto de vista ecológico se considerarmos o cosmopolitismo do grupo e o registro de muitas espécies endêmicas. Acrescente-se também a grande dificuldade de aplicar o conceito de espécie do ponto de vista morfológico neste grupo, que apresenta ampla variação morfológica, levando à existência de muitas morfoespécies e muitas espécies crípticas.

Com base estritamente na morfologia, espécies são definidas como grupos de indivíduos morfologicamente idênticos ou muito similares (Futuyma 1998). Este conceito tem dominado a sistemática das algas, pois a grande maioria das espécies podem ser reconhecidas através de suas diferenças morfológicas (Maggs 1997). No caso das desmídias, devido à grande variação morfológica documentada em uma mesma população, a direta influência das variáveis ambientais pode resultar em elevada plasticidade fenotípica ou ecomorfia e à possível existência de um grande número de espécies crípticas ou pseudocrípticas, o que tem feito que o uso irrestrito desse conceito se torne, muitas vezes, um grande problema para taxonomia tradicional, pois, pode frequentemente mascarar ou subestimar o real número de espécies presentes (Kouwets 2008, Šťastný 2013).

No que tange ao conceito ecológico, espécies podem ser definidas como grupos de indivíduos que ocupam a mesma zona adaptativa ou nicho ecológico (Valen 1976). Apesar das desmídias ocorrerem em ambientes dulcícolas, suas assembleias podem ser distintas em vários ambientes e, muitas vezes, restritas a hábitats particulares, apresentando especificidade e ótimo ecológico de acordo com determinada variável ambiental, o que influencia, diretamente, sua distribuição e delimitação (Coesel & Krienitz 2008). Fato como este pode ser constatado observando a classificação sugerida por Coesel (1996) ao dividir as desmídias em 10 regiões de acordo com sua distribuição geográfica.

O conceito biológico caracteriza a espécie através de seu isolamento reprodutivo (Mayr 1949) e, consequentemente, genético. Especial para as desmídias, tem sido evidenciada por

alguns autores a existência de espécies morfologicamente similares ou idênticas, entretanto, completamente isoladas reprodutivamente. Podem ser citadas como exemplos os casos de *Micrasterias thomasiana* W.Archer (Blackburn & Tyler 1987), o complexo de espécies *Closterium moniliferum*/*Closterium ehrenbergii* (Denboh *et al*. 2003) e *Micrasterias truncata* Brébisson *ex* Ralfs (Nemjová *et al*. 2011), entre outros.

Indivíduos que compartilham uma mesma e única história evolutiva podem ser considerados espécies a partir do conceito filogenético ou evolutivo de espécie. Neste conceito, são utilizados marcadores moleculares para testar a hipótese da ancestralidade e as relações filogenéticas entre táxons (Wheeler & Platnick 2000). Apesar dos importantes avanços moleculares e filogenéticos (McCourt *et al*. 2000, Gontcharov *et al*. 2003, Gontcharov & Melkonian 2004, 2005, 2008, 2010, Gontcharov 2008, Hall *et al*. 2008) que têm gerado mudanças na taxonomia das desmídias, muitos resultados ainda permanecem controversos e inconclusos, especialmente porque a grande maioria destes estudos têm se concentrado na reconstrução filogenética de linhagens correspondentes a famílias e ordens, sendo poucos os que abordaram ou abordam a validação do conceito de espécie (Neustupa *et al*. 2010, Nemjová *et al*. 2011, Neustupa *et al*. 2011, Šťastný 2013), principalmente, em táxons conhecidos como "complexo" de espécies.

De forma a buscar uma identificação e a delimitação correta de muitas espécies de desmídias e evitar a proposição de novas espécies, variedades e formas taxonômicas artificiais e destituídas de critérios, a abordagem polifásica vem sendo bastante utilizada em estudos de taxonomia, ecologia e filogenia, visando a juntar e validar toda informação existente nos mais diversos conceitos de espécie (Kouwets 2008).

O gênero *Micrasterias* foi proposto por C. Agardh (1827), validado por Ralfs (1848) e encontra-se classificado na família Desmidiaceae. Está composto por indivíduos unicelulares, solitários, coloniais ou que formam falsos filamentos simples, como é o caso de *Micrasterias foliacea* Bailey *ex* Ralfs. Os indivíduos considerados representantes de *Micrasterias* apresentam grandes dimensões celulares quando comparados com os demais componentes da família, além de uma morfologia altamente complexa (Neustupa *et al.* 2010). Esses indivíduos apresentam, ainda, simetria bilateral e incisões celulares que fazem com que suas semicélulas sejam divididas em lobos e lóbulos e os indivíduos sejam, nesta condição, divididos em trilobados (três lobos e duas incisões) e pentalobados (cinco lobos e quatro incisões) (Sormus 1980).

Atualmente, o gênero apresenta distribuição cosmopolita e compreende cerca de 70 morfoespécies, além de muitas categorias infraespecíficas descritas (Bicudo & Menezes 2017).

No entanto, devido à classificação das espécies estar baseada, exclusivamente, em dados morfológicos tradicionais, tal número pode ser considerado subestimado e possível de mascarar a real diversidade específica existente no grupo (Poulíèkova *et al.* 2014).

A história taxonômica de *Micrasterias* foi registrada, principalmente, nos trabalhos de Krieger (1939), Prescott *et al*. (1977) e Růžička (1981), a partir de materiais coletados basicamente no Hemisfério Norte. No entanto, desde sua descrição original e devido ao tamanho e às formas ornamentadas de várias de suas espécies, ao seu padrão de crescimento e à elevada sensibilidade ambiental, o gênero tem sido alvo de vários estudos fisiológicos, morfogenéticos e experimentais (Meindl 1993, Škaloud *et al*. 2011) desde a década dos anos 50 do século passado.

O crescente uso da abordagem polifásica na taxonomia de microalgas vem permitindo o desenvolvimento de inúmeros trabalhos, muitos dos quais abordando espécies pertencentes ao gênero *Micrasterias* (Kallio 1949, 1951, 1953, 1954, 1960, 1969, Kiermayer 1970, Pickett-Heaps 1975, Meindl 1993, Weiss *et al.* 1999, Holzinger 2000, Holzinger & Lütz-Meindl 2001, Neustupa & Šťastný 2006, Neustupa & Škaloud 2007, Neustupa *et al.* 2008, 2009, Savriama *et al.* 2010, Neustupa *et al.* 2010, Škaloud *et al*. 2011, Jurdíkova *et al.* 2014, Neustupa 2013, 2016, 2017, Neustupa *et al.* 2014, Poulíèkova *et al.* 2014, Lütz-Meindl 2016, Neustupa & Šťastný 2018) e utilizando técnicas de cultivo, experimentos de radiação UV, morfometria geométrica, padrões de simetria e assimetria ao longo do desenvolvimento ontogenético e evolutivo, biologia molecular e filogenia, além da avaliação da influência de variáveis ambientais sobre a plasticidade fenotípica de espécies do gênero. Entretanto, vale ressaltar que a maioria das *Micrasterias* estudadas nesses trabalhos são espécies pentalobadas e muitas encontram-se disponíveis em coleções de cultivo ao redor do mundo.

Hall *et al*. (2008) estudaram a posição filogenética de alguns gêneros de Zygnematophyceae utilizando dois marcadores moleculares, o plastidial *rbc*L e o mitocondrial *Cox*III. Conforme esse estudo, *Triploceras gracile* Bailey, uma espécie morfologicamente bastante distinta de todas as descritas para o gênero *Micrasterias* situou-se na mesma linhagem evolutiva deste gênero. Foi então sugerido que *T. gracile* possa ter surgido a partir de uma *Micrasterias* ancestral ou através de uma possível hibridização acompanhada por diversas transformações morfológicas as quais resultaram em diferentes níveis de complexidade dos padrões de ramificação celular como respostas às variáveis ambientais (Gontcharov 2008, Škaloud *et al*. 2011).

Ao desenvolver um estudo sobre a filogenia multigênica de *Micrasterias* utilizando três marcadores moleculares, o nuclear SSU rDNA e os dois plastidiais *psa*A e *rbc*L, Škaloud *et al.*

(2011) mostraram que o gênero inclui, pelo menos, oito linhagens e possui origem monofilética. Além disso, a possibilidade de ter ocorrido uma substancial transformação morfológica de suas linhagens durante a diversificação das espécies, o que permitiu sugerir a origem de diferentes expressões morfológicas dentro do gênero (Gontcharov 2008, Neustupa *et al.* 2010, Gontcharov & Melkonian 2010). Ness estudo, foi mostrada a presença de *T. gracile* junto ao ramo de *Micrasterias* e, ainda, a proximidade filogenética de outras espécies de desmídias, as quais são morfologicamente bastante distintas, como *Cosmarium ralfsii* Brébisson *ex* Ralfs e *Staurodesmus dickiei* (Ralfs) S.Lillieroth. Os dois estudos previamente citados, Hall *et al*. (2008) e Škaloud *et al*. (2011), assim como os de Neustupa *et al*. (2010) e Goncharov & Melkonian (2011) confirmaram a origem monofilética do gênero *Micrasterias*.

No que diz respeito às abordagens morfológicas e moleculares em nível específico, como as que ocorreram com *M. crux-melitensis* Ralfs e *M. radians* W.B.Turner (Neustupa *et al.* 2010), *Micrasterias rotata* Ralfs e *M. fimbriata* Ralfs (Neustupa *et al*. 2011) e o complexo *M. truncata* Brébisson *ex* Ralfs (Nemjová *et al.* 2011), tais estudos revelaram que espécies tradicionais podem representar unidades taxonômicas significativas e que algumas das variedades documentadas em literatura podem, aparentemente, ser consideradas espécies independentes. Isto sugere que muitos táxons infraespecíficos identificados via morfologia clássica devam ser eminentemente artificiais e, com isto, mascarar seu real valor taxonômico e a real diversidade existente no gênero (Neustupa *et al.* 2011).

Para o Brasil, não foi reportado até o momento qualquer estudo que aborde a biologia molecular, a filogenia e/ou a ecofisiologia de uma espécie de *Micrasterias*, nem de representantes de outros gêneros das Desmidiaceae. No entanto, há uma grande quantidade de trabalhos desenvolvidos a partir da taxonomia morfológica clássica de nível α sobre as desmídias brasileiras, inclusive para o gênero em questão. Segundo Santos (2016), existem 21 trabalhos que abordam somente o gênero *Micrasterias* no Brasil, sendo que a grande maioria deles foi realizada a partir de material coletado no Estado de São Paulo, contabilizando 12 publicações.

Excetuado o trabalho de Bicudo & Sormus (1977) sobre a tipificação do nome genérico *Micrasterias*, todos os demais versaram sobre polimorfismo, autecologia e taxonomia clássica. Assim, Bicudo & Senna (1975) avaliaram o uso de medidas celulares na delimitação de táxons infraespecíficos de *M. laticeps*; Bicudo (1978) discutiu qual nome utilizar, *M. furcata* ou *M. radiata*; Bicudo & Sophia (1981) examinaram o polimorfismo em *M. simplex* e suas implicações taxonômicas; Bicudo & Sormus (1982) inventariaram taxonomicamente as *Micrasterias* do Estado de São Paulo; Sormus & Bicudo (1974) estudaram o polimorfismo em

M. pinnatifida e suas implicações taxonômicas; Sormus (1975) deu a conhecer as *Micrasterias* trilobadas do Brasil; Sormus (1980) reviu taxonomicamente *M. arcuata* Bailey e *M. laticeps* Nordstedt; Sormus & Bicudo (1997) inventariaram as *Micrasterias* do Parque Estadual das Fontes do Ipiranga, São Paulo, SP; por fim, é importante considerar o trabalho de Gil-Gil & Bicudo (2000) sobre a ecologia de *M. arcuata* var. *arcuata* e *M. arcuata* var. *expansa* e o de Bicudo & Gil-Gil (2003) sobre a variação morfológica em *M. arcuata.*

Micrasterias arcuata Bailey foi proposta por Bailey (1851) a partir de material coletado no Estado da Flórida, Estados Unidos da América. Assim como outras espécies do gênero, *M. arcuata* tem preferência por ambientes dulcícolas oligotróficos a mesotróficos e levemente ácidos, ocorrendo em lagos rasos, açudes e pântanos da região temperada e em ecossistemas aquáticos tropicais (Sormus 1980, Coesel 1982, Gil-Gil & Bicudo 2003, Neustupa *et al.* 2010), com presença ou não de *Sphagnum*.

De acordo com sua descrição original, *M. arcuata* apresenta semicélulas trilobadas e célula de tamanho mediano e contorno quadrangular. Os lobos basais são lunados, estreitos, atenuados para o ápice e curvados no sentido do lobo polar. Os lobos polares são estreitos, possuem as margens laterais paralelas entre si e são bífidos no ápice, com as expansões estiradas horizontalmente dando ao conjunto a forma de um "T". A margem apical é sutilmente arqueada, mostrando uma depressão mediana suave ou mais acentuada, além de ângulos acuminados. A parede celular é lisa e o cloroplastídio axial acompanha o contorno celular (Bailey 1851, Sormus 1975, 1980).

Além da forma típica da espécie, *M. arcuata* Bailey var. *arcuata* f. *arcuata*, existem atualmente outros 23 táxons entre variedades e formas taxonômicas (Guiry & Guiry 2019) registrados a partir de materiais coletados na América do Norte, América do Sul, Ásia, África, Cuba e a extinta União das Repúblicas Socialistas Soviéticas (Kossinskaja 1960, Prescott *et al*. 1977), sendo a proposição da grande maioria desses 23 táxons há pouco mencionados baseada em materiais brasileiros (Förster 1964, 1969). No entanto, tais registros jamais foram verificados de modo consistente e vários desses táxons constituem atualmente sinônimos. Considera-se, ainda, que tais táxons foram propostos nas décadas dos anos 60, 70 e 80 do século passado, na ausência de coletas sistematizadas (Sormus 1975, Bicudo & Sormus 1980, Guiry & Guiry 2019), o que faz com que *M. arcuata* seja uma espécie problemática e complexa no que diz respeito às suas taxonomia e nomenclatura.

As primeiras ocorrências de *M. arcuata* no Brasil datam do século XIX, quando Börgesen (1890) identificou *M. arcuata* var. *expansa* (Bailey) Nordstedt e Borge (1918) registrou a ocorrência de *M. arcuata* f. Nordstedt no Estado de São Paulo. Atualmente, há documento da

existência de representantes da referida espécie em todas as regiões do Brasil a partir de trabalhos exclusivamente de taxonomia clássica de nível α. Em suma, foram registradas nove variedades taxonômicas oriundas de material coletado nos estados do Amazonas, Bahia, Goiás, Mato Grosso, Minas Gerais, Pará, Paraná, Rio de Janeiro, Santa Catarina, São Paulo, Rio Grande do Sul e no Distrito Federal (Borge 1918, Förster 1964, 1969, Bittencourt-Oliveira & Mecenas 1994, Fonseca & Estrela 2015, Oliveira *et al*. 2017, Santos *et al*. 2018, Ramos *et al*. 2019).

Especialmente para o Estado de São Paulo foram mencionadas na literatura as três variedades seguintes: *M. arcuata* var. *arcuata*, *M. arcuata* var. *robusta* Borge (inclui *M. arcuata* var. *expansa* (Bailey) Nordstedt f. Borge, até então identificada por alguns autores como sinônimo) e *M. arcuata* var. *subpinnatifida* West & West. Essas variedades foram identificadas de materiais coletados em ecossistemas aquáticos dos municípios de Conchal, Itirapina, Moji Guaçu, Pedregulho, Pirassununga, São Bernardo do Campo, São Carlos e São Simão (Gil-Gil 1996, Bicudo *et al.* 2015).

Com exceção dos registros provenientes de estudos de taxonomia clássica, as únicas informações referentes à sinonímia e ao polimorfismo e suas implicações taxonômicas na sistemática e na nomenclatura de *M. arcuata*, além de sua relação com as variáveis ambientais constam nos trabalhos de Sormus (1975, 1980), Gil-Gil (1996), Gil-Gil & Bicudo (2000) e Bicudo & Gil-Gil (2003), todos realizados a partir de materiais coletados no Estado de São Paulo e os únicos existentes em nível mundial para a referida espécie. Não existe, pois, um estudo que tenha sido realizado utilizando a abordagem polifásica ou mesmo análises moleculares e filogenéticas sobre esta espécie.

1.1. Justificativa

Apesar dos numerosos estudos de taxonomia clássica de nível α e do crescente aumento do número dos subsidiados pela taxonomia polifásica de nível ω, ainda são verificadas lacunas no que tange ao conhecimento de *M. arcuata*, especialmente pelo fato desta espécie apresentar ampla variação morfológica, problemas do ponto de vista taxonômico e nomenclatural, escassez de informação referente às suas ecofisiologia e autecologia, além da inexistência de informação molecular e filogenética.

O presente estudo é pioneiro em escala mundial, pois além de ampliar o conhecimento e esclarecer possíveis implicações taxonômicas e nomenclaturais em *M. arcuata* visa, principalmente, a partir do uso de ferramentas da taxonomia polifásica de nível ω, a suprir a carência de informação sobre o polimorfismo e a plasticidade fenotípica em populações de *M.*

arcuata e suas variedades infraspecíficas, além de realizar o primeiro estudo filogenético para a espécie.

1.2. Perguntas

1. Até que ponto a variação morfológica documentada e amplamente registrada em *M. arcuata* é válida para separar espécies em variedades e formas taxonômicas?
2. A classificação morfológica de *M. arcuata* representa sua classificação filogenética?
3. Qual o posicionamento filogenético de *M. arcuata* em relação ao gênero *Micrasterias*, assim como ante gêneros e espécies próximas?

1.3. Hipóteses

H1. A variabilidade morfológica é uma resposta genética.

H2. A variabilidade morfológica documentada e o desenvolvimento populacional de *M. arcuata* e suas variedades taxonômicas são os mesmos na natureza e em condições de cultivo.

H3. A variabilidade morfológica representa uma unidade taxonômica bem definida do ponto de vista filogenético e molecular.

2. OBJETIVOS

2.1. Objetivo geral

Ampliar o conhecimento sobre a caracterização, a variabilidade morfológica, a autecologia e inferir o posicionamento filogenético de *M. arcuata* e suas categorias infraespecíficas.

2.2. Objetivos específicos

- Identificar e isolar os morfotipos associados a *M. arcuata* encontrados na área de estudo e acompanhar o desenvolvimento de suas cepas.
- Utilizar as morfometrias clássica e geométrica derivadas de dados da literatura e da natureza para avaliar e diferir os caracteres morfológicos descritivos e os diagnósticos em morfotipos de *M. arcuata.*
- Utilizar as morfometrias clássica e geométrica derivadas de dados obtidos em condições de cultivo para avaliar e diferir os caracteres morfológicos em cepas contendo os morfotipos de *M. arcuata.*
- Analisar a filogenia de *M. arcuata* e suas categorias infraspecíficas.
- Revisar a taxonomia e a nomenclatura de *M. arcuata* e de suas categorias

infraspecíficas, com base em evidências morfológicas e moleculares.

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Capítulo 1

Assessment of traditional and geometric morphometric analyses to discriminate populations of *Micrasterias arcuata* species complex (Desmidiaceae, Zygnematophyceae)

ABSTRACT

The taxonomy of *Micrasterias arcuata* Bailey is mainly based on traditional morphological characters, such as cell shape and dimensions. Regarding taxonomic and the nomenclatural points of view, *M. arcuata* is considered a species complex comprising several infraspecific taxa. In this study, seven morphological characters and traditional measurements were obtained from 921 morphotypes from natural populations and literature data. Morphological attributes such as average cell length, maximum cell width, width of polar lobes, and distance between basal lobes were the key discrimination features in the species. Considering the observations and remarks on classical taxonomy, the total of 24 *M. arcuata* taxa are registered in the literature, from which the taxonomical value of some infraspecific taxa remains unclear, and further research is needed to reveal their true nature, especially the closest morphologically ones and the similar taxa. According to the present data, three varieties (var. *arcuata*, var. *expansa* and var. *perforata*) have their morphological attributes clearly delimited, and their taxonomic splitting into infraspecific taxa is not justified or recommended. Present results also indicate that the real hidden diversity of *M. arcuata* is probably unknown, and that is extremely necessary to broaden the *M. arcuata* concept with a combination of morphological, physiological and, especially, molecular information to reveal wheather the individuals traditionally identified as *M. arcuata* infraspecific taxa represent taxonomically meaningful units and perhaps even independent species.

Key words: desmid, infraspecific taxa, morphology, Streptophyta, traditional taxonomy

INTRODUCTION

Micrasterias arcuata was described by Bailey (1851) from material collected in the state Florida, United States of America. The species has unicellular 3-lobed organisms with basal lobes curved upward, concave apical margin, and polar lobe transversely projected (Bailey 1851, Prescott *et al.* 1977). Its presence was already documented in the United States, Canada, Cuba, West Africa, Asia and South America (Prescott *et al.* 1977, Sormus 1980, Fonseca *et al.* 2019), and in the former Union of Soviet Socialist Republics too (Kossinskaja 1960). As the same as other species of *Micrasterias*, current specimens prefer oligotrophic and slightly acidic environments, occurring in shallow lakes, dams and marshes of temperate and tropical aquatic ecosystems (Sormus 1980, Gil-Gil & Bicudo 2003) populated or not by *Sphagnum* (Bailey 1851, Prescott & Scott 1943).

Micrasterias arcuata is a morphologically very diverse species (Thomasson 1966, 1971, Martins & Bicudo 1987, Kastovysky *et al.* 2011, Fonseca & Estrela 2015, Oliveira *et al.* 2017, Santos *et al.* 2018, Ramos *et al.* 2019). Furthermore, it displays a large amount of documented morphological variation (e.g. Nordstedt 1877, Borge 1896, Sormus 1980), that means that it is a complex taxon worthy of interest from both taxonomic and nomenclatural points of view. Besides the typical variety, this species comprises nowadays 23 infraspecific taxa represented by varieties and taxonomic forms (Bailey 1851, Nordstedt 1877, West & West 1896, 1897, Borge 1899, Prescott & Scott 1943, 1952, Förster 1964, 1969, Guiry & Guiry 2019).

From the taxonomic entities above, seven varieties were proposed based on Brazilian material (Borge 1899, Förster 1964, 1969), representing most taxa proposed for the species. The first occurrence of *M. arcuata* in Brazil dates from the 19th century represented by the proposition of *M. arcuata* var. *expansa* (Bailey) Nordstedt (Börgesen 1890) and *M. arcuata* var. *robusta* Borge (Borge 1889). Currently, the species was frequently recorded in all Brazilian regions (Borge 1899, 1918, Förster 1964, 1969, Bittencourt-Oliveira & Mecenas 1994, Fonseca & Estrela 2015, Oliveira *et al.* 2017, Santos *et al.* 2018, Ramos *et al.* 2019).

The great majority of the studies on *M. arcuata* made in Brazil focused, exclusively, on classical α taxonomy. A few studies were dedicated, at the same time, to its taxonomic revision and distribution (Sormus 1975, 1980), polymorphism and ecology (Gil-Gil & Bicudo 2000, Bicudo & Gil-Gil 2003), and a single one to the relationship between ecological characteristics of specific sites and the morphological features of *M. arcuata* populations using the geometric morphometric approach (Fonseca *et al.* 2019).

According to the literature, features used to identify *M. arcuata* and to split it into varieties

and taxonomic forms are the cell measurements, morphology of the polar and basal lobes (e.g. angles), presence and number of spines and, sometimes the cell wall sculpture (Bailey 1851, West & West 1896). Cell measurements are the most useful feature and an important morphological attribute used as diagnostic criterion to discriminate infraspecific taxa in *M. arcuata*. However, similarly to other microalgal groups and especially other desmid species, in many circumstances this morphological attribute may be extremely variable even within natural populations, dependent on the environment conditions (Bicudo & Sormus 1972, Sormus & Bicudo 1974).

The cell size was obtained up to now for *M. arcuata* studies exclusively by conventional or linear procedures, and are represented by the cell length, cell width represented by the width got from a basal lobe to its opposite similar, isthmus width, and sometimes the polar lobe width (Förster 1964). Measurement overlapping are somewhat frequently documented in the literature, even for infraspecific taxa (Sormus 1980), thus showing that the use of that characteristic as the only diagnostic one, considering that they are sometimes taken from just a few cells, surely is insufficient to capture the whole geometry of the species, making impossible differentiation of some taxa and causing many taxonomic problems for the traditional morphological species-level taxonomy.

Geometric morphometry is a multivariate approach based on the configuration of homologous points (landmarks coordinates) (Booksterin 1997). Contrary to the traditional morphometric measurements (which are based on linear distances, ratios and angular measurements), that analysis is used to capture and quantify more information about the shape of interspecific and/or intraspecific specimens with inherent separation of size differences and statistical analysis, that reflect shape variation of size of the cells standardized to a unit dimension (Zelditch *et al*. 2004).

Currently, analysis of shape coordinates is widely used in almost every branch of biology and is considered the most powerful method for biological shape analysis (Dryden & Mardia 1998, Zelditch *et al.* 2012). Geometric morphometric methods are used in desmids research for taxonomy (Neustupa & Šťastný 2006), phylogeny (Neustupa & Škaloud 2007, Neustupa *et al*. 2010, Nemjová *et al.* 2011, Šťastný *et al.* 2013, Neustupa et al. 2014), ecology applications (Neustupa *et al*. 2011, Neustupa *et al.* 2013, Fonseca *et al*. 2019), experimental studies of phenotypic plasticity such as pH and temperature (Neustupa *et al.* 2008, Černá & Neustupa 2009, respectively), and patterns of symmetric and asymmetric components (Neustupa 2016, Neustupa & Šťastný 2018, Savriama *et al*. 2010).

Present paper aimed at figuring out whether the wide morphological variation described

for *M. arcuata* is adequate for its separation into distinct infraspecific taxa and even species; also at providing a revision of the species complex comparing our current data with the up-to-date delineation using traditional morphometric criteria and geometric morphometric analyses in natural populations and literature data.

MATERIAL AND METHODS

This study is based on natural population of *M. arcuata* species complex obtained from samples collected monthly, from March 2017 to February 2018, at a small pond covered to a large extent by *Sphagnum* located at the "Reserva Experimental de Itirapina" (47º00'52.12" W, 22º12'39" S), municipality Itirapina, São Paulo state, southeast Brazil. A total of 36 samples were collected using a 20 µm mesh plankton net, at three different sites located at 5 m distant from each other. Algal material was fixed with 4% formalin solution immediately after they were sampled. All samples were deposited in the "Herbário Científico do Estado Maria Eneyda P. Kauffmann Fidalgo" (SP), São Paulo Municipality, São Paulo state, Brazil. The access numbers of the samples are listed in the table in the Supplementary material 1.

Figure 1A-C. Small pond at the "Reserva Experimental de Itirapina", municipality Itirapina, São Paulo state, southeast Brazil. **A**. Sampling station 1; **B**. Sampling station 2; **C.** Sampling station 3.

Traditional morphometric analyses

Altogether, 875 individual specimens of the *M. arcuata* species complex were analyzed during the traditional morphometric analyses. In addition, 32 illustrations from the literature (iconotypes) were analyzed (Supplementary material 2). Because several iconotypes did not include measurements of their morphological attributes currently investigated, they were measured using the scale bar of their publications. In this case, measurements were obtained

with a ruler in 1/100 mm, and the results obtained transformed to µm using the conversion factor of the scale bar of each illustration.

Morphological attributes were investigated using a light microscope (Axioskop 2, Zeiss) and an inverted one (Zeiss Axio Observer D1 with a 2.5 optovar and image capture Axiocam MRc Rev. 3, Imaging Systems 4.7.2), under 400x magnification. The ZEN Zeiss software v. 2012, with picture analyses tools, was used for the examination of the morphological attributes in each individual and to obtain their microphotographs.

Measurements were taken from individual cells as follows (Fig. 2A): maximum cell length (MCL), the distance of polar lobes of opposite semicells; average cell length (ACL), the distance of polar lobes midpoints of opposite semicells; maximum cell width (MCW), the distance between basal lobes of the same semicell; polar lobes distance (BPL), the distance between polar lobes of the same semicell; and basal lobes distance (BLD), the distance between the tips of basal lobes of opposite semicells. All these measurements were the same used in Bicudo & Gil-Gil (2003). In addition, the isthmus width (IW) and the height of polar lobe (HPL), i.e. the distance between the basal and polar lobes of the same semicell were also measured.

Geometric morphometric analyses

For the geometric morphometric analyses, photomicrographs were taken from 812 individual cells of *M. arcuata* (779 from natural populations and 33 from iconotypes). To conduct the analyses, software TpsUtil 1.61 (Rohlf 2015) was used to transform the photomicrographs of specimens into a TPS files format. Then, the software TpsDig 2.32 (Rohlf 2005) was used to digitize landmarks and semi-landmarks on scaled TIFF images.

A total of 49 equidistant landmarks were depicted in the front view of the semicell of *M. arcuata* (Fig. 2B), according to the previous study concerning this species (Fonseca *et al.* 2019). One landmark was positioned on the bilateral symmetry axis of semicell, and the other 48 were bilaterally symmetrically positioned on each side (left and right as mirror images) of the semicell. Thirteen landmarks were considered fixed points, as follows: point number 1, 49 - margin of the isthmus; 7, 43 - basal lobes extremities; 13, 37 - base of basal lobes; 14, 36 - middle point of polar lobes height; 15, 35 - base of polar lobes lateral margins; 19, 31 - extremities of polar lobe lateral margins; 25 - polar lobe central incision (apical margin). The remaining 36 landmarks represented semi-landmarks and were positioned along the semicell outline to minimize Procrustes distances and any apparent shape difference (Zelditch *et al.* 2012).

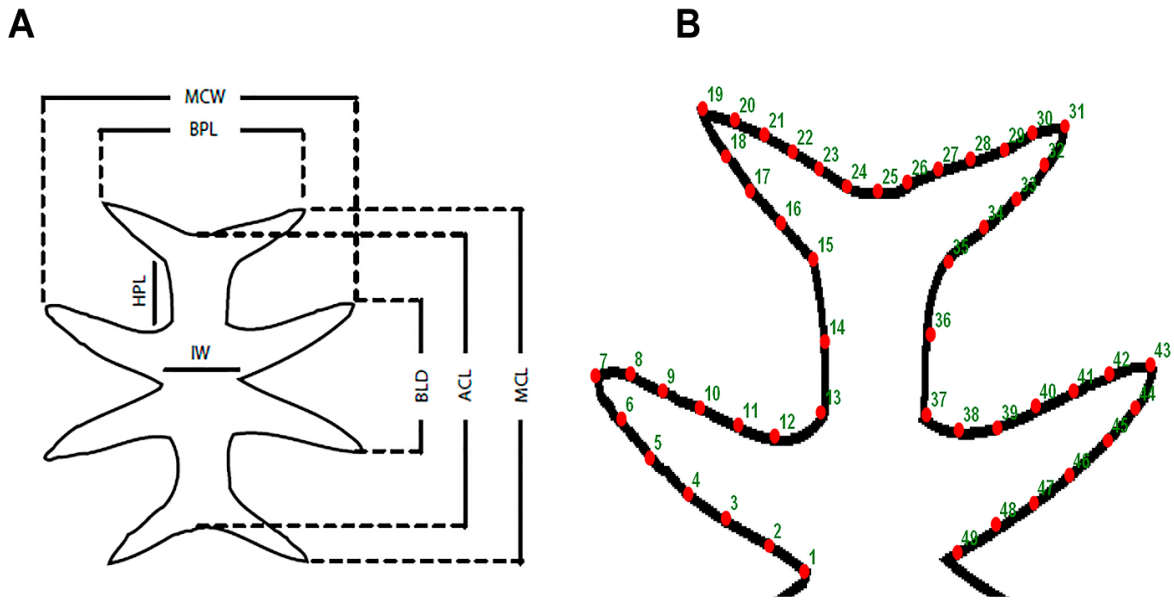

Figure 2. Morphological attributes of *M. arcuata* analyzed in traditional and geometric morphometric analyses. **A.** Morphological attributes analyzed in traditional analyses: Maximum cell length (MCL); Average cell length (ACL); Maximum cell width (MCW); Width of polar lobe or minimum cell width (BPL); Distance between basal lobes (BLD); Height of polar lobe (HPL); Isthmus width (IW); Linear measurements were proposed in Gil-Gil & Bicudo (2000), Bicudo & Gil-Gil (2003); Figure was modified in this study; **B.** Positions of the forty-nine equidistant landmarks depicted on front semicells outline in geometric morphometric analyses

Landmarks data were imported into RStudio v. 1.2 (Core Team 2019) and opened in the *geomorph* R-package (Adams & Otárola-Castillo 2013). Then, data were aligned using the Generalized Procrustes Analysis (GPA), that standardizes the size and optimizes the translation and rotation into a common shape space, thus minimizing the distances between corresponding landmarks and all-non shape information (Zelditch *et al*. 2012) using the "*gpagen*" function.

Cells of *M. arcuata* are symmetric with respect to two perpendicular axes of symmetry. The first axis separates the cell, in the region of the isthmus, into juvenile and adult semicells, and the second one divides the semicells into left and right sides (Savriama *et al*. 2010). Due to this, landmarks data obtained from semicells in front view were considered an object symmetric and they were symmetrized by calculating the mean of the original coordinates, and their reflected relabeled copies by using the "*bilat.symmetry*" function in *geomorph* package. The

Procrustes shape coordinates were used for subsequent statistical analysis.

To visualize the shape variation among individuals in the morphospace, a Principal Component Analysis (PCA) on shape variability was generated by the "*plotTangentSpace*" function. Procrustes coordinates for the mean shape of each morphotype/strain were calculated by "*mshape*" function, and the Thin-Plate spline deformation grids (TPS-grids) were generated and plotted, considering the first two PCA axes, to allow visualization of the shape changes of the morphotypes studied. The Linear Discriminant Analysis (LDA) was conducted using all the non-zero PC axes in the MASS R-Package (Venables & Ripley 2002) to evaluate groups discrimination by the reassignment probabilities (McLachlan 2004) that were evaluated by leave-one-out cross-validation. The non-parametric MANOVA (Anderson 2001) was also performed to test significant differentiation between morphotypes on the matrix of Euclidean distances in Past v. 2.17 (Hammer *et al*. 2001).

Scanning Electron Microscope (SEM) analysis

For the scanning electron microscope (SEM) analysis, a drop of the formaldehyde fixed cell suspension was transferred into 30% acetone, and dehydrated using an acetone series: 30%, 50%, 70%, 90% and 100% (twice at the last one). Subsequently, the cells were placed on cover glasses and dried out at room temperature. Then, the cover glasses were attached to aluminum stubs with double-side carbon tape. Finally, they were sputter coated with gold and examined using a Philips 20XL scanning electron microscope.

All photomicrographs were digitally manipulated and plates containing LM and SEM pictures were mounted using Adobe Creative Cloud Photoshop software v. 2013.

RESULTS

Traditional morphometric analyses

The Principal component analysis (PCA) divided the morphotypes into four partially defined groups (Fig. 3A-B). The first PCA axis described 72.2% of total variability and divided the morphotypes according to their length (MCL and ACL) from the shorter or compact forms, and the morphotypes 2 and 5 were separated form the remaining ones (morphotypes 1, 3, 6 and 7). The second PCA axis described 17.3% of total variability and separated morphotype 2 from 5. The main differences between these both morphotypes were in the isthmus width (IW, Fig. 3C) and the maximum cell length (MCL, Fig. 3D). The morphotype 2 was strongly correlated with the distance between basal lobes (BLD). The two PCA axes grouped together morphotypes 1, 3, 4, 6 and 7.

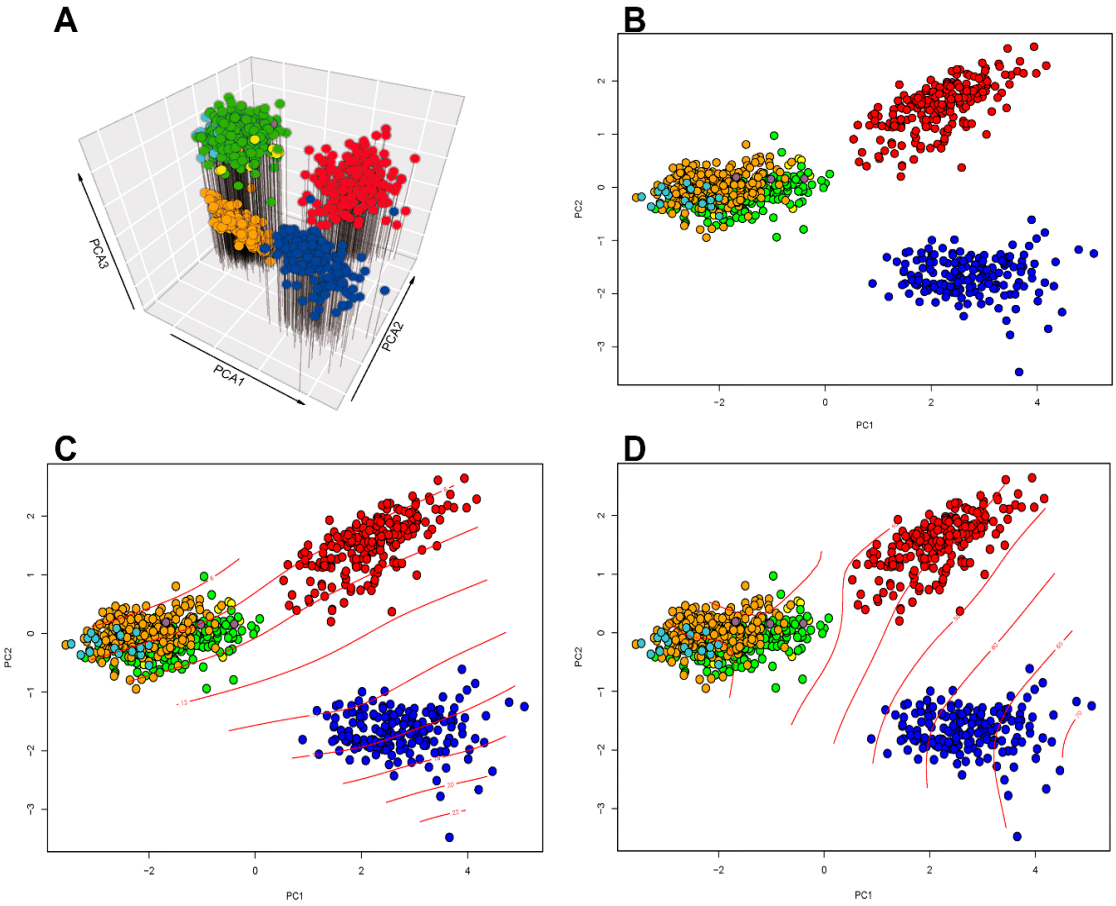

Figure 3. Principal component analysis (PCA) of natural populations of *M. arcuata* morphotypes. **A.** PCA 3D, scatter plot; **B.** PCA 2D, PC1 x PC2; **C.** PCA 2D, PC1 x PC2, surface plot of isthmus width (IW); **D.** PCA 2D, PC1 x PC2, surface plot of maximum cell length (MCL); PC1 axis = 72.2% and PC2 axis = 17.3%; M1, yellow circles; M2, red circles; M3, green circles; M4, orange circles; M5, blue circles; M6, light blue circles; M7, lilac circles.

The third PCA axis (7% of data variability) exhibited the differences between morphotypes 1, 3, 4, 6 and 7 (Fig. 4A-D). According to this PCA configuration, morphotypes 1, 3, 6 and 7 were grouped at the positive side of the third axis, and morphotype 4 was separated from all these others and ordered at the negative side of first axis. The main difference between both clusters mentioned above is in the MCL, HPL and BLD values (Fig. 4B-D).

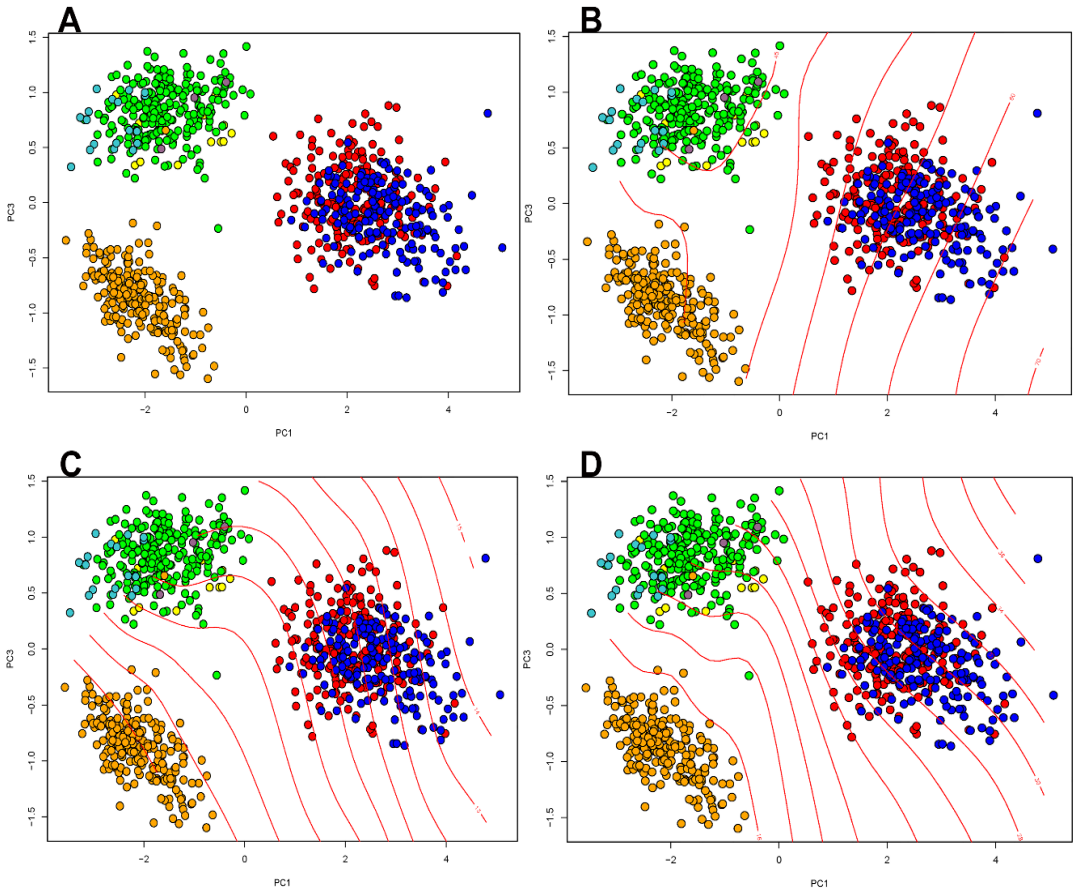

Figure 4. Principal component analysis (PCA) of natural populations of *M. arcuata* morphotypes. **A.** PCA, 2D PC1 x PC3; **B.** PCA, 2D PC1 x PC3, surface plot of maximum cell length (MCL); **C.** PCA, 2D PC1 x PC3, surface plot of height polar lobe (HPL); **D.** PCA, 2D PC1 x PC3, surface plot of basal lobe distance (BLD); PC1 axis = 72.2% and PC3 axis = 7%; M1, yellow circles; M2, red circles; M3, green circles; M4, orange circles; M5, blue circles; M6, light blue circles; M7, purple circles.

Considering the Principal Component Analysis (PCA) applied to both literature and natural morphotypes of *M. arcuata* (Fig. 5A), the first principal component axis described 57.5% of total variability, and the second one 20.5% of total variability. This analysis allowed depicting how morphological diversity in *M. arcuata* complex was distributed, and the relation between natural morphotypes and literature data ones according to the morphological attributes analyzed.

Linear discriminant analysis (LDA, Fig. 5B) plot applied to *M. arcuata* natural morphotypes showed four separated groups assigned as morphotypes 2, 4, 5 and the morphotypes 1, 3, 6 and 7 that were grouped together. The Results obtained from NPMANOVA

showed statistically significant differences among the seven morphotypes currently evaluated according to the traditional morphometric analysis ($F = 1130$, $p < 0.0001$).

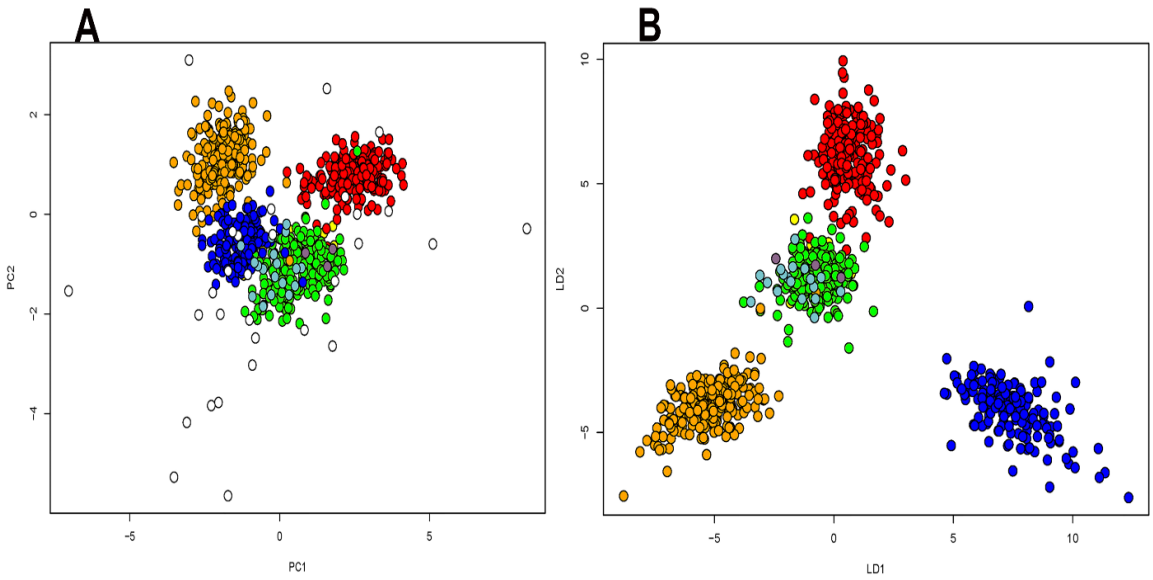

Figure 5. A. Principal component analysis (PCA) of natural populations and literature data of *M. arcuata* morphotypes. PCA1 axis = 57.6% and PCA2 axis = 20.5%; **B.** Linear discriminant analysis (LDA) of natural populations and literature data of *M. arcuata* morphotypes; M3, green circles; M4, orange circles; M5, blue circles; M6, light blue circles; M7, purple circles; White circles in PCA ordination represent the position of the morphotypes from the literature data (see supplementary material 1).

The correct classification/ prediction (Supplementary material 3) based on natural population data and literature taxa showed the relation among the morphotypes and the iconotypes analysed. According to these results, morphotype 1 was closely related with *Micrasterias acuata* var. *robusta* f. *goyazensis* Förster. Morphotype 2 was related to taxa identified as *Micrasterias arcuata* Bailey, morphotype 5 was classificated within taxa identified as *Micrasterias arcuata* var. *perforata* Förster and all their taxonomic forms. Morphotype 4 was related to *Micrasterias expansa* Bailey, *Micrasterias arcuata* var. *minuta* Förster, *Micrasterias arcuata* var. *robusta* f. *recurvata* Prescott & Scott that comprising taxa morphologically very closer, and it was related to one taxon identified as *Micrasterias borgei* Förster, especially due to the shape of apical margin and the ends of polar lobe. Morphotype 3 and 6 were related to individuals taxa identified in their greater majority as *Micrasterias arcuata*

var. *subpinnatifida* West & West, or *Micrasterias arcuata* var. *cornuta* Förster or *Micrasterias arcuata* var. *subcornuta,* or even so, as some individuals of *Micrasterias arcuata* var. *robusta* due the specimens comprising these both morphotypes have shorter length, basal lobes straight and ain many cases, basal lobes and the lateral extremities of polar lobe swollen at the base.

Geometric morphometric analyses

The PCA analysis of the symmetrized dataset divided morphotypes into four groups (Fig. 6). The first PCA axis spanned 65.3% of total variation and the second one 21.4%. Average of shape variation of individual groups may be seen on the thin plate splines of grid deformation (Fig. 6B-J).

In the negative side of the first PCA axis were located morphotypes 4 (Fig. 6B-C) and 5 (Fig. 6D). They had narrow polar lobes, with concave apical margin. Lateral extension of polar lobes was faintly straight to curve upward, and in some cases slightly curved downward (M5) to strongly curved upward (M4). Basal lobes were narrowly straight to slight curved upward (M4) or narrow and straight with an intumescence at the base (M5).

At the positive side of the first PCA axis, morphotypes were grouped according to the following attributes: compact cell body, relative longer polar lobes with little straight to strong curved downward apices, and sometimes with an elevation on either side of the mid region. Morphotype 2 (Fig. 6I-J) presented its basal lobes slender and curved upward with a marked depression or concavity at the apical margin. Moreover, morphotypes M3 had basal lobes straight (Figs. 6E, 6G-H). Morphotype 3 exhibited basal lobes narrow and straight curved towards the apex of the polar lobe. Lateral extension of the polar lobe was narrow and slightly curved downward and presented sometimes an elevation on either side of the mid region. And morphotype 1 (Fig. 6E) exhibited both basal and polar lobes apices slightly curved upward, the latter sometimes slight to straight curved down, with a marked depression in the apical margin.

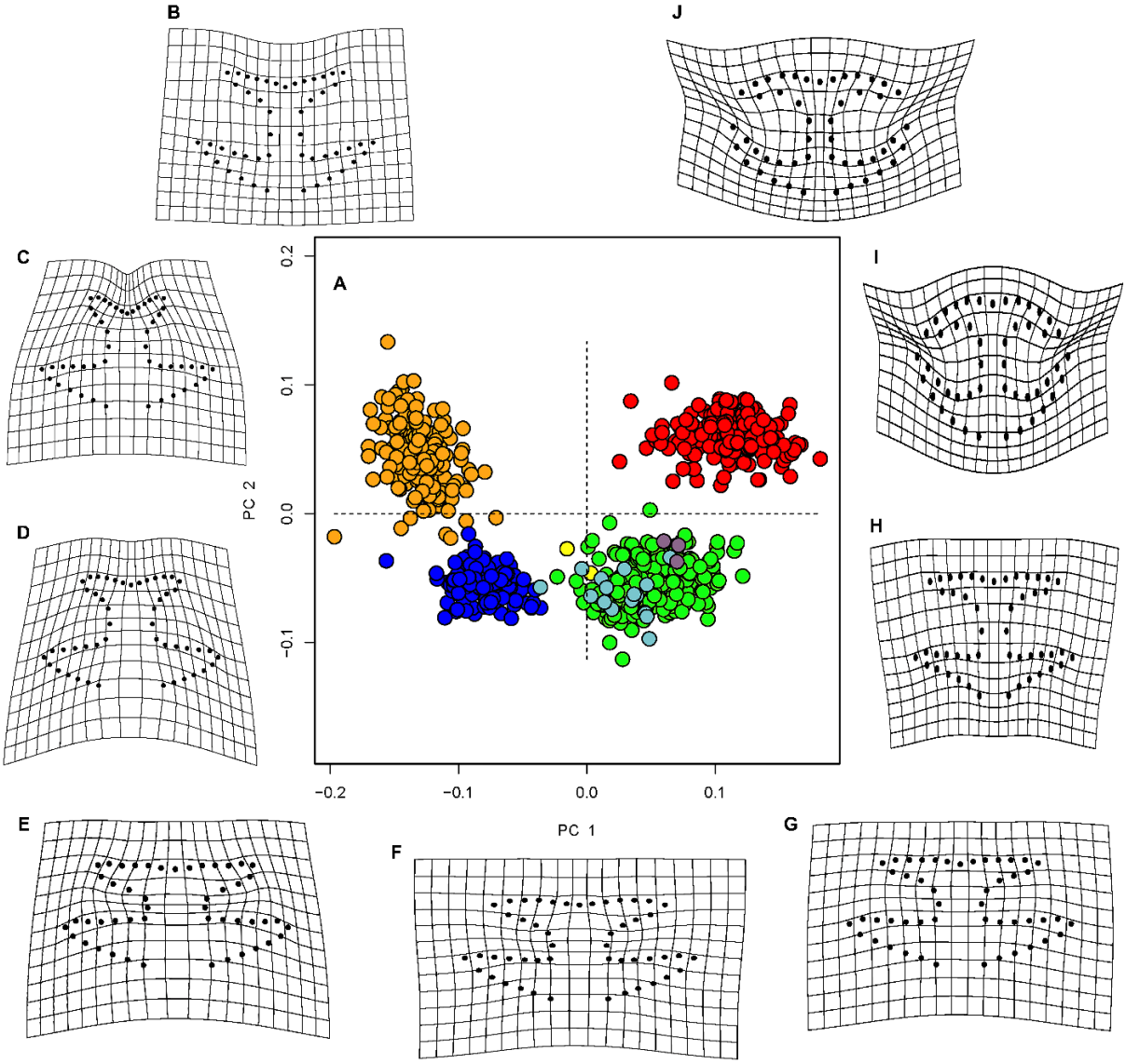

Figure 6. A. Principal component analysis showing the separation of *Micrasterias arcuata* morphotypes across morphospace and its relationship with grid deformation or thin plate splines. PCA1 axis = 65.3% and PCA2 axis = 21.4%; **F.** M1: yellow circles; **I-J.** M2: red circles; **E.** M3: green circles; **B-C.** M4: orange circles; **D.** M5: blue circles; **G.** M6: light blue circles; **H.** M7: purple circles.

According to the geometric morphometric analyses carried out using both *M. arcuata* literature data and natural populations morphotypes, first PCA axis spanned 63.8% and the second axis 21.9% of total variability (Fig. 7). Similarly, examining the ordination scheme that considered only natural populations, PCA divided the natural morphotypes into four groups, the iconotypes clustered in each morphotype group depending on the cell shape characteristics.

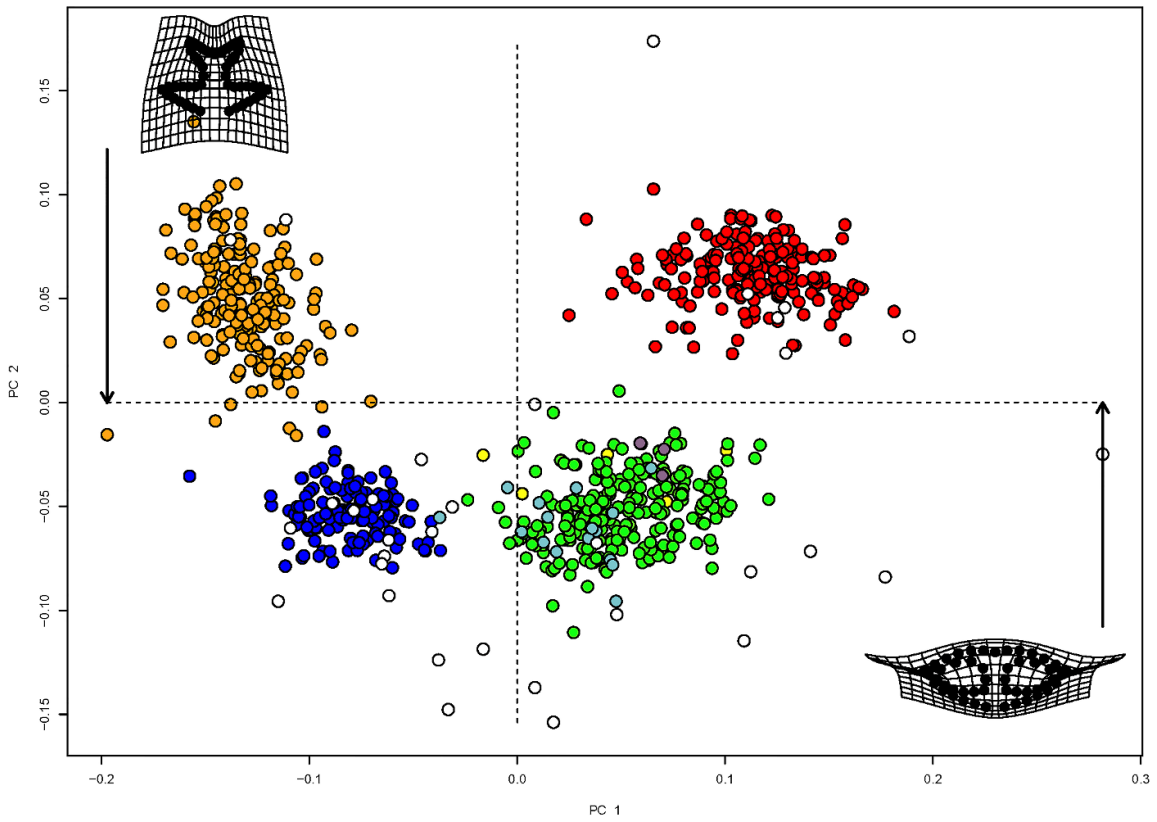

Figure 7. Principal component analysis of *Micrasterias arcuata* morphotypes across morphospace. PCA1 axis = 63.8% and PCA2 axis = 21.9%; M1, yellow circles; M2, red circles; M3, green circles; M4, orange circles; M5, blue circles; M6, light blue circles; M7, lilac circles; White circles represent the position of the iconotypes from the literature data.

Linear discriminant analysis (LDA) plot performed only on natural population dataset (Fig. 8), showed the morphotype 2 as the most dissimilar one and morphotypes 1, 3, 6 and 7 weere grouped together. Morphotype 2 followed by morphotype 4 and 5 presented 100% of correct classification values. Morphotype 1 had one individual morphologically similar to those classified as morphotype 3, the same thing occurred with morphotype 7 too. As in the traditional morphometric analysis, moprhotypes 3 and 6 shared presented many individuals with similar morphological characters (Supplementary material 4). Results obtained from NPMANOVA showed statistically significant differences among the seven morphotypes currently evaluated during the geometric morphometric analysis ($F = 676.9$, $p < 0.0001$).

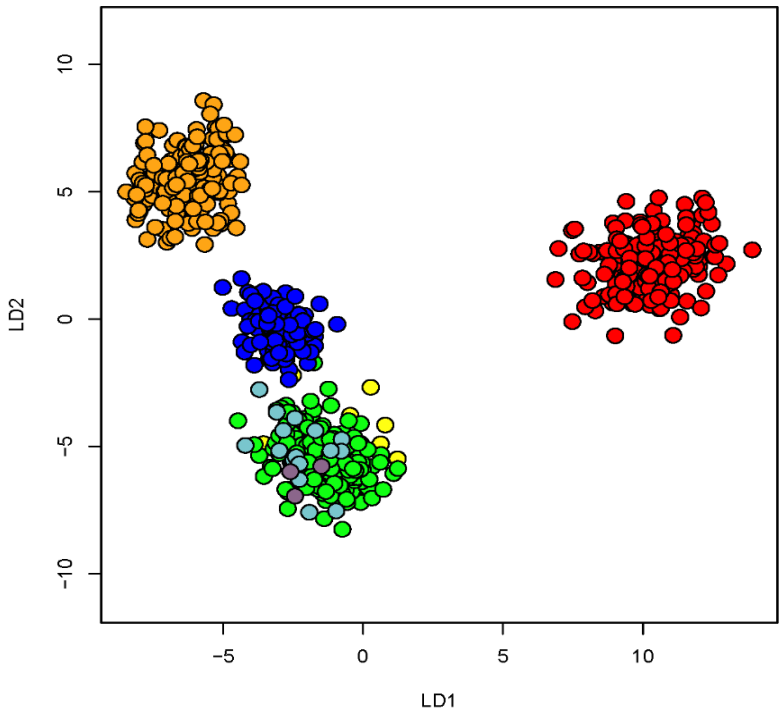

Figure 8. Linear discriminant analysis (LDA) of natural populations and literature data on geometric morphometric analyses. M1, yellow circles; M2, red circles; M3, green circles; M4, orange circles; M5, blue circles; M6, light blue circles; M7, purple circles.

Considering the correct classification/ prediction of natural population and literature taxa according to the geometric morphometric analyses (Supplementary material 5), morphotypes 1 and 7 were not classified/ clustered with any iconotypes. The morphotypes 2 and 5 were related with specimens of literature that presented longer length such as *Micrasterias arcuata* Bailey or *Micrasterias arcuata* var. *perforata* Förster or even *Micrasterias arcuata* var. *robuta* Borge, this latter one, specifically in the case of morphotype 5 which had big individuals and swollen cells. Morphotype 4 was related with *Micrasterias expansa* Bailey and *Micrasterias arcuata* var. *minuta* Förster. Moprhotype 3 and 6 were related with shorter lenght individuals or those individuals that presented basal lobes and lateral extremities of polar lobe swollen at the base, such as *Micrasterias arcuata* var. *subpinnatifida* West & West or some individuals of *Micrasterias arcuata* var. *robusta* Borge.

Morphotypes 1, 3, 6 and 7 (compact morphotypes)

Geometric morphometric analyses were carried out exclusively for the morphotypes 1, 3, 6 and 7 totaling 266 individual specimens. These analyses aimed to look for differences in the shape of these compact and morphologically remarkably similar morphotypes.

The first two axes of PCA spanned 34% and 21.6%, respectively. Morphotype 3 was present in all PCA quadrants, despite of some morphological differences be registered in the shape of the individual specimens. As a result, the greater majority of morphotype 1 was plotted in the fourth quadrant or positive side of the first PCA axis and the individuals of morphotype 7 was plotted at the positive side of the first PCA axis whereas the great majority of morphotype 6 was plotted in the negative side o the first and the second PCA axes (Figs. 19A-L).

The mainly difference observed in morphotype 1 (Figs. 9G-I) and the other ones was in the shape of the basal lobes that it has its extremities slightly curved upward whereas the other three morphotypes have the shape of their basal lobes completely straight. The morphotype 7 (Figs.9K-L) had highest polar lobe height and the basal lobes thinner at the base than the morphotypes 3 and 6. Besides that, it presented the lateral extremities of the polar lobe slightly longer than the other ones. The morphotypes 3 and 6 had their shape similar each other. They presented the basal lobes swollen at the base and the apical margin slightly concave to sloightly straight at the middle region. The main difference observed in these both morphotypes were in the shape of the lateral extremities of their polar lobe where morphotype 6 (Figs. 9C-D) had shorter and slightly sitaight ones whereas morphotype 3 (Figs. 9B, E-F, J**)** had the lateral end of its polar lobe slightly curved downward.

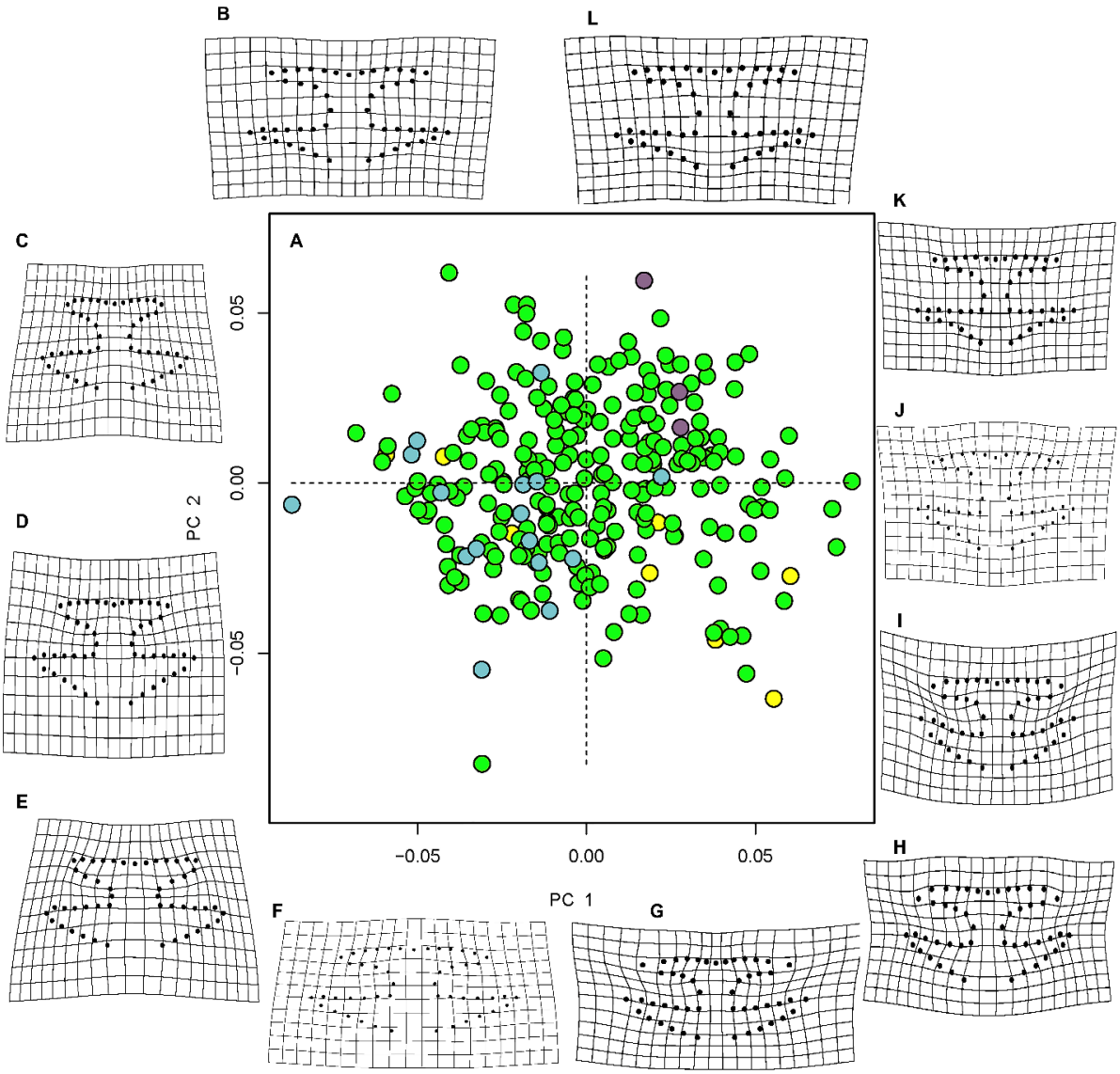

Figure 9. A. Principal component analysis showing the separation of *Micrasterias arcuata* morphotypes across morphospace and its relationship with grid deformation or Thin-Plate Splines. PCA1 axis = 34% and PCA2 axis = 21.6; **G-I.** M1: yellow circles; **B, E-F, J.** M3: green circles; **C-D.** M6: light blue circles; **K-L.** M7: purple circles.

Linear discriminant analysis (LDA, fig. 10) divided the morphotypes into three groups. Morphotype 1 was the most dissimilar of them followed by morphotype 7. And both morphotypes 3 and 6 were grouped together. However, according to the classification/ prediction (Supplementary material 6) based exclusively on the natural population data, morphotype 1 presented 99% of correct classification, and morphotype 7 presented 100%. Results obtained from NPMANOVA showed statistically significant differences among M1

and M3 ($F = 5.867$, $p < 0.0001$).

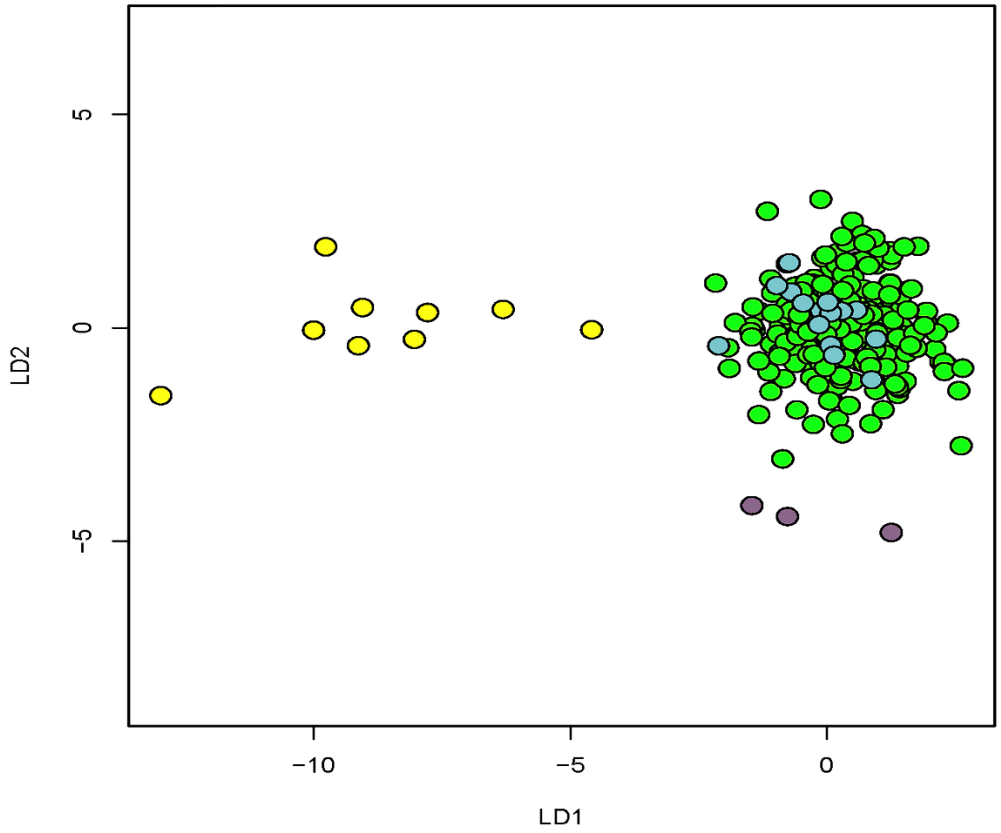

Figure 10 - Linear discriminant analysis (LDA) of natural populations on geometric morphometric analyses; M1, yellow circles; M3, green circles; M6, light blue circles; M7, purple circles.

Also, some taxa with remarkably similar shapes that were considered to be closely related in terms of traditional taxonomy were clustered or not in both analyses, the photomicrographs or iconotypes used in the present study reflected the cell morphology. And due to this, the analyses of the previously published morphological data and the correlation between these figures with our present data was possible with a well supported morphological and metric differentiation. Together, they produced a rather interesting pattern of morphological delimitation of infraspecific taxa of *M. arcuata*.

Due the morphological characteristics observed from the natural population data and the literature ones in both morphological analyses, and in accordance with the descriptions of the classical literature, the morphotypes were identified as follows: morphotype 1*: Micrasterias arcuata* morphotype 1; morphotype 2: *Micrasterias arcuata* var. *arcuata* f. *arcuata* Bailey; morphotype 3: *Micrasterias arcuata* morphotype 3; morphotype 4: *Micrasterias arcuata* var.

expansa (Bailey) Nordstedt; morphotype 5: *Micrasterias arcuata* var. *perforata* Förster; morphotype 6: *Micrasterias arcuata* morphotype 6; morphotype 7: *Micrasterias arcuata* morphotype 7.

DISCUSSION

Micrasterias arcuata was proposed based on solely morphological characteristics and conventional measurements (Bailey 1851). The species includes several varieties and taxonomic forms that were proposed on the same morphometric grounds. Such propositions led to serious problems of taxonomic circumscriptions and sometimes to severe difficulties in their separation.

According to classical taxonomy, *M. arcuata* is considered a species complex for the morphological and nomenclatural point of view and includes 24 infraspecific taxa (Guiry & Guiry 2020). However, if all the literature available about this species is considered, the number of taxa moves to more than 65 taxa (including taxonomic varieties and taxonomic forms), as well as the classical references to the protologue of taxa (Bailey 1851, Nordstedt 1877, West & West 1896, 1897, Borge 1899, 1918, Krieger 1939, Prescott & Scott 1943, 1952b, Prescott *et al.* 1977, Förster 1964, 1969, Thomasson 1966, 1971, Förster 1981b, Martins & Bicudo 1987, Kastovyski *et al.* 2011, Fonseca & Estrela 2015, Oliveira *et al*. 2017, Santos *et al*. 2018, Guiry & Guiry 2020, Ramos *et al.* 2019).

An extensive dataset from natural population and literature data were used for both conventional and geometric morphometric approaches aiming to producing tools for the identification, and to distinguish infraspecific taxa in a qualitatively and quantitatively way in the *M. arcuata* species complex. Results currently obtained indicated that the criteria adopted for both morphological analyses were effective. However, some taxonomic relationship suggested for some infraspecific taxa of *M. arcuata* and their relation to the classical taxonomy was not supported using only one or all these morphological analyses performed.

In the present study, the type material of *M. arcuata* proposed in Bailey (1851) and some other infraspecific taxa presented in Förster's studied (1964, 1969) were examined. The two latter publications include the greatest number of *M. arcuata* infraspecific taxa available in the classical literature, including some that could not be measured using their own official protologue information (Borge 1899, 1918, Prescott & Scott 1952, West & West 1896, 1897). Photomicrographs or iconotypes circumscriptions easily exhibited the specimens morphology, thus making possible their proper taxonomic identification and morphological delimitation.

According to the Thin-Plate Splines (grids of shape deformation) results revealed by the

geometric morphometric approach such as: polar lobes width, apical margin incision depth, and basal lobes curvature were the most important trends, and MCL, BLD, IW and HPL were important features as conventional criteria for traditional morphometric analysis. All these patterns obtained from the current dataset were in good agreement with some already recorded attempts in the classical literature referring to *M. arcuata* infraspecific taxa and their current descriptions (West & West 1897, Borge 1899, Förster 1964).

The morphotypes of *Micrasterias arcuata* species complex presently studied had their cell length ranging from 30 µm to 71 µm and their cell width from 31 µm to 76 µm, despite the specialized literature will include greater length and greater width values (Prescott *et al.* 1977).

Morphotypes 2, 4 and 5 were quite distinct in relation to both conventional and geometric morphometric analyses, as well as in the analyse performed by light microscopy (LM) and by scanning electron microscopy (SEM) observations. According to the Thin-Plate Splines grids deformation (Figs. 6B-C, I-J), nevertheless, certain morphological variation was observed within populations of those three morphotypes, that should possibly be related to the physical and chemical environment variables throughout the studied period. However, it is important to comment that all these morphotypes presented from 99% to 100% of posterior probabilities in the linear discriminant analysis (LDA), especially when just natural populations were considered.

Morphotype 2 exhibited cells as long as broad, deep median constriction, sinus acute opening widely, polar lobe narrows with lateral extensions slight curved downward. Apical margin was somewhat flattened, sometimes slight curved, often somewhat prominent on either side of the middle region. Basal lobes were sometimes slight straight at the base, curving upward towards the lateral projections of polar lobes. Both basal and polar lobes ending in a short tooth or spine (Bailey 1851, Prescott *et al.* 1977). These features were in agreement with the *M. arcuata* type description (Bailey 1851), as well as with the other morphotypes identified as var. *arcuata* or even var. *gracilis* (Förster 1964, 1969). However, in the present study, morphotype 2 was identified as *Micrasterias arcuata* Bailey (Figs. 11D-F, figs. 14A-F).

According to the classical taxonomy, *M. arcuata* includes considerable morphological variation, with several distinct morphotypes recorded, e.g. forms of small cell dimensions and a tendency to be broader than long, as well as some other morphotypes with greater cell dimensions, consistently longer than broad (Bicudo & Gil-Gil 2003). The original material examined by Bailey (1851) was always broader than long and presents slender and upward curved basal lobes, which sometimes can resemble specimens of *Micrasterias arcuata* var. *gracilis* West & West.

Thomasson (1971) questioned the existence of *M. arcuata* var. *gracilis* stating that according to him, it should be better considered as a morphological expression of *M. arcuata* var. *arcuata* due the existence of some specimens that were identified as var. *arcuata* despite of showing characteristics of var. *gracilis*, and some other specimens were identified as var. *gracilis* in despite of having characteristics of var. *arcuata*.

Micrasterias arcuata var. *gracilis* was formally proposed by West & West (1896), the authors referring to the material identified by Nordstedt (1877) that presented morphological features and circumscription of *M. arcuata* var. *arcuata* species proposed by Bailey (1851). The var. *gracilis* had cells very slender and the basal lobes straight and facing up towards the ends of polar lobe. And the extremities of polar lobe were curved downward towards the ends of basal lobes. However, it was impossible to know or to guess at this moment if the var. *gracilis* represent an ecomorpha of *M. arcuata* var. *arcuata* or even a heterotypical synonym of the typical species *M. arcuata* Bailey.

Nevertheless, further analyses based on molecular, phylogenetic and ecological approaches must be carried out to know the real diversity and taxonomic status of both entities above.

Morphotype 4 (Figs. 11G-I, figs. 15A-G) presented a narrow and slender shape, with the apical margin concave. The ends of polar lobe were straight and curved upwards, and the basal lobes vary from straight to slight curved upwards. These features agree with *Micrasterias arcuata* var. *expansa* Nordstedt or *Micrasterias expansa* Bailey (1851: 37, pl. 1, fig. 7) circumscriptions because of its long-sized cells and marked upward curved basal lobes, and due to this, morphotype 4 was consequently identified as so. According to our data, *M. arcuata* var. *expansa* should be erected again as a separate species: *Micrasterias expansa* (Bailey emend. Nordstedt) C.B.Araújo, stat. nov.

Micrasterias expansa was described by Bailey (1851) therefore, it is now considered at the varietal level of *M. arcuata* in Nordstedt (1877: 23). According to some authors, individual specimens of var. *expansa* resemble those of var. *arcuata* and intermediate forms can occur in natural populations (Nordstedt 1877, Sormus 1980).

Although var. *arcuata* and var. *expansa* may occur simultaneously in nature, including morphological expressions and intermediate forms such as some broad more compact specimens, with basal lobes straight or slight upwardly curved or long-sized individuals with greater HPL values and notably upward curved basal lobes, consequently with greater BLD values (Bicudo & Gil-Gil 2003). Both above taxa showed different ecological strategies to avoid possible inter competition (Gil-Gil & Bicudo 2000). These evidence agree with current results, i.e. both taxonomic varieties were clearly different in their morphological features and

measurements, thus representing different taxonomic entities.

Specimens of *M. expansa* resemble some of *M. arcuata* var. *robusta* (Borge 1899, 1918, Krieger 1939). Borge (1899: 27, pl. 2, fig. 36) proposed *M. arcuata* var. *expansa* f. *minor* that Krieger (1939: 12) incorrectly considered synonymous with var. *robusta*. This misinterpretation possibly happened because Borge (1918: 65, pl. 5, fig. 18) identified specimens morphologically similar with *M. arcuata* var. *expansa*, which fitted alright into the circumscription of *M. arcuata* var. *robusta*.

Also, incorrectly, Prescott *et al.* (1977: pl. 87, fig. 7) illustrated some material they identified with *M. arcuata var. robusta* f. *protuberans*, their figure 7 including features belonging to *M. expansa* such as basal lobes longer than the polar, parallel margins and fairly slender cells. Same thing happened to *M. arcuata* var. *robusta* f. *recurvata* described by Prescott & Scott (1952: 232, pl. 5, fig. 5) and *M. arcuata* var. *robusta* f. *recurvata* in Prescott *et al.* (1977: 144, 145, pl. 86, fig. 6). The last two taxa were compared to Förster (1969: 39, pl. 10, fig. 11) material collected in the state Pará, Brazil. Although *M. arcuata* var. *robusta* f. *recurvata* did not have widely upward curved basal lobes, it fits nicely in the circumscription of *M. expansa* including all diagnostic features of the species.

Micrasterias arcuata var. *minuta* was described by Förster (1964: 373, pl. 13, fig. 9) from material collected in Rio das Fêmeas, Porta Azul, Goiás state, central Brazil. In its description, Förster (1964) included two different materials: *M. arcuata* var. *expansa* Borge (1899) and *M. arcuata* var. *robusta* f. *recurvata* Scott & Prescott (1961: 47, pl. 18, fig. 7, 8), the latter being the same material described 12 years before by Prescott & Scott (1952). Observing the metric limits and the morphological features of *M. arcuata* var. *minuta*, we concluded that the variety fits perfectly in the *M. expansa* circumscription.

Morphotype 5 (Figs. 12A-F, figs. 16A-I) differ from all other *M. arcuata* infraspecific taxa currently evaluated by traditional and geometric morphometric, LM and SEM analyses. It has the greatest size and the most swollen cells of all the morphotype presently analysed by natural population data. It presented, moreover, apical margin concave and prominent on either side of the mid region. Lateral extensions of polar lobe were shorter and sometimes slight straight to slight curved upward. Basal lobes were swollen and straight, or sometimes slightly curved upward and strongly punctate cell wall and some very conspicuous pores. This morphotype was identified as *Micrasterias arcuata* var. *perforata* Förster. According to our data, *M. arcuata* var. *perforata* should be erected as a separate species: *Micrasterias perforata* (Förster) C.B.Araújo, comb. nov.

In addition, some morphological variation was recorded in some specimens, and despite of

this, all specimens showed their morphology in accordance with the descriptions of the two forms proposed by Förster (1964). This author proposed two taxonomic forms of var. *perforata*: f. *robustior* collected in Serra das Almas, Bahia, Brazil, that was frequently recorded together with the typical variety, and f. *magniperforata*, a rare taxon recorded from material collected in Rio das Fêmeas, Goiás state, Brazil.

The main difference among the two forms above mentioned and the typical one was in the shape of the polar lobe. Therefore, the typical variety had the polar lobes apices straight whereas the f. *magniperforata* has the polar lobes curved upward, and f. *robustior* comprises smaller and compact individuals, with polar lobes (HPL values) short and robust.

Sometimes specimens of var. *perforata* remind representative individuals of *M. arcuata* var. *robusta*. This similarity was attributed to the shape of their polar lobes and the swollen and robust basal and polar lobes. However, according to literature (Förster 1964), specimens of var. *perforata* display greater cell length and width and cell wall punctate.

During the present study, all three morphological variations were observed simultaneously in all populations recorded as var. *perforata*. Considering the almost impossibility to differ between those morphotypes, we opted to consider ecomorphas the two taxonomic forms documented by Förster (1964). However, further studies are necessary to confirm whether the differences in Förster (1964) are enough for the material studied represent different taxonomic entities.

Morphotype 1 was identified as *Micrasterias arcuata* morphotype 1 (Figs. 11A-C). It exhibited more robust specimens with their basal lobes and lateral extensions of polar lobe swollen at the base. The easily visible difference between morphotype 1 and morphotype 3 was in the curvature degree of their basal lobes that is curved upward in morphotype 1, in addition to the BLD greater values. Differences between morphotype 1 and morphotype 6 were in the cell size, the shape of the apical margin and the polar lobe lateral extension.

Morphotypes 3, 6 and 7 exhibited their basal lobes straight, parallel to each other or sometimes slight directed at the ends of the polar lobe. However, when LM and SEM images and both traditional and geometric morphometric analyses were considered, some differences were noted among them. Morphotype 6 (Figs. 13D-F, figs. 18A-I) had the smallest size currently recorded. It presented, sometimes, straight apical margin and shorter lateral extension of polar lobe, that it is straight and swollen at the base. Morphotypes 3 (Figs. 13 A-C, figs. 17A-F) and morphotype 7 were quite similar each other in their morphology, although morphotype 7 (Figs. 13 G-I, figs. 19A-F) showed slightly larger, thinner and elongate cells. All characteristics of these three morphotypes resemble those of the circumscription of the

Micrasterias arcuata var. *subpinnatifida* West & West. However, at this moment, we choose named them as morphotypes of *M. arcuata* species.

Although the four compact morphotypes (M1, M3, M6 and M7) presently evaluated in this study were different from the morphotype 2, currently identified as the typical species *M. arcuata* Bailey. And these four morpotypes presented some distinguishiable features, it was impossible to know their real taxonomic and nomenclatural value, or even if they represent an ecomorpha or different entities. And due to this, they were treated and identified in this study as *Micrasterias arcuata* morphotypes. Nevertheless, it is essential the use of other approaches, such as molecular and phylogenetic studies to confirm the real nature and taxonomic status of these four morphotypes.

As well as other protist groups, desmids may exhibit high phenotypic variation affected by factors like changing environmental conditions and, among others by temperature (Neustupa *et al.* 2008), pH (Černá & Neustupa 2009) and ontogenetic factors (Neustupa 2016). Concerning the *M. arcuata* species complex, variation was observed in all sample unities analyzed, and all morphotypes now analyzed included high morphological variability at the population level. Our current results are in agreement with those in Bicudo & Gil-Gil (2003), who showed that cell elongation values increased consistently together with the length:width ratio ones and the clear tendency of increase of the angle between the lateral lobes (BLD) of the same semicell, that turned into widely open.

According to the present results from natural populations and the careful analysis of the literature data, it is possible to state that some infraespecific taxa presently evaluated were proposed arbitrarily, without any specific criteria. Due to the notorious problematic nomenclatural situation documented for the species, a taxonomic review of the *M. arcuata* species complex was carried out bringing taxonomic changes based on the morphological species criterion (Maggs 1997). This review reduced the number of varieties and taxonomic forms either by considering some of them synonyms, taxonomical irrelevant phenotypes or by transferring some of them to the species level.

Some remarks on classical taxonomy of *M. arcuata* species

Considering all observations and remarks about *M. arcuata* classical taxonomy, two taxa originally published as new combinations (*M. arcuata* var. *eckertii* Förster 1981: 245, and *M. arcuata* var. *subparallela* Förster 1981: 245) corresponded, respectively, to *M. arcuata* var. *subpinnatifida* f. *eckertii* Förster (1964: 375) and *M. arcuata* var. *subpinnatifida* f. *gracilis* Förster (1964: 376). And *M. arcuata* var. *robusta* f. *semilunata* Förster & Eckert shall be

considered just a reference to the material collected in northern Brazil for the proposition of *M. arcuata* var. *robusta* f. *goyazensis* Förster (1964: 374, 375), but unfortunately with not much information about f. *semilunata*.

Micrasterias arcuata var. *subpinnatifida* f. *gracilis* Förster and *Micrasterias arcuata* var. *subparallela* Förster (synonym) were currently considered a new combination (variety status) of the present *M. subpinnatifida* (see nomenclatural changes and table 1).

Two taxa will remain their indefinite taxonomic values. Thus, *M. arcuata* var. *compacta* described by Förster (1969: 39, pl. 10, fig. 8, 10) on the basis of material collected in the state Pará, Brazil, has metrical limits and morphological characteristics (small cells, apical margin straight to slight concave and basal lobes conical and slight curved upward) that also fit into the circumscription of the typical var. *subpinnatifida*. However, figure 8 above of the same material revealed cells with basal lobes curved upward and a concave apical margin, i.e. an iconotype with characteristics similar to those of var. *arcuata*.

Micrasterias arcuata var. *subcornuta* recorded by Förster (1964: 375, pl. 13, fig. 7, 8) was characteristic by having small cells with polar lobes slight curved downward, the ends of basal lobes slight curved downward toward each other, and yellow zygospore covered with conical papillae. The only difference between var. *subcornuta* and var. *subpinnatifida* was in their zygospore. Therefore, also according to Förster (1964) last variety has grey zygospores with smooth cell wall. However, considering the adult cells of both varieties and var. *subcornuta* illustration in Förster (1964: fig. 7), they were very similar in their measurements and morphological features.

Nordstedt (1877: 22) proposed *M. arcuata* var. *expansa* f. *intermedia* Nordstedt and *M. arcuata* f. *intermedia* Nordstedt that correspond, respectively, to *M. arcuata* var. *expansa* Nordstedt and *M. arcuata* var. *arcuata* Bailey. Therefore, both taxonomic forms above have no taxonomic value.

Micrasterias arcuata var. *lata* Prescott & Scott (1943: 70, pl. 1, fig. 14, pl. 5, fig. 2) is a very wide-ranging variety with both polar and basal lobes prominently greatly extended. This variety, however, was not presently evaluated. On the other hand, we do agree with the original authors' comments, that the morphological characteristics of this variety remind those of *M. pinnatifida* Ralfs (Ralfs 1848: 77).

Nomenclatural changes

Micrasterias expansa (Bailey emend. Nordstedt) C.B.Araújo, **stat. nov**.

Figs. 11G-I, fig. 15A-G

Basionym - *Micrasterias expansa* Bailey, Smithsonian Contributions to Knowledge 2(8): 37, pl. 1, fig. 7. 1851.

Synonyms - *Micrasterias arcuata* var. *expansa* Nordstedt (1877: 23); *Micrasterias arcuata* var. *robusta* f. *recurvata* Prescott & Scott (1952: 232); *Micrasterias arcuata* var. *minuta* Förster (1964: 373).

Micrasterias perforata C.B.Araújo, **comb. nov**.

Figs. 12A-F, fig. 16A-I

Basionym - *Micrasterias arcuata* Bailey var. *perforata* Förster, Hydrobiologia 23: 373, pl. 13, fig. 14, 15, pl. 44, fig. 10. 1964.

Synonyms - *Micrasterias arcuata* var. *perforata* f. *magniperforata* Förster (1964: 373); *Micrasterias arcuata* var. *perforata* f. *robustior* Förster (1964: 373, 374).

CONCLUSION

Our results revealed that most *M. arcuata* taxa are perfectly apart according to their morphological features and may therefore represent different taxonomic entities. Moreover, the real diversity of the infraspecific taxa derived from the *M. arcuata* species complex probably is much greater than is recently known. Especially in the case of the close morphological taxa showing similar shape and measurements overlapping, such as the present *M. subpinnatifida* species complex, they were currently considered ecomorphas, with unclear taxonomic value.

Consequently, biological delimitation to enhance the species concept in *M. arcuata* complex is of the most importance, and extremely necessary especially with the application of molecular and phylogenetic methods or, even better, with the combination of different approaches, including ecological or experimental studies too. These investigations may consequently help to reveal which of these traditional taxa will indeed mean taxonomic meaningful units (independent taxa) or conversely will lack taxonomic value.

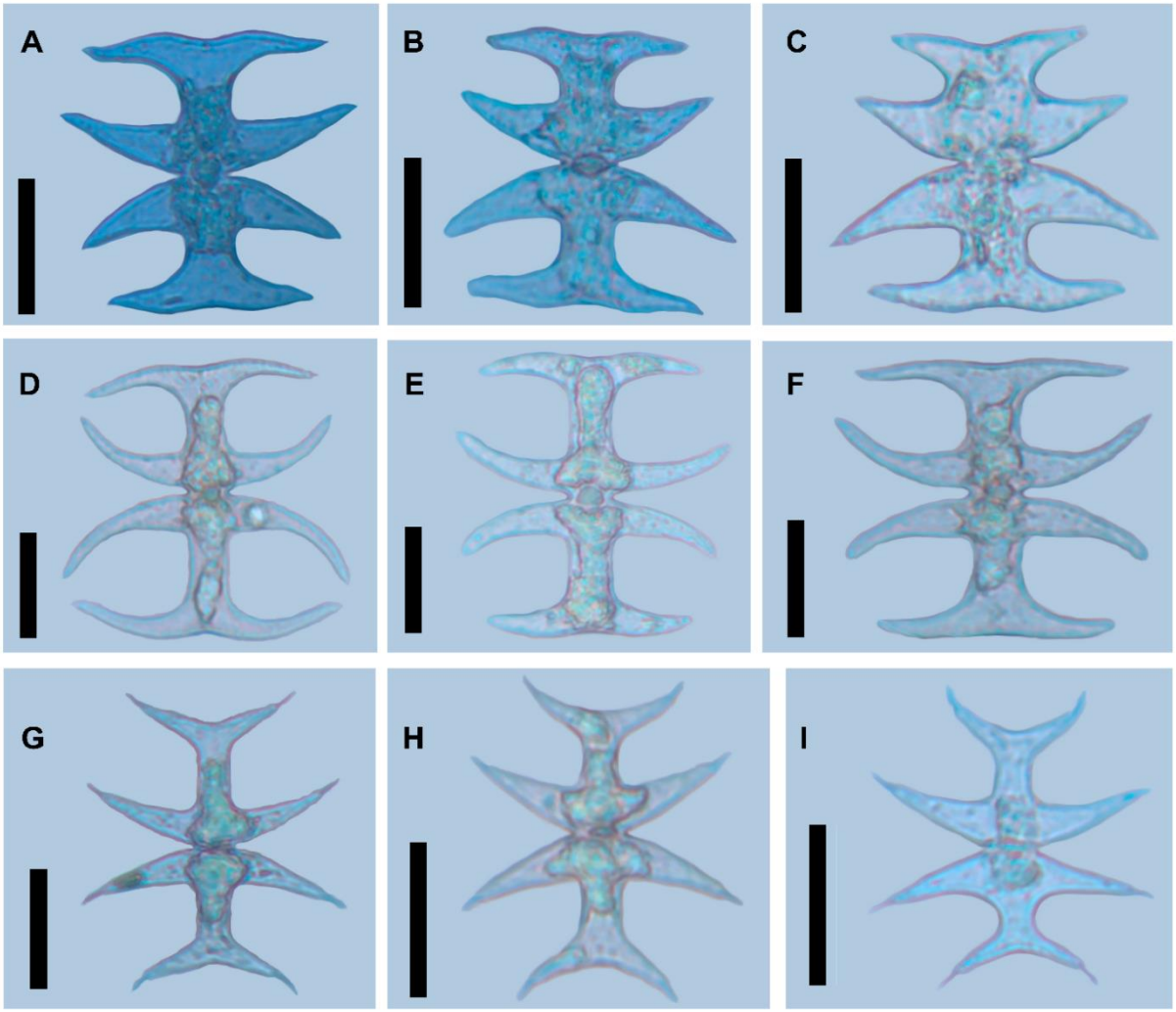

Figure 11. A-C. *Micrasterias arcuata*, morphotype 1; **D-F.** *Micrasterias arcuata*, morphotype 2; **G-I.** *Micrasterias expansa*, morphotype 4; Scale bars: 50 µm.

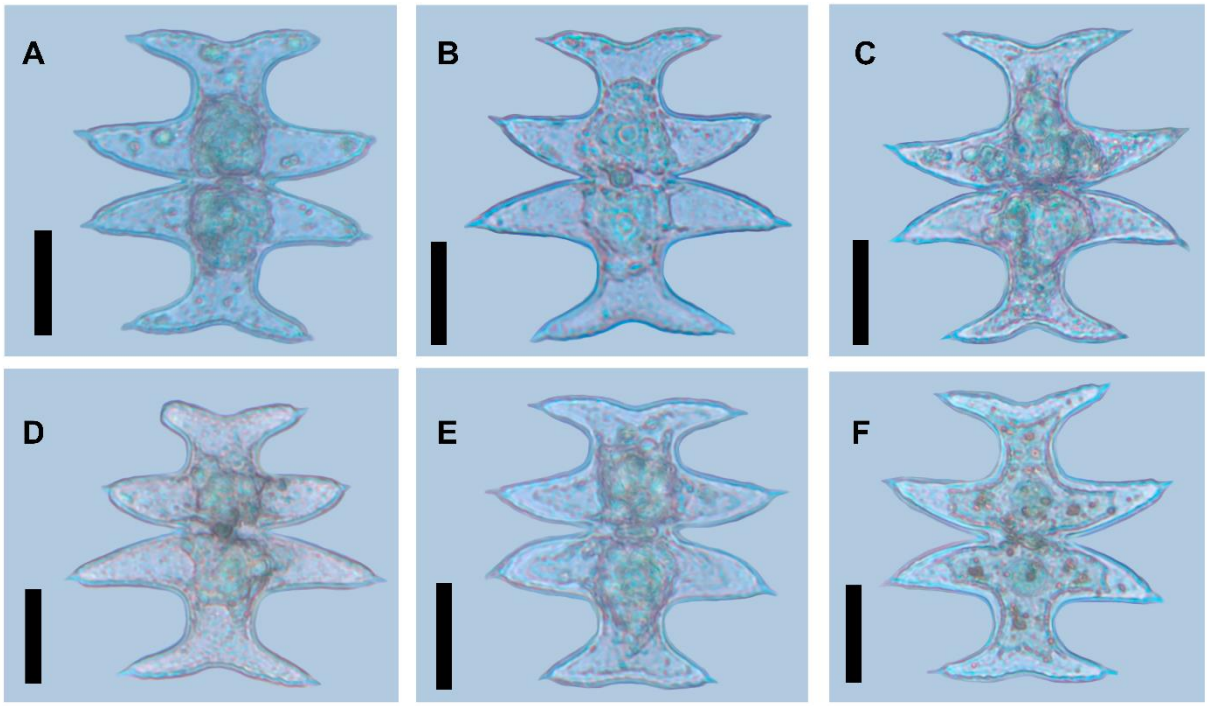

Figure 12. A-F. *Micrasterias perforata*; Scale bars: 50 µm.

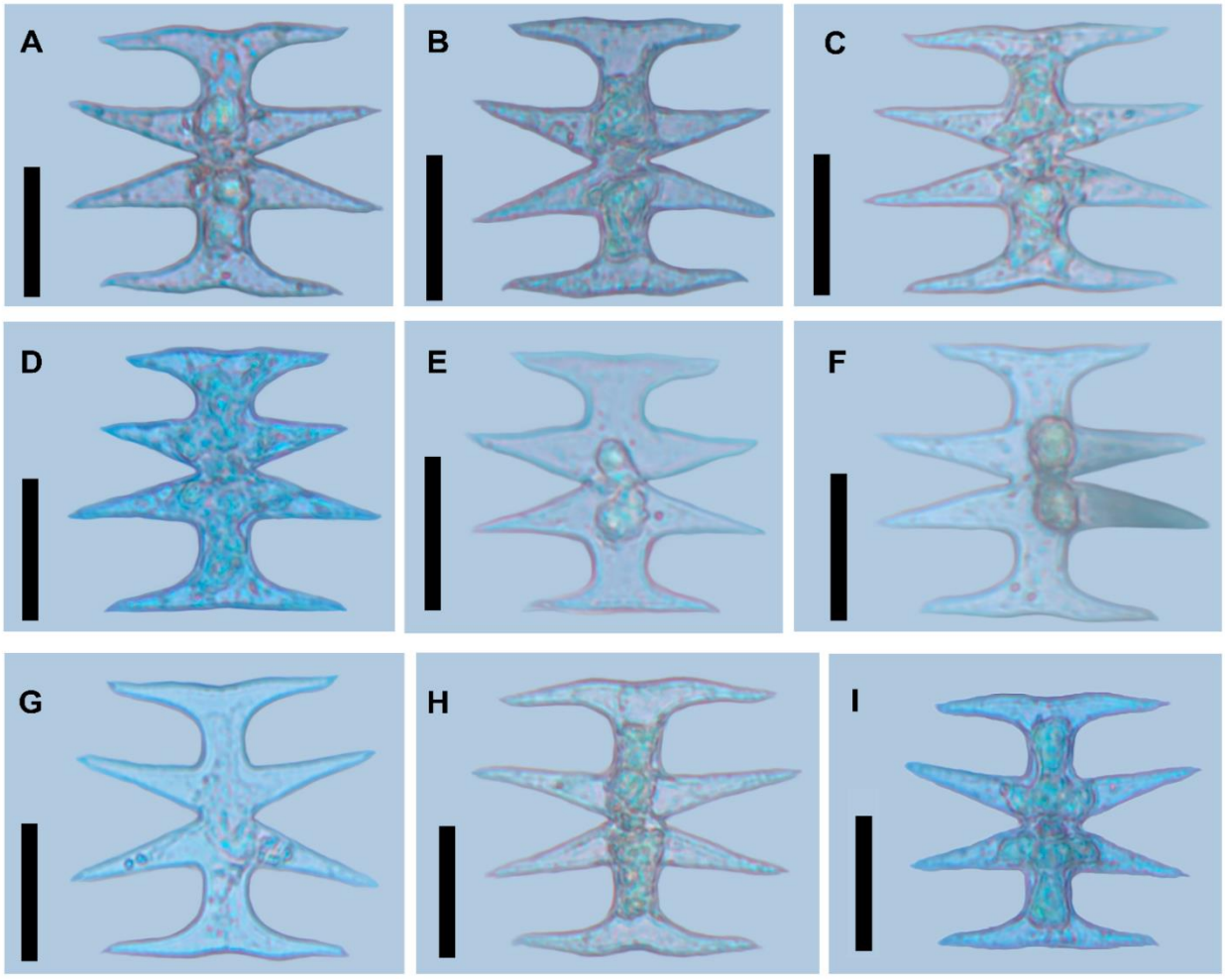

Figure 13. A-C. *Micrasterias arcuata*, morphotype 3; **D-F.** *Micrasterias arcuata*, morphotype 6; **G-I.** *Micrasterias arcuata*, morphotype 7; Scale bars: 50 µm.

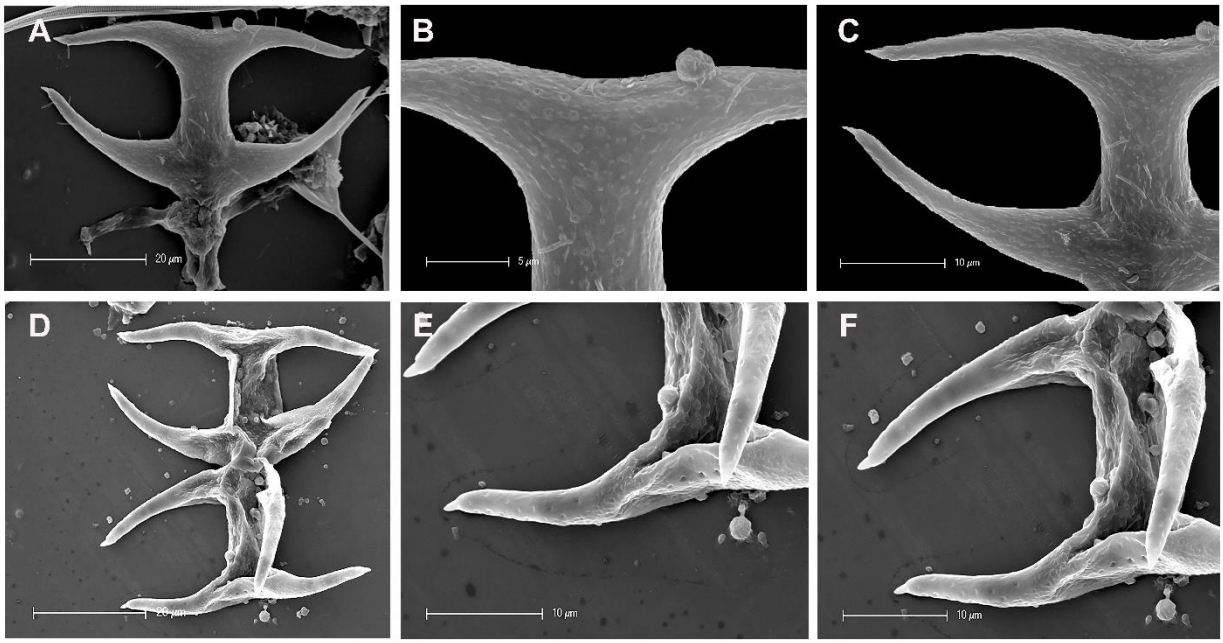

Figure 14. *Micrasterias arcuata*, morphotype 2; **A, D, G.** Details of semicell; **B, E, H.** Details of apical margin and polar lobe; **C, F, I.** Details of basal lobes and lateral extension of polar lobe.

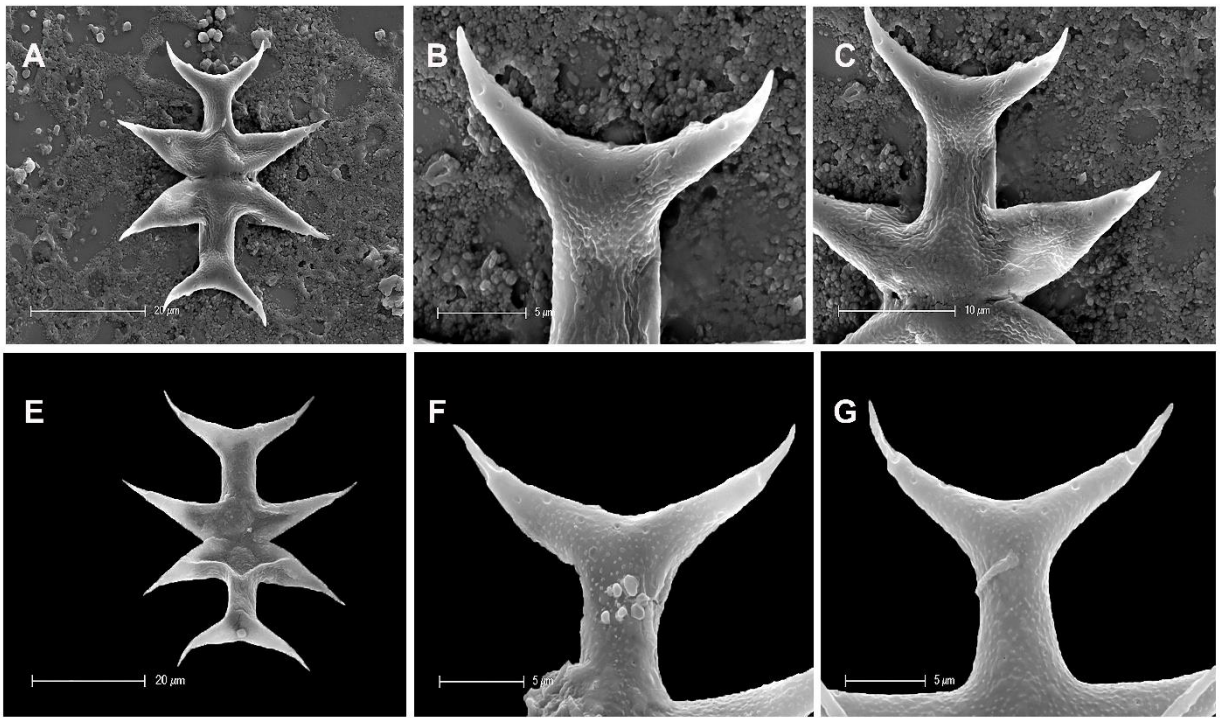

Figure 15. *Micrasterias expansa*, morphotype 4; **A, D.** Details semicells; **B, E-F.** Details of polar lobe and apical margin; **C.** Details of lateral extension of polar lobe and basal lobes.

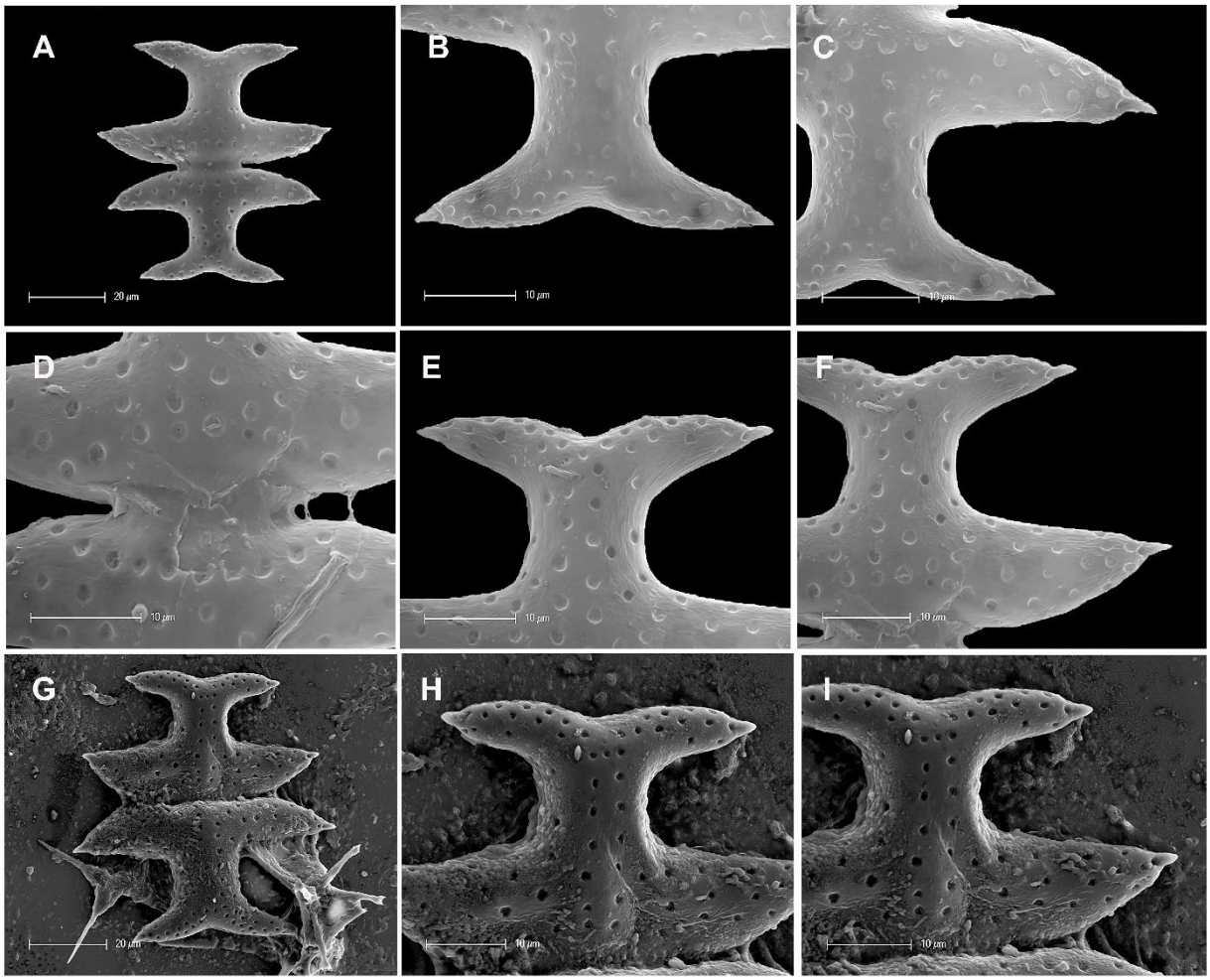

Figure 16. *Micrasterias perforata*, morphotype 5; **A, G.** Details of semicell; **B, E, H.** Details of apical margin; **C, F, I.** Details of lateral extension of polar and basal lobes; **D.** details of isthmus region.

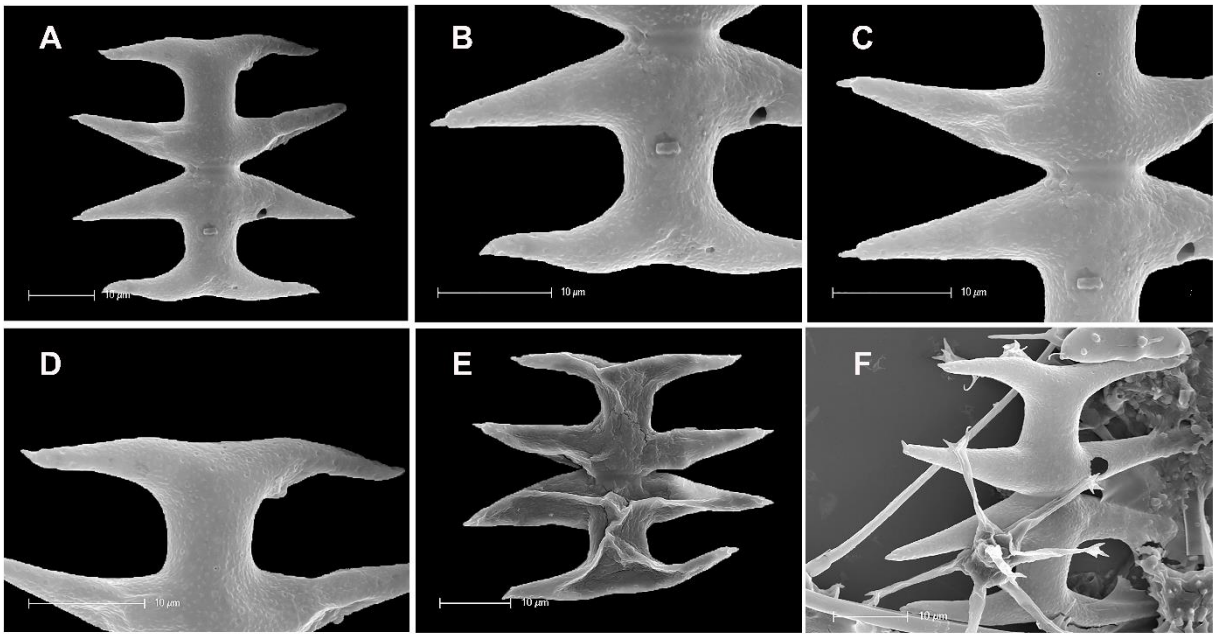

Figure 17. *Micrasterias arcuata*, morphotype 3; **A, E, F.** Details of semicells; **B, D.** Details of polar lobe and apical margin; **C.** Details of basal lobes.

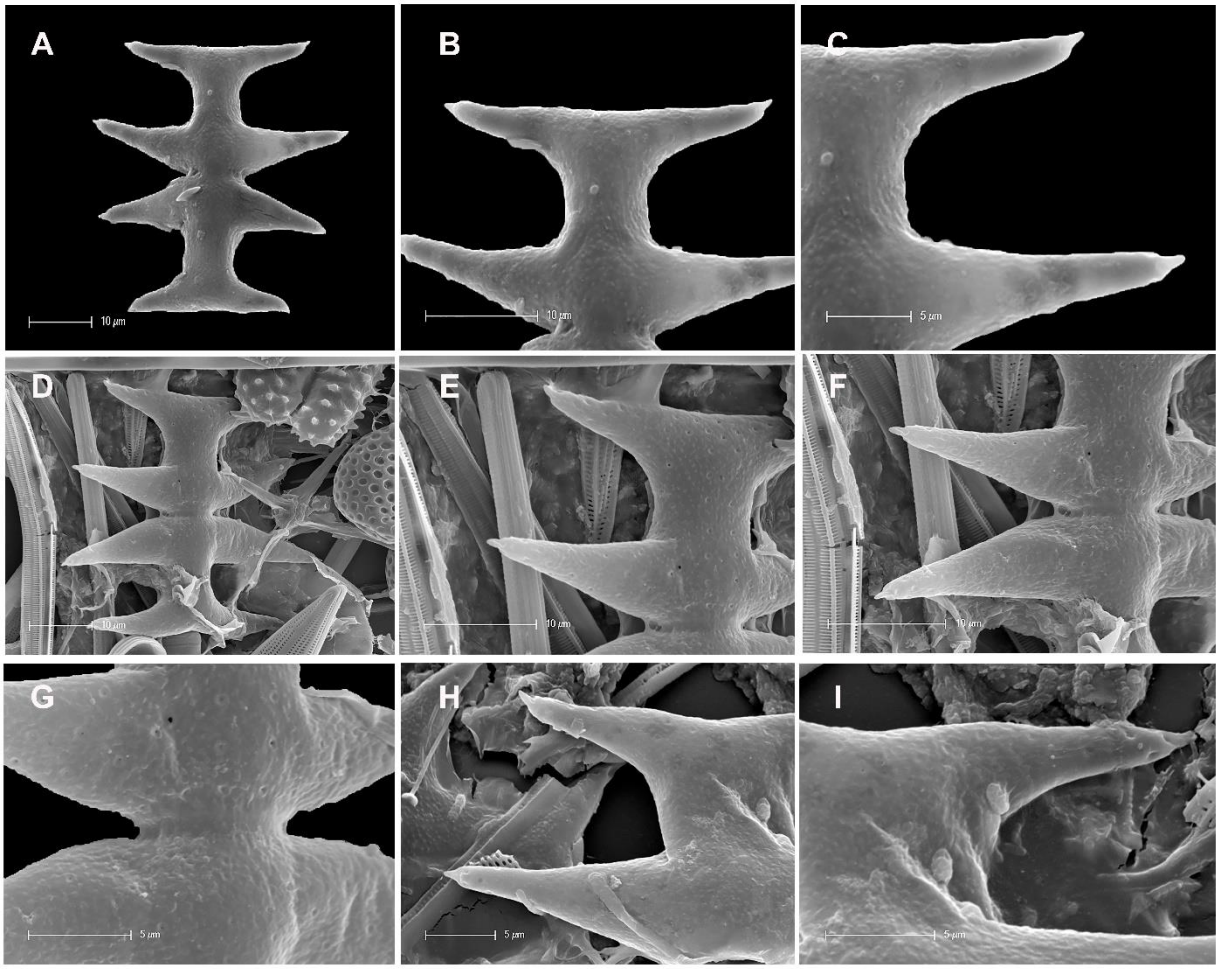

Figure 18. *Micrasterias arcuata*, morphotype 6; **A, D.** Details of semicell; **B, E, H.** Details of apical margin and polar lobe; **C, E, H, I.** Details of lateral extension of polar and basal lobes; **F.** Details of basal lobes; **G.** Details of isthmus region.

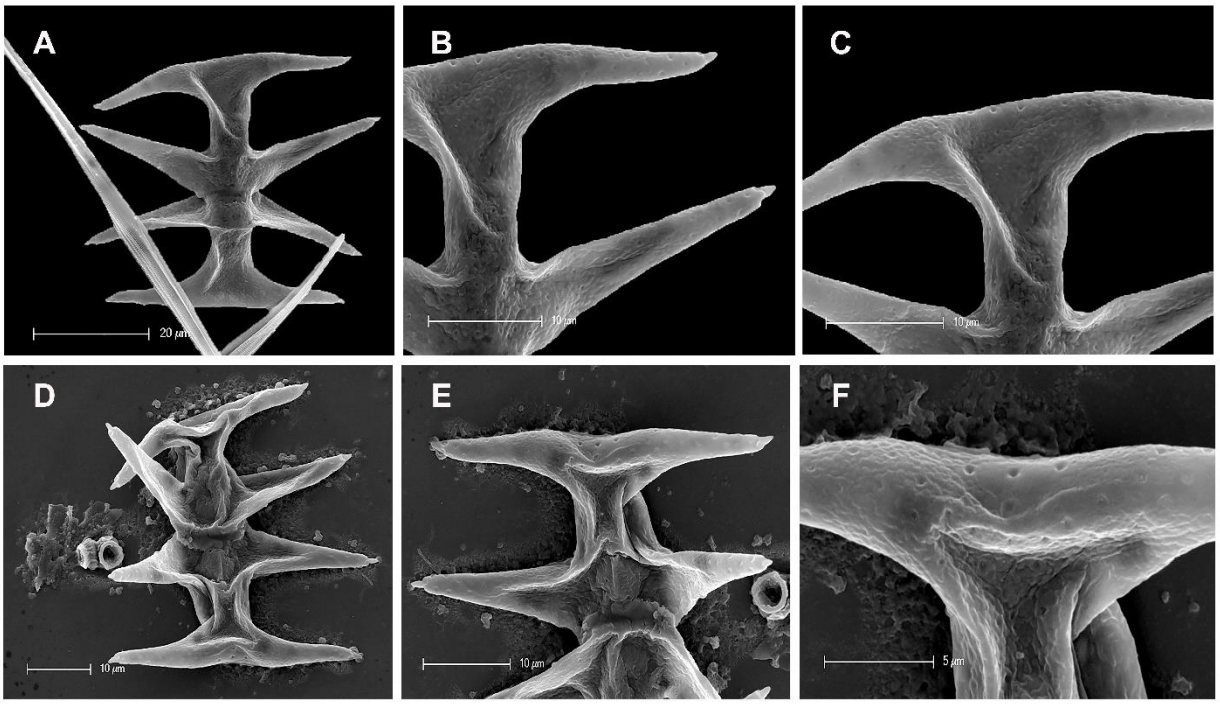

Figure 19. *Micrasterias arcuata*, morphotype 7; **A, D.** Details of semicell; **B, E.** Details of polar and basal lobes; **C, F.** Details of polar lobe and apical margin.

SUPPLEMENTARY DATA

(1). List of material examined and Herbarium accession number.

(2). Literature taxa used in traditional and geometric morphometric analyses.

(3). Classification of the morphotypes according to the correct classification/prediction in linear discriminant analysis (LDA) of *Micrasterias arcuata* morphotypes. Data set of traditional morphometric analyses and literature data in traditional morphometric analyses.

(4). Classification of the morphotypes according to the correct classification/prediction in linear discriminant analysis (LDA) of *Micrasterias arcuata* morphotypes. Data set of geometric morphometric analyses of natural population and literature data.

(5). Classification of the morphotypes according to the correct classification/prediction in linear discriminant analysis (LDA) of *Micrasterias arcuata* morphotypes. Data set of geometric morphometric analyses of natural population and literature data of compact morphotypes of *M. arcuata* species complex.

(6). Classification of morphotypes according to the correct classification/prediction. Dataset of geometric morphometric analyses of natural population.

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Supplementary material 1: List of material examined and Herbarium Acession number registration.

Month/year	Acession number	Sample	Municpality	Locality
Mar-17	SP428480	1	Itirapina/SP	Reserva Experimental de Itirapina
Mar-17	SP428481	2	Itirapina/SP	Reserva Experimental de Itirapina
Mar-17	SP428482	3	Itirapina/SP	Reserva Experimental de Itirapina
Apr-17	SP428483	4	Itirapina/SP	Reserva Experimental de Itirapina
Apr-17	SP428484	5	Itirapina/SP	Reserva Experimental de Itirapina
Apr-17	SP428485	6	Itirapina/SP	Reserva Experimental de Itirapina
May-17	SP428486	7	Itirapina/SP	Reserva Experimental de Itirapina
May-17	SP428487	8	Itirapina/SP	Reserva Experimental de Itirapina
May-17	SP428488	9	Itirapina/SP	Reserva Experimental de Itirapina
Jun-17	SP428489	10	Itirapina/SP	Reserva Experimental de Itirapina
Jun-17	SP428490	11	Itirapina/SP	Reserva Experimental de Itirapina
Jun-17	SP428491	12	Itirapina/SP	Reserva Experimental de Itirapina
Jul-17	SP428492	13	Itirapina/SP	Reserva Experimental de Itirapina
Jul-17	SP428493	14	Itirapina/SP	Reserva Experimental de Itirapina
Jul-17	SP428494	15	Itirapina/SP	Reserva Experimental de Itirapina
Aug-17	SP428495	16	Itirapina/SP	Reserva Experimental de Itirapina
Aug-17	SP429020	17	Itirapina/SP	Reserva Experimental de Itirapina
Aug-17	SP429021	18	Itirapina/SP	Reserva Experimental de Itirapina
Sep-17	SP429022	19	Itirapina/SP	Reserva Experimental de Itirapina
Sep-17	SP429023	20	Itirapina/SP	Reserva Experimental de Itirapina
Sep-17	SP429024	21	Itirapina/SP	Reserva Experimental de Itirapina
Oct-17	SP429025	22	Itirapina/SP	Reserva Experimental de Itirapina
Oct-17	SP429026	23	Itirapina/SP	Reserva Experimental de Itirapina
Oct-17	SP429027	24	Itirapina/SP	Reserva Experimental de Itirapina
Nov-17	SP429028	25	Itirapina/SP	Reserva Experimental de Itirapina
Nov-17	SP429029	26	Itirapina/SP	Reserva Experimental de Itirapina
Nov-17	SP429030	27	Itirapina/SP	Reserva Experimental de Itirapina
Dec-17	SP469907	28	Itirapina/SP	Reserva Experimental de Itirapina
Dec-17	SP469908	29	Itirapina/SP	Reserva Experimental de Itirapina
Dec-17	SP469909	30	Itirapina/SP	Reserva Experimental de Itirapina
Jan-18	SP469910	31	Itirapina/SP	Reserva Experimental de Itirapina
Jan-18	SP469911	32	Itirapina/SP	Reserva Experimental de Itirapina
Jan-18	SP469912	33	Itirapina/SP	Reserva Experimental de Itirapina
Feb-18	SP469913	34	Itirapina/SP	Reserva Experimental de Itirapina
Feb-18	SP469914	35	Itirapina/SP	Reserva Experimental de Itirapina
Feb-18	SP469915	36	Itirapina/SP	Reserva Experimental de Itirapina

Supplementary material 2: Literature taxa used in traditional and geometric morphometric analyses.

Taxon	Code	Publication
M. arcuata Bailey	Lit1	Bailey (1851)
M. expansa Bailey	Lit2	Bailey (1851)
M. arcuata var. *borgei* Förster	Lit10	Förster (1969)
M. arcuata var. *compacta* Förster	Lit11a	Förster (1969)
M. arcuata var. *compacta* Förster	Lit11b	Förster (1969)
M. arcuata var. *cornuta* Förster	Lit12	Förster (1964)
M. arcuata var. *minuta* Förster	Lit13	Förster (1964)
M. arcuata var. *perforata* Förster	Lit14	Förster (1964)
M. arcuata var. *perforata* f. *magniperforata* Förster	Lit15	Förster (1964)
M. arcuata var. *perforata* f. *robustior* Förster	Lit16	Förster (1964)
M. arcuata var. *perforata* f. *robustior* f. *minor* Förster	Lit17	Förster (1964)
M. arcuata var. *robusta* f. *goyazensis* Förster	Lit18	Förster (1964)
M. arcuata var. *subcornuta* Förster	Lit19	Förster (1964)
M. arcuata var. *subpinnatifida* f. *gracilis* Förster	Lit20	Förster (1964)
M. arcuata var. *subpinnatifida* f. *eckertii* Förster	Lit21	Förster (1964)
M. arcuata morpha minor Bailey	Lit22a	Förster (1969)
M. arcuata morpha minor Bailey	Lit22b	Förster (1969)
M. arcuata var. *robusta* f. *recurvata* Prescott & Sott	Lit 23	Förster (1969)
M. arcuata var. *robusta* f. *goyazensis* Förster	Lit24a	Förster (1969)
M. arcuata var. *robusta* f. *goyazensis* Förster & Eckert	Lit24b	Förster (1969)
M. arcuata var. *robusta* Borge	Lit25a	Förster (1964)
M. arcuata var. *robusta* Borge	Lit25b	Förster (1964)
M. arcuata var. *subpinnatifida* f. Borge	Lit26a	Förster (1964)
M. arcuata var. *subpinnatifida* f. Borge	Lit26b	Förster (1964)
M. arcuata var. *subpinnatifida* f. Förster	Lit27	Förster (1964)
M. arcuata Bailey	Lit28	Förster (1964)
M. arcuata f. Förster	Lit29	Förster (1964)
M. arcuata var. *gracilis* f. 1 Förster	Lit30	Förster (1964)
M. arcuata var. *gracilis* f. 2 Förster	Lit31	Förster (1964)

Supplementary material 3: Classification of the morphotypes according to the correct classification/prediction in linear discriminant analysis (LDA) of *Micrasterias arcuata* morphotypes. Data set of traditional morphometric analyses and literature data in traditional morphometric analyses.

Literature taxa	ID	M1	M2	M3	M4	M5	M6	M7
M. arcuata	Lit1	1,9E-05	3,8E-09	5,5E-07	1,7E-29	3,3E-51	2,8E-19	0,99998
M. expansa	Lit2	1,4E-23	0,00106	1E-28	0,99894	1,8E-26	6,5E-34	4,5E-29
M. arcuata var. *borgei*	Lit10	9,9E-40	3,8E-62	1E-41	1	2,5E-27	4,3E-43	2,7E-45
M. arcuata var. *compacta*	Lit11a	6,4E-11	1	8,7E-17	1,5E-27	2,1E-28	7,6E-24	5,3E-17
M. arcuata var. *compacta*	Lit11b	0,3675	8,7E-20	0,63182	1,8E-16	9,2E-10	0,00059	8,8E-05
M. arcuata var. *cornuta*	Lit12	5,3E-07	6E-10	0,9729	3,9E-25	2,8E-09	5,9E-05	0,02704
M. arcuata var. *minuta*	Lit13	2,8E-13	9,4E-27	8,8E-13	1	8,7E-40	8,3E-08	3,5E-19
M. arcuata var. *perforata*	Lit14a	1E-25	4,9E-30	1,6E-24	4E-35	1	1,6E-30	3,1E-28
M. arcuata var. *perforata*	Lit14b	1,7E-41	5,8E-41	2,2E-40	8,4E-50	1	1,9E-47	5,7E-44
M. arcuata var. *perforata* f. *magniperforata*	Lit15	2,7E-54	2,6E-56	8E-53	1,9E-67	1	3,5E-62	4,6E-56
M. arcuata var. *perforata* f. *robustior*	Lit16	3,1E-33	2,3E-30	8,4E-22	1,5E-50	1	3,1E-26	3,7E-28
M. arcuata var. *perforata* f. *robustior* fa. *minor*	Lit17a	3,6E-12	1E-25	6,6E-16	1E-44	1	8,1E-19	5,4E-22
M. arcuata var. *perforata* f. *robustior* fa. *minor*	Lit17b	2,2E-10	2,8E-20	4,7E-08	2,6E-26	1	1,9E-11	7,5E-13
M. arcuata var. *perforata* f. *robustior* fa. *minor*	Lit17c	5,4E-14	2,6E-24	1,1E-10	2,8E-23	1	2,3E-12	5,8E-17
M. arcuata var. *robusta* f. *goyazensis*	Lit18	1,8E-07	1,2E-16	0,3087	2,6E-16	8,3E-15	0,6913	1,2E-07
M. arcuata var. *subcornuta*	Lit19	1,5E-09	4,9E-27	0,99845	1,1E-32	1,3E-38	0,00085	0,0007
M. arcuata var. *subpinnatifida* f. *gracilis*	Lit20	1,1E-27	1	8,8E-31	8,3E-21	1,4E-12	1,2E-38	4,2E-32
M. arcuata var. subpinnatifida f. *eckertii*	Lit21	6,3E-22	1E-21	5,7E-06	2,4E-38	0,99999	8,2E-08	8,3E-14
M. arcuata morpha minor Bailey	Lit22a	2E-08	1	3,6E-12	1,1E-23	5,8E-33	2E-17	8,7E-13
M. arcuata morpha minor Bailey	Lit22b	4,6E-57	1	5,2E-64	3,4E-65	1,1E-79	5,7E-64	9E-70
M. arcuata var. *robusta* f. *recurvata*	Lit23	2,2E-16	1,2E-22	2,4E-18	1	8,5E-26	4,8E-20	2,6E-20
M. arcuata var. *robusta* f. *goyazensis*	Lit24a	0,9998	0,00018	1,7E-05	4,6E-15	5,2E-14	4,2E-06	3,1E-10
M. arcuata var. *robusta* f. *goyazensis*	Lit24b	0,99873	7,2E-09	0,00098	8,9E-05	1,4E-10	0,0002	2,5E-08
M. arcuata var. *robusta* Borge	Lit25a	1E-11	5,5E-18	0,00136	5E-15	8,6E-29	0,99864	4,8E-11
M. arcuata var. *robusta* Borge	Lit25b	0,00077	3,3E-18	0,27111	4,1E-06	2E-22	0,72811	1,8E-06

M. arcuata var. *subpinnatifida* West & West form Borge 1918	Lit26a	8,9E-16	1,3E-19	0,32081	3,6E-24	0,00429	0,6749	1,4E-10
M. arcuata var. *subpinnatifida* West & West form Borge 1918	Lit26b	1,1E-28	1E-23	0,01749	9,9E-30	3,4E-09	0,98251	1,1E-14
M. arcuata var. *subpinnatifida* f. Förster 1964	Lit27	2,5E-10	6,5E-58	0,15245	0,83402	5,2E-05	1,8E-09	0,01348
M. arcuata Bailey 1851	Lit28	2,4E-53	1,9E-44	2,3E-54	2,6E-47	1	1,4E-66	1,7E-54
M. arcuata f. Förster 1964	Lit29	2,9E-71	1	3,1E-81	1,8E-84	5,9E-27	1,9E-90	2E-83
M. arcuata var. *gracilis* f.1 Förster 1964	Lit30	6E-123	1	2E-140	2E-162	6,1E-87	1E-148	2E-142
M. arcuata var. *gracilis* f.2 Förster 1964	Lit31	4,3E-80	0,00025	8,8E-92	0,99975	2,7E-49	3E-105	7,8E-86

Supplementary material 4: Classification of the morphotypes according to the correct classification/prediction in linear discriminant analysis (LDA) of *Micrasterias arcuata* morphotypes. Data set of geometric morphometric analyses of natural population and literature data.

Literature taxa	ID	M1	M2	M3	M4	M5	M6	M7
M. arcuata	Lit1	4,85876E-12	1	1E-11	3,5E-53	1E-29	3,4E-18	2,8E-23
M. expansa	Lit2	1,00903E-70	2,2E-81	6,6E-53	1	1,5E-56	1E-50	4,2E-58
M. arcuata var. *borgei*	Lit10	6,28161E-26	1,5E-55	4,7E-15	4,6E-09	1	1,6E-11	1,9E-31
M. arcuata var. *compacta*	Lit11a	3,18364E-20	1	2,6E-27	1,9E-41	3,6E-31	6,7E-30	5,5E-46
M. arcuata var. *compacta*	Lit11b	3,96977E-12	6E-16	0,88221	4E-30	9,4E-15	0,11779	1,8E-16
M. arcuata var. *cornuta*	Lit12	6,85486E-30	6,6E-61	0,99852	1,6E-63	3,6E-38	2E-05	0,00146
M. arcuata var. *minuta*	Lit13	1,04499E-23	2,4E-51	1E-06	0,99733	4,8E-07	0,00267	5,6E-19
M. arcuata var. *perforata*	Lit14a	3,81145E-30	1	1,4E-34	1,8E-43	3,1E-39	1,8E-37	6E-49
M. arcuata var. *perforata*	Lit14b	1,01194E-30	1	1E-22	3,3E-38	2,3E-35	1,1E-26	8,3E-39
M. arcuata var. *perforata* f. *magniperforata*	Lit15	6,47898E-53	3,5E-71	4,8E-41	1	3,9E-42	2,3E-40	1,6E-46
M. arcuata var. *perforata* f. *robustior*	Lit16	2,72236E-34	1	1,5E-28	2,1E-32	7,4E-16	2,2E-28	2,7E-52
M. arcuata var. *perforata* f. *robustior* f. *minor*	Lit17a	3,08864E-20	5,7E-16	1,4E-12	6,4E-12	1	3,6E-12	2,9E-31
M. arcuata var. *perforata* f. *robustior* f. *minor*	Lit17b	1,71208E-35	2,8E-56	1,7E-19	1,6E-29	1	5,9E-19	2,8E-34
M. arcuata var. *perforata* f. *robustior* f. *minor*	Lit17c	2,70918E-27	3E-52	1,3E-17	4,5E-25	1	4,3E-16	2,7E-33
M. arcuata var. *robusta* f. *goyazensis*	Lit18a	8,47011E-32	7,4E-47	2,6E-19	3,7E-20	1	8,9E-19	3,6E-34
M. arcuata var. *robusta* f. *goyazensis*	Lit18b	1,0953E-23	3,4E-56	0,00715	3,7E-35	0,06713	0,92572	8,3E-17
M. arcuata var. *subcornuta*	Lit19	2,54407E-14	6,4E-51	0,08575	3,4E-37	0,63579	0,27846	2,4E-15
M. arcuata var. *subpinnatifida* f. *gracilis*	Lit20	3,67713E-24	7,3E-61	4E-15	2,1E-47	1	7,5E-13	1,5E-30
M. arcuata var. subpinnatifida f. *eckertii*	Lit21	2,6566E-29	9,4E-56	8,4E-25	1,7E-33	1	1,1E-20	3,5E-45
M. arcuata morpha minor Bailey	Lit22a	5,61739E-27	1,8E-33	3,2E-06	2,8E-24	0,99997	2,6E-05	1E-24
M. arcuata morpha minor Bailey	Lit22b	2,89123E-20	1,1E-26	2,3E-05	9,9E-18	0,99994	3,9E-05	9,2E-23
M. arcuata var. *robusta* f. r*ecurvata*	Lit 23	2,77658E-31	5,2E-57	0,99993	4,7E-51	4,8E-19	7,3E-05	4E-07
M. arcuata var. *robusta* f. *goyazensis*	Lit24a	1,10162E-30	7,7E-77	0,99552	8,7E-54	3,5E-36	0,00147	0,00302
M. arcuata var. *robusta* f. *goyazensis*	Lit24b	2,96605E-42	6E-75	0,02174	1,4E-55	1,1E-13	0,97826	6,6E-11
M. arcuata var. *robusta* Borge	Lit25a	3,16852E-21	5,7E-44	0,00271	1,8E-16	0,37992	0,61737	4,1E-16

M. arcuata var. *robusta* Borge	Lit25b	4,23133E-20	1,4E-52	1,7E-09	1,6E-24	1	6,9E-08	3,9E-26
M. arcuata var. *subpinnatifida* West & West form Borge 1918	Lit26a	1,41914E-30	4,9E-67	0,86416	8,6E-27	6,1E-06	0,13583	4,6E-07
M. arcuata var. *subpinnatifida* West & West form Borge 1918	Lit26b	5,28846E-33	1,2E-73	0,85138	2,5E-38	7E-16	0,14862	3,8E-09
M. arcuata var. *subpinnatifida* f. Förster 1964	Lit27	3,92231E-22	5,6E-61	0,97754	2,1E-28	1,2E-16	0,02246	2,4E-08
M. arcuata Bailey 1851	Lit28	1,0237E-22	2,2E-34	0,99966	3,4E-53	1,5E-27	0,00034	9,7E-09
M. arcuata f. Förster 1964	Lit29	1,05867E-21	1	6,1E-22	1,3E-39	7,5E-26	3,3E-25	4,1E-38
M. arcuata var. *gracilis* f.1 Förster 1964	Lit30	4,48962E-53	1	2,4E-54	1,4E-38	3,9E-52	2,7E-59	6,6E-68
M. arcuata var. *gracilis* f.2 Förster 1964	Lit31	1,62342E-17	0,95769	0,04231	1E-59	4,3E-35	3,1E-09	3,8E-15

Supplementary material 5: Classification of the morphotypes according to the correct classification/prediction in linear discriminant analysis (LDA) of *Micrasterias arcuata* morphotypes. Data set of geometric morphometric analyses of natural population and literature data of compact morphotypes of *M. arcuata* species complex.

ID	M1	M3	M6	M7
M1	1	1,84E-14	1,31E-14	7,54E-18
M1	0,999998	1,09E-07	1,91E-06	1,77E-12
M1	0,649737	0,326166	0,024093	4,02E-06
M1	1	1,76E-13	6,93E-14	3,05E-18
M1	1	1,93E-18	1,41E-19	2,41E-23
M1	1	4,4E-22	3,69E-21	3,84E-25
M1	1	1,13E-33	2,99E-33	3,74E-33
M1	1	1,01E-18	4,83E-19	1,3E-21
M1	1	2,91E-21	7,01E-22	1,16E-28
M3	8,72E-26	0,993229	0,006771	8,92E-08
M3	2,55E-13	0,683938	0,316061	3,4E-07
M3	8,11E-26	0,996797	0,003203	2,2E-08
M3	3E-20	0,997385	0,002538	7,69E-05
M3	1,59E-20	0,996323	0,003677	4,3E-10
M3	4,51E-23	0,886261	0,113739	1,24E-10
M3	8,09E-14	0,804478	0,195522	7,31E-12
M3	5,88E-22	0,98594	0,01406	4,04E-10
M3	5,41E-23	0,997008	0,002992	1,46E-10
M3	1,48E-18	0,997693	0,002307	5,73E-10
M3	4,1E-25	0,998021	0,001979	2,08E-11
M3	5,64E-20	0,996148	0,003847	5,68E-06
M3	6,49E-16	0,87517	0,124829	2,08E-07
M3	8,91E-13	0,97963	0,020369	1,69E-06
M3	1,04E-14	0,996398	0,003602	5,17E-07
M3	1,62E-17	0,802578	0,197422	3,64E-08
M3	3,35E-23	0,963789	0,036211	1,55E-10
M3	1,67E-21	0,974005	0,025995	4,54E-08
M3	1,06E-17	0,997983	0,002017	3,87E-13
M3	8E-16	0,996603	0,003397	5,9E-09
M3	1,8E-27	0,953415	0,046585	1,28E-07
M3	3,73E-14	0,882126	0,117864	9,44E-06
M3	7,82E-18	0,989873	0,010127	4,04E-07
M3	6,57E-16	0,97455	0,02545	7,45E-08
M3	5,23E-20	0,986372	0,013628	3,77E-08
M3	7,22E-19	0,997485	0,002513	1,35E-06
M3	1,53E-14	0,988033	0,011965	1,7E-06
M3	1,01E-20	0,9643	0,0357	5,15E-08
M3	2,76E-17	0,987935	0,012065	3,01E-09
M3	6,74E-17	0,978045	0,021946	9,2E-06
M3	2,23E-21	0,998766	0,001234	1,45E-07
M3	3,63E-18	0,99963	0,000352	1,8E-05
M3	2,76E-15	0,501793	0,006324	0,491883
M3	4,97E-23	0,993952	0,006048	1,85E-09
M3	7,81E-17	0,730161	0,269832	7,51E-06
M3	9,65E-20	0,773762	0,226229	8,83E-06
M3	1,25E-16	0,988443	0,011531	2,65E-05

M3	9,86E-17	0,999077	0,000923	7,17E-09
M3	7,08E-19	0,997317	0,002683	1,06E-08
M3	1,2E-18	0,993389	0,006611	4,68E-07
M3	3,3E-20	0,993088	0,006911	1,47E-07
M3	3,85E-20	0,994616	0,005384	3,76E-12
M3	2,01E-18	0,995256	0,004678	6,59E-05
M3	1,75E-19	0,998866	0,001133	1,06E-06
M3	1,57E-19	0,985599	0,014401	1,25E-08
M3	7,61E-24	0,99776	0,002238	1,35E-06
M3	1,44E-23	0,9988	0,0012	4,93E-07
M3	6,39E-24	0,998726	0,001273	1,11E-07
M3	1,61E-19	0,999581	0,000419	2,88E-07
M3	3,14E-23	0,981175	0,018782	4,25E-05
M3	4,5E-24	0,996303	0,003697	3,04E-11
M3	8,23E-22	0,999127	0,000871	2,56E-06
M3	5,03E-16	0,986851	0,013149	6,67E-07
M3	2,19E-27	0,996035	0,003959	5,98E-06
M3	2,59E-21	0,997313	0,002687	2,59E-07
M3	3,5E-21	0,983076	0,016924	1,38E-08
M3	1,45E-22	0,975152	0,024848	6,14E-08
M3	6,69E-19	0,774398	0,225602	4,37E-08
M3	1,2E-18	0,979988	0,019995	1,69E-05
M3	9,32E-19	0,994622	0,005377	6,62E-07
M3	4,04E-17	0,99021	0,00979	4,81E-09
M3	6,27E-22	0,999493	0,000507	6,39E-08
M3	9,76E-22	0,947481	0,052519	3,45E-10
M3	7,05E-10	0,875198	0,124802	2,48E-08
M3	1,08E-20	0,829637	0,170363	5,54E-10
M3	2,1E-19	0,944711	0,055289	9,64E-08
M3	1,7E-14	0,412115	0,586598	0,001287
M3	1,28E-12	0,974168	0,025831	1,16E-06
M3	5,75E-19	0,970969	0,029031	1,3E-08
M3	3,24E-19	0,754902	0,245098	2,82E-10
M3	9,55E-21	0,998099	0,001892	8,48E-06
M3	2,23E-16	0,806297	0,193703	1,51E-07
M3	5,7E-24	0,981074	0,018926	5,42E-07
M3	8,16E-20	0,943447	0,056553	4,38E-08
M3	2,98E-23	0,998801	0,001198	5,54E-07
M3	4,58E-19	0,857297	0,142687	1,59E-05
M3	5,04E-27	0,87986	0,12013	1,01E-05
M3	1,47E-21	0,970187	0,029798	1,52E-05
M3	2,14E-20	0,926474	0,073525	1,24E-06
M3	1,52E-22	0,998616	0,000606	0,000778
M3	1,33E-18	0,997987	0,00149	0,000523
M3	3,62E-20	0,998029	0,001964	6,91E-06
M3	8,84E-20	0,996976	0,003022	2,2E-06
M3	1,18E-21	0,96321	0,036788	2,07E-06
M3	3,73E-21	0,994293	0,005707	9,08E-08
M3	1,14E-23	0,991462	0,008524	1,36E-05
M3	2,88E-20	0,943494	0,056506	2,68E-09
M3	1,34E-12	0,99927	0,00073	5,62E-08
M3	2,73E-28	0,99913	0,000819	5,07E-05
M3	8,44E-19	0,950074	0,049918	8,4E-06
M3	1,82E-17	0,912303	0,087697	1,34E-07
M3	8,49E-19	0,998902	0,001098	1,89E-07

M3	1,9E-26	0,999381	0,000619	7,54E-07
M3	2,55E-24	0,998417	0,001582	1,88E-06
M3	5,05E-21	0,932162	0,067837	1,08E-06
M3	4,13E-24	0,991547	0,008453	4,1E-09
M3	9,2E-23	0,99693	0,00307	1,23E-08
M3	2,26E-19	0,995011	0,004988	6,68E-07
M3	3,46E-17	0,998363	0,00161	2,68E-05
M3	4,81E-19	0,997762	0,002091	0,000146
M3	6,04E-21	0,982008	0,017992	3,07E-10
M3	1,03E-11	0,44099	0,559009	1,26E-06
M3	5,95E-23	0,96868	0,03132	1,37E-08
M3	3,05E-19	0,999221	0,000779	9,93E-09
M3	1,03E-18	0,987425	0,012561	1,42E-05
M3	1,28E-15	0,999592	0,000408	1,5E-07
M3	3,8E-27	0,998593	0,001404	3,37E-06
M3	1,48E-19	0,989663	0,010335	2,32E-06
M3	8,71E-19	0,822932	0,177068	1,46E-07
M3	5,92E-20	0,782609	0,217391	3,77E-08
M3	8,52E-21	0,997237	0,002763	1,31E-07
M3	4,05E-19	0,9852	0,007807	0,006993
M3	1,95E-19	0,895703	0,104105	0,000192
M3	6,57E-18	0,988109	0,011891	3,14E-10
M3	1,66E-12	0,970355	0,029643	2,15E-06
M3	8,23E-15	0,90193	0,098051	1,95E-05
M3	6,07E-16	0,943841	0,056157	2,37E-06
M3	5,12E-19	0,983678	0,016322	6,14E-10
M3	7,45E-22	0,999359	0,000641	4,6E-09
M3	1,11E-16	0,997311	0,002688	1,97E-06
M3	9,67E-17	0,999024	0,000973	2,46E-06
M3	1,07E-23	0,982174	0,017495	0,000331
M3	1,42E-17	0,919388	0,080611	4,56E-07
M3	8,43E-19	0,993564	0,006435	1,23E-07
M3	1,55E-18	0,959278	0,039088	0,001634
M3	3,97E-23	0,997424	0,002576	1,68E-07
M3	7,37E-21	0,999126	0,000874	8,41E-12
M3	9,33E-20	0,959975	0,040011	1,48E-05
M3	4,5E-25	0,988171	0,011827	1,8E-06
M3	9,1E-20	0,947064	0,052936	7,15E-11
M3	1,59E-19	0,758409	0,241591	4,98E-11
M3	3,32E-15	0,974873	0,025127	2,61E-07
M3	9,98E-23	0,998549	0,001448	2,68E-06
M3	7,97E-17	0,781888	0,218112	9,9E-09
M3	5,99E-16	0,997772	0,002228	1,2E-07
M3	8,5E-18	0,991219	0,008759	2,17E-05
M3	3,95E-14	0,975713	0,024287	2,74E-08
M3	2,22E-19	0,984776	0,015141	8,27E-05
M3	3,78E-22	0,968373	0,031627	8,13E-11
M3	1,97E-21	0,991538	0,008462	6,23E-07
M3	7,86E-17	0,999541	0,000459	1,52E-08
M3	2,5E-18	0,9993	0,000699	3,59E-07
M3	1,9E-16	0,837295	0,162705	2,42E-08
M3	5,3E-21	0,998058	0,001942	3,36E-09
M3	5,59E-19	0,943418	0,056582	1,86E-08
M3	2,51E-20	0,934393	0,065607	2,45E-07
M3	1,65E-21	0,946071	0,053929	7,45E-08

M3	2,18E-21	0,995005	0,004995	3,47E-10
M3	6,56E-24	0,952547	0,047453	4,97E-08
M3	1,61E-19	0,943228	0,056772	2,24E-09
M3	4,11E-18	0,993814	0,006186	1,88E-10
M3	4,87E-22	0,99909	0,00091	2,67E-07
M3	2,65E-18	0,99386	0,00614	1,76E-07
M3	1,48E-26	0,997326	0,002674	1,87E-07
M3	8,14E-19	0,996883	0,003111	5,85E-06
M3	8,87E-24	0,999533	0,000405	6,22E-05
M3	7,55E-22	0,997186	0,002759	5,51E-05
M3	6,07E-17	0,998722	0,001278	5,44E-07
M3	3,55E-27	0,999343	0,00065	7,22E-06
M3	7,01E-24	0,985612	0,014388	8,4E-09
M3	1,26E-15	0,996251	0,003744	5,02E-06
M3	1,19E-23	0,996368	0,003632	5,05E-07
M3	3,32E-15	0,997383	0,002617	4,41E-08
M3	6E-23	0,954484	0,045516	4,32E-09
M3	8,35E-21	0,994785	0,005214	1,57E-07
M3	1,27E-22	0,993174	0,006826	6,31E-08
M3	6,68E-11	0,983024	0,016858	0,000118
M3	5,09E-23	0,980472	0,019528	3,91E-09
M3	3E-18	0,997553	0,002447	5,55E-08
M3	6,69E-22	0,86307	0,13693	4,06E-11
M3	3,9E-13	0,987072	0,00453	0,008398
M3	5,98E-14	0,996378	0,003622	3,69E-09
M3	3,46E-21	0,913745	0,086231	2,4E-05
M3	4,17E-15	0,999426	0,000573	1,62E-06
M3	4,72E-23	0,995557	0,004443	1,8E-09
M3	5,55E-22	0,99926	0,000631	0,000109
M3	7,46E-22	0,999839	0,00016	9,97E-07
M3	6,39E-18	0,98686	0,01314	5,39E-09
M3	1,23E-19	0,998794	0,001206	4,93E-08
M3	4,45E-16	0,943925	0,049752	0,006323
M3	5,48E-21	0,950827	0,049173	8,27E-08
M3	2,82E-17	0,782175	0,217591	0,000234
M3	5,93E-25	0,980222	0,019777	4,32E-07
M3	1,21E-24	0,997735	0,002265	2,14E-09
M3	9,51E-22	0,990759	0,009241	5,78E-09
M3	4,02E-21	0,938556	0,061444	6,37E-11
M3	3,11E-20	0,970803	0,029195	2,14E-06
M3	4,1E-15	0,926968	0,072742	0,00029
M3	4,14E-20	0,993464	0,006536	2,07E-07
M3	5,32E-20	0,980105	0,019895	4,87E-09
M3	1,12E-14	0,999193	0,000798	8,46E-06
M3	2,7E-23	0,986728	0,013272	1,18E-07
M3	3,19E-18	0,866857	0,133141	2,41E-06
M3	1,35E-17	0,995372	0,004441	0,000187
M3	4,02E-22	0,392097	0,594203	0,0137
M3	5,6E-20	0,997572	0,001521	0,000907
M3	5,71E-14	0,884729	0,115087	0,000185
M3	5,74E-16	0,88653	0,113414	5,63E-05
M3	2,56E-17	0,961683	0,018689	0,019628
M3	1,69E-28	0,981698	0,000838	0,017464
M3	6,51E-18	0,997355	0,002645	8,86E-08
M3	2,47E-21	0,986578	0,013414	8,15E-06

M3	7,5E-11	0,885274	0,114705	2,1E-05
M3	1,66E-15	0,990679	0,009321	1,13E-07
M3	2,72E-24	0,970064	0,029849	8,68E-05
M3	1,24E-23	0,998499	0,001427	7,49E-05
M3	5,03E-23	0,965189	0,034811	1,07E-08
M3	1,25E-25	0,998412	0,001588	8,59E-08
M3	4,86E-16	0,993806	0,006192	1,48E-06
M3	2,55E-19	0,863031	0,136968	9,57E-07
M3	3,68E-18	0,992885	0,007083	3,16E-05
M3	1,67E-23	0,95814	0,04172	0,000141
M3	3,95E-13	0,987522	0,012441	3,66E-05
M3	1,21E-12	0,886544	0,113451	5,23E-06
M3	3,32E-18	0,982195	0,017805	3,47E-07
M3	1,65E-19	0,965401	0,00599	0,028609
M3	1,3E-19	0,994058	0,005942	3,18E-08
M3	9,54E-23	0,997774	0,002214	1,16E-05
M3	3,36E-15	0,997508	0,002492	5,54E-10
M3	1,45E-28	0,99918	0,000815	4,45E-06
M3	3,37E-20	0,686284	0,313715	4,32E-08
M3	9,66E-20	0,958958	0,041042	5,16E-10
M3	1,93E-24	0,9665	0,0335	4,36E-08
M3	1,12E-21	0,992386	0,007614	6,7E-08
M3	9,51E-15	0,891223	0,108747	3,06E-05
M3	1,72E-18	0,997294	0,002706	2E-07
M3	2,56E-16	0,941153	0,058846	7,42E-07
M3	1,96E-19	0,994243	0,005757	6,71E-09
M3	6,75E-17	0,984321	0,015666	1,24E-05
M3	1,42E-21	0,998092	0,001896	1,21E-05
M3	4,83E-21	0,989589	0,010411	9,04E-08
M3	3,12E-18	0,973178	0,02682	1,36E-06
M3	6,37E-17	0,903437	0,096562	1,63E-06
M3	2,55E-17	0,987025	0,012965	1,02E-05
M3	2,04E-18	0,993039	0,006961	6,45E-08
M3	3,91E-16	0,997887	0,002113	1,08E-07
M3	1,21E-24	0,963302	0,036696	1,89E-06
M3	3,8E-14	0,984911	0,015089	2,07E-07
M6	4,24E-18	0,605722	0,394278	1,69E-07
M6	1,68E-20	0,992462	0,007538	5,14E-08
M6	9,41E-19	0,906587	0,093413	7,45E-08
M6	4,99E-22	0,976935	0,023064	1,23E-06
M6	1,92E-15	0,908267	0,091733	1,24E-09
M6	5,84E-16	0,415851	0,584149	2,59E-08
M6	2,14E-10	0,524172	0,475809	1,92E-05
M6	1,56E-20	0,014107	0,985892	5,11E-07
M6	1,49E-19	0,760715	0,239285	1,42E-07
M6	8,38E-17	0,508589	0,491411	8,37E-08
M6	4,55E-19	0,638607	0,361376	1,68E-05
M6	1,07E-14	0,626477	0,373523	1,81E-08
M6	1,3E-18	0,920844	0,079156	1,63E-07
M6	1,31E-22	0,150095	0,849835	6,96E-05
M6	9,41E-18	0,883263	0,116737	6,99E-07
M6	1,45E-18	0,640626	0,359374	6,31E-08
M7	5,1E-15	0,004566	8,63E-05	0,995348
M7	6,62E-18	0,002954	1,52E-05	0,99703
M7	2,35E-26	0,000884	3,77E-05	0,999078

Supplementary material 6: Classification of the morphotypes according to the correct classification/prediction in linear discriminant analysis (LDA) of *Micrasterias arcuata* morphotypes. Data set of geometric morphometric analyses of natural population and literature data of compact morphotypes of *M. arcuata* species complex.

ID	M1	M3	M6	M7
M1	1	1,841E-14	1,305E-14	7,543E-18
M1	0,999998	1,087E-07	1,91E-06	1,765E-12
M1	0,6497374	0,326166	0,0240926	4,023E-06
M1	1	1,758E-13	6,933E-14	3,054E-18
M1	1	1,928E-18	1,409E-19	2,413E-23
M1	1	4,402E-22	3,687E-21	3,843E-25
M1	1	1,13E-33	2,991E-33	3,739E-33
M1	1	1,01E-18	4,831E-19	1,303E-21
M1	1	2,912E-21	7,014E-22	1,155E-28
M3	8,718E-26	0,9932285	0,0067714	8,922E-08
M3	2,555E-13	0,6839382	0,3160614	3,399E-07
M3	8,108E-26	0,9967971	0,0032029	2,203E-08
M3	2,998E-20	0,9973847	0,0025384	7,694E-05
M3	1,591E-20	0,9963233	0,0036767	4,299E-10
M3	4,506E-23	0,8862607	0,1137393	1,237E-10
M3	8,091E-14	0,8044784	0,1955216	7,312E-12
M3	5,88E-22	0,9859398	0,0140602	4,043E-10
M3	5,409E-23	0,9970077	0,0029923	1,46E-10
M3	1,478E-18	0,9976925	0,0023075	5,735E-10
M3	4,101E-25	0,9980214	0,0019786	2,079E-11
M3	5,636E-20	0,9961477	0,0038466	5,684E-06
M3	6,489E-16	0,8751703	0,1248295	2,076E-07
M3	8,911E-13	0,9796297	0,0203686	1,688E-06
M3	1,042E-14	0,9963978	0,0036017	5,169E-07
M3	1,616E-17	0,8025783	0,1974217	3,636E-08
M3	3,347E-23	0,963789	0,036211	1,552E-10
M3	1,672E-21	0,974005	0,0259949	4,544E-08
M3	1,061E-17	0,9979831	0,0020169	3,865E-13
M3	7,995E-16	0,996603	0,003397	5,9E-09
M3	1,799E-27	0,9534149	0,046585	1,276E-07
M3	3,732E-14	0,8821261	0,1178645	9,436E-06
M3	7,816E-18	0,9898727	0,0101269	4,04E-07
M3	6,575E-16	0,9745499	0,02545	7,445E-08
M3	5,231E-20	0,9863722	0,0136277	3,768E-08
M3	7,22E-19	0,9974852	0,0025134	1,352E-06
M3	1,532E-14	0,988033	0,0119653	1,697E-06
M3	1,012E-20	0,9643004	0,0356996	5,154E-08
M3	2,764E-17	0,9879346	0,0120654	3,007E-09
M3	6,737E-17	0,9780453	0,0219455	9,198E-06
M3	2,232E-21	0,9987659	0,001234	1,446E-07
M3	3,629E-18	0,9996295	0,0003525	1,8E-05
M3	2,76E-15	0,5017931	0,0063236	0,4918833
M3	4,97E-23	0,9939523	0,0060477	1,849E-09
M3	7,808E-17	0,7301609	0,2698316	7,509E-06
M3	9,648E-20	0,7737623	0,2262289	8,829E-06

M3	1,253E-16	0,9884429	0,0115306	2,652E-05
M3	9,856E-17	0,9990772	0,0009228	7,173E-09
M3	7,081E-19	0,9973171	0,0026829	1,065E-08
M3	1,2E-18	0,9933889	0,0066106	4,677E-07
M3	3,301E-20	0,9930884	0,0069114	1,475E-07
M3	3,85E-20	0,9946164	0,0053836	3,756E-12
M3	2,014E-18	0,9952562	0,004678	6,585E-05
M3	1,749E-19	0,9988657	0,0011332	1,056E-06
M3	1,572E-19	0,9855989	0,0144011	1,249E-08
M3	7,614E-24	0,9977602	0,0022384	1,354E-06
M3	1,439E-23	0,9988	0,0011995	4,925E-07
M3	6,39E-24	0,9987264	0,0012735	1,113E-07
M3	1,613E-19	0,9995807	0,000419	2,88E-07
M3	3,143E-23	0,9811752	0,0187824	4,247E-05
M3	4,497E-24	0,9963032	0,0036968	3,037E-11
M3	8,235E-22	0,9991267	0,0008707	2,562E-06
M3	5,028E-16	0,9868507	0,0131486	6,673E-07
M3	2,194E-27	0,9960349	0,0039592	5,978E-06
M3	2,591E-21	0,9973129	0,0026868	2,594E-07
M3	3,497E-21	0,9830757	0,0169243	1,378E-08
M3	1,451E-22	0,9751522	0,0248477	6,142E-08
M3	6,687E-19	0,7743981	0,2256019	4,366E-08
M3	1,204E-18	0,9799877	0,0199954	1,686E-05
M3	9,319E-19	0,9946223	0,005377	6,617E-07
M3	4,045E-17	0,9902103	0,0097897	4,808E-09
M3	6,271E-22	0,9994927	0,0005072	6,387E-08
M3	9,759E-22	0,9474806	0,0525194	3,447E-10
M3	7,05E-10	0,8751983	0,1248017	2,475E-08
M3	1,078E-20	0,8296374	0,1703626	5,543E-10
M3	2,103E-19	0,9447107	0,0552893	9,64E-08
M3	1,7E-14	0,4121152	0,586598	0,0012869
M3	1,276E-12	0,9741679	0,025831	1,164E-06
M3	5,751E-19	0,970969	0,029031	1,296E-08
M3	3,245E-19	0,7549024	0,2450976	2,817E-10
M3	9,549E-21	0,9980991	0,0018924	8,478E-06
M3	2,23E-16	0,8062968	0,1937031	1,508E-07
M3	5,705E-24	0,9810738	0,0189256	5,418E-07
M3	8,162E-20	0,9434467	0,0565532	4,378E-08
M3	2,977E-23	0,9988011	0,0011984	5,537E-07
M3	4,583E-19	0,8572975	0,1426866	1,589E-05
M3	5,039E-27	0,8798595	0,1201304	1,01E-05
M3	1,469E-21	0,9701865	0,0297982	1,525E-05
M3	2,141E-20	0,9264737	0,073525	1,242E-06
M3	1,517E-22	0,9986156	0,0006061	0,0007784
M3	1,329E-18	0,997987	0,0014903	0,0005227
M3	3,622E-20	0,9980293	0,0019638	6,911E-06
M3	8,84E-20	0,9969757	0,0030221	2,203E-06
M3	1,176E-21	0,9632099	0,036788	2,073E-06
M3	3,73E-21	0,9942932	0,0057067	9,085E-08
M3	1,137E-23	0,9914621	0,0085243	1,358E-05
M3	2,884E-20	0,9434944	0,0565056	2,676E-09
M3	1,345E-12	0,9992696	0,0007303	5,623E-08
M3	2,732E-28	0,9991299	0,0008194	5,068E-05
M3	8,442E-19	0,950074	0,0499176	8,403E-06

M3	1,815E-17	0,9123032	0,0876966	1,344E-07
M3	8,488E-19	0,998902	0,0010978	1,89E-07
M3	1,902E-26	0,9993806	0,0006186	7,54E-07
M3	2,554E-24	0,9984166	0,0015815	1,876E-06
M3	5,047E-21	0,9321618	0,0678371	1,076E-06
M3	4,126E-24	0,9915469	0,0084531	4,098E-09
M3	9,199E-23	0,9969295	0,0030704	1,229E-08
M3	2,264E-19	0,9950113	0,0049881	6,682E-07
M3	3,463E-17	0,9983634	0,0016099	2,676E-05
M3	4,814E-19	0,9977622	0,0020914	0,0001464
M3	6,043E-21	0,982008	0,017992	3,066E-10
M3	1,025E-11	0,4409897	0,559009	1,264E-06
M3	5,948E-23	0,9686804	0,0313196	1,371E-08
M3	3,047E-19	0,9992212	0,0007788	9,926E-09
M3	1,029E-18	0,9874245	0,0125613	1,418E-05
M3	1,285E-15	0,9995917	0,0004082	1,497E-07
M3	3,799E-27	0,9985929	0,0014037	3,369E-06
M3	1,484E-19	0,9896627	0,010335	2,324E-06
M3	8,705E-19	0,8229318	0,1770681	1,462E-07
M3	5,917E-20	0,7826085	0,2173914	3,774E-08
M3	8,52E-21	0,9972367	0,0027632	1,314E-07
M3	4,049E-19	0,9852001	0,0078073	0,0069926
M3	1,948E-19	0,8957034	0,1041046	0,000192
M3	6,574E-18	0,9881093	0,0118907	3,136E-10
M3	1,658E-12	0,9703545	0,0296433	2,155E-06
M3	8,233E-15	0,90193	0,0980505	1,951E-05
M3	6,074E-16	0,9438407	0,0561569	2,366E-06
M3	5,123E-19	0,9836776	0,0163224	6,14E-10
M3	7,454E-22	0,9993595	0,0006405	4,596E-09
M3	1,106E-16	0,9973105	0,0026875	1,974E-06
M3	9,67E-17	0,9990243	0,0009733	2,456E-06
M3	1,066E-23	0,9821741	0,0174952	0,0003307
M3	1,416E-17	0,9193884	0,0806111	4,561E-07
M3	8,432E-19	0,9935644	0,0064355	1,226E-07
M3	1,549E-18	0,9592785	0,0390877	0,0016339
M3	3,966E-23	0,9974239	0,0025759	1,678E-07
M3	7,367E-21	0,9991263	0,0008737	8,414E-12
M3	9,326E-20	0,9599746	0,0400106	1,476E-05
M3	4,502E-25	0,9881711	0,0118271	1,8E-06
M3	9,103E-20	0,9470645	0,0529355	7,154E-11
M3	1,589E-19	0,7584094	0,2415906	4,982E-11
M3	3,318E-15	0,9748732	0,0251265	2,613E-07
M3	9,983E-23	0,9985493	0,001448	2,68E-06
M3	7,969E-17	0,7818884	0,2181116	9,898E-09
M3	5,986E-16	0,9977716	0,0022283	1,201E-07
M3	8,498E-18	0,9912188	0,0087595	2,172E-05
M3	3,949E-14	0,9757134	0,0242866	2,736E-08
M3	2,22E-19	0,984776	0,0151413	8,27E-05
M3	3,776E-22	0,9683727	0,0316273	8,127E-11
M3	1,97E-21	0,9915377	0,0084617	6,225E-07
M3	7,857E-17	0,9995414	0,0004585	1,518E-08
M3	2,498E-18	0,9993004	0,0006992	3,593E-07
M3	1,904E-16	0,8372948	0,1627052	2,422E-08
M3	5,295E-21	0,9980584	0,0019416	3,363E-09

M3	5,592E-19	0,9434184	0,0565816	1,864E-08
M3	2,509E-20	0,9343927	0,0656071	2,449E-07
M3	1,651E-21	0,9460713	0,0539287	7,451E-08
M3	2,184E-21	0,9950047	0,0049953	3,466E-10
M3	6,556E-24	0,9525467	0,0474532	4,966E-08
M3	1,606E-19	0,943228	0,056772	2,241E-09
M3	4,11E-18	0,9938139	0,0061861	1,876E-10
M3	4,87E-22	0,9990898	0,00091	2,672E-07
M3	2,651E-18	0,9938596	0,0061403	1,764E-07
M3	1,477E-26	0,9973256	0,0026742	1,865E-07
M3	8,136E-19	0,9968832	0,003111	5,849E-06
M3	8,868E-24	0,9995328	0,0004051	6,216E-05
M3	7,549E-22	0,9971855	0,0027594	5,505E-05
M3	6,072E-17	0,9987219	0,0012776	5,44E-07
M3	3,55E-27	0,999343	0,0006498	7,224E-06
M3	7,011E-24	0,9856117	0,0143883	8,404E-09
M3	1,265E-15	0,996251	0,0037439	5,016E-06
M3	1,192E-23	0,9963679	0,0036316	5,055E-07
M3	3,32E-15	0,997383	0,002617	4,405E-08
M3	6E-23	0,9544837	0,0455163	4,318E-09
M3	8,349E-21	0,9947854	0,0052145	1,565E-07
M3	1,27E-22	0,9931736	0,0068263	6,31E-08
M3	6,678E-11	0,9830238	0,0168579	0,0001184
M3	5,092E-23	0,9804716	0,0195284	3,914E-09
M3	3,004E-18	0,9975529	0,002447	5,55E-08
M3	6,693E-22	0,8630703	0,1369297	4,055E-11
M3	3,898E-13	0,9870724	0,0045299	0,0083977
M3	5,983E-14	0,9963784	0,0036216	3,688E-09
M3	3,459E-21	0,9137451	0,0862309	2,399E-05
M3	4,165E-15	0,9994256	0,0005728	1,615E-06
M3	4,722E-23	0,9955568	0,0044432	1,804E-09
M3	5,554E-22	0,9992605	0,0006306	0,0001089
M3	7,463E-22	0,9998393	0,0001597	9,971E-07
M3	6,389E-18	0,9868598	0,0131402	5,389E-09
M3	1,232E-19	0,9987936	0,0012064	4,934E-08
M3	4,453E-16	0,9439248	0,0497517	0,0063235
M3	5,481E-21	0,9508269	0,049173	8,27E-08
M3	2,824E-17	0,7821751	0,2175914	0,0002335
M3	5,925E-25	0,9802224	0,0197772	4,325E-07
M3	1,212E-24	0,9977348	0,0022652	2,137E-09
M3	9,508E-22	0,9907586	0,0092414	5,778E-09
M3	4,016E-21	0,9385561	0,0614439	6,37E-11
M3	3,107E-20	0,9708027	0,0291952	2,142E-06
M3	4,102E-15	0,9269677	0,0727418	0,0002904
M3	4,141E-20	0,9934639	0,0065359	2,072E-07
M3	5,316E-20	0,9801047	0,0198953	4,87E-09
M3	1,121E-14	0,9991931	0,0007984	8,462E-06
M3	2,703E-23	0,986728	0,0132719	1,176E-07
M3	3,185E-18	0,8668568	0,1331408	2,406E-06
M3	1,347E-17	0,9953725	0,0044407	0,0001868
M3	4,022E-22	0,3920966	0,5942034	0,0137
M3	5,604E-20	0,9975717	0,0015212	0,0009071
M3	5,706E-14	0,8847287	0,1150867	0,0001847
M3	5,738E-16	0,8865298	0,113414	5,626E-05

M3	2,56E-17	0,9616833	0,0186891	0,0196276
M3	1,685E-28	0,9816982	0,0008378	0,017464
M3	6,513E-18	0,9973552	0,0026447	8,858E-08
M3	2,471E-21	0,9865776	0,0134142	8,147E-06
M3	7,496E-11	0,8852743	0,1147046	2,102E-05
M3	1,66E-15	0,9906791	0,0093208	1,134E-07
M3	2,724E-24	0,9700643	0,0298489	8,682E-05
M3	1,235E-23	0,9984985	0,0014265	7,494E-05
M3	5,027E-23	0,9651891	0,0348109	1,068E-08
M3	1,252E-25	0,9984117	0,0015882	8,588E-08
M3	4,861E-16	0,9938061	0,0061925	1,478E-06
M3	2,546E-19	0,8630311	0,136968	9,569E-07
M3	3,68E-18	0,9928855	0,0070829	3,16E-05
M3	1,665E-23	0,9581396	0,0417198	0,0001406
M3	3,951E-13	0,987522	0,0124414	3,662E-05
M3	1,206E-12	0,8865439	0,1134509	5,233E-06
M3	3,319E-18	0,982195	0,0178047	3,469E-07
M3	1,651E-19	0,9654005	0,00599	0,0286095
M3	1,295E-19	0,9940578	0,0059421	3,181E-08
M3	9,543E-23	0,9977745	0,0022139	1,16E-05
M3	3,36E-15	0,9975084	0,0024916	5,542E-10
M3	1,449E-28	0,9991804	0,0008152	4,449E-06
M3	3,375E-20	0,6862845	0,3137155	4,32E-08
M3	9,656E-20	0,9589583	0,0410417	5,16E-10
M3	1,932E-24	0,9664998	0,0335001	4,362E-08
M3	1,117E-21	0,9923863	0,0076136	6,702E-08
M3	9,509E-15	0,8912227	0,1087467	3,065E-05
M3	1,716E-18	0,9972939	0,0027059	1,998E-07
M3	2,561E-16	0,941153	0,0588463	7,424E-07
M3	1,965E-19	0,9942434	0,0057566	6,713E-09
M3	6,752E-17	0,9843213	0,0156663	1,237E-05
M3	1,419E-21	0,9980921	0,0018958	1,209E-05
M3	4,834E-21	0,9895886	0,0104113	9,041E-08
M3	3,12E-18	0,9731784	0,0268202	1,359E-06
M3	6,369E-17	0,9034366	0,0965617	1,63E-06
M3	2,551E-17	0,9870246	0,0129652	1,019E-05
M3	2,035E-18	0,9930393	0,0069606	6,453E-08
M3	3,913E-16	0,9978874	0,0021125	1,081E-07
M3	1,208E-24	0,9633025	0,0366956	1,895E-06
M3	3,805E-14	0,9849108	0,015089	2,071E-07
M6	4,236E-18	0,6057219	0,3942779	1,688E-07
M6	1,679E-20	0,9924621	0,0075378	5,138E-08
M6	9,412E-19	0,9065873	0,0934127	7,455E-08
M6	4,993E-22	0,9769351	0,0230637	1,229E-06
M6	1,918E-15	0,9082668	0,0917332	1,242E-09
M6	5,836E-16	0,4158509	0,5841491	2,591E-08
M6	2,141E-10	0,5241716	0,4758092	1,92E-05
M6	1,561E-20	0,014107	0,9858925	5,112E-07
M6	1,487E-19	0,7607146	0,2392852	1,421E-07
M6	8,378E-17	0,5085889	0,491411	8,367E-08
M6	4,549E-19	0,6386074	0,3613759	1,676E-05
M6	1,072E-14	0,6264771	0,3735229	1,81E-08
M6	1,297E-18	0,9208441	0,0791558	1,629E-07
M6	1,312E-22	0,150095	0,8498354	6,962E-05

M6	9,415E-18	0,8832628	0,1167365	6,994E-07
M6	1,445E-18	0,6406261	0,3593738	6,31E-08
M7	5,096E-15	0,004566	8,632E-05	0,9953477
M7	6,616E-18	0,0029544	1,525E-05	0,9970304
M7	2,348E-26	0,0008844	3,767E-05	0,9990779

Capítulo 2

Elucidating the hidden genetic diversity of *Micrasterias arcuata* (Desmidiaceae, Zygnematophyceae) species complex

ABSTRACT

Micrasterias arcuata Bailey has some extremely problematic taxonomy and nomenclature and presents some wide morphological variation and a great number of often poorly circumscribed infraspecific categories. This study aimed at evaluating the phylogenetic position of strains traditionally assigned to *M. arcuata* based on molecular and phylogenetic approaches and morphometric analyses. Multigene analysis was performed using the nuclear SSU rDNA and both chloroplast *rbc*L and *psa*A markers. Genetic analyses revealed that all three investigated strains of *M. arcuata* are separate within a distinct and monophyletic clade, with considerable support from all species of *Micrasterias* lineage and closely related to *Euastrum* 2 clade. Morphometric analyses exhibited differences among strains, revealing important trends for morphological differentiation and delimitation of infraspecific taxa assigned to *M. arcuata*. All three strains investigated are separate according to their morphological features and represent different taxonomic entities from the molecular and phylogenetic points of view. Current data demonstrated that proposition of *Pseudomicrasterias* gen. nov. is adequate, and that a new lineage belonging to family Desmidiaceae is well recognized. Two infraspecific taxa were erected to the species level and classified as new combinations of *Pseudomicrasterias*, and a new species is recognized.

Keywords: alpha taxonomy, Desmids, geometric morphometric, new genus, phylogeny, polyphasic approaches.

INTRODUCTION

Desmids although does not represent a taxonomic status, is a term or name used to designate a morphologically diverse group of green algae belong the class Zygnematophyceae Round ex Guiry (Streptophyta) (Edward 1869, Brook 1981).

Desmidiaceae is one of the most species-rich families of the Streptophyta, as well as its greatest morphologically diversity (Škaloud *et al*. 2011, Nemjová *et al*. 2011). Its taxonomy and classification are based on solely morphological characters of cell (Gontcharov 2008). Thus, genera and species are differentiated based mostly on cell organization (filaments/ unicells), size and shape, cell symmetry, projection and ornamentations of cell wall and chloroplast structure. For the great majority of the taxa, there are no stability of these taxonomic characters for the classification schemes (Gontcharov & Melkonian 2008, 2010, Hall *et al.* 2008, McCourt *et al.* 2000).

Nowadays, Desmidiaceae family englobe 34 genera and more than 2.500 species validly described, proposed in its great majority during the XIX century, based on exclusively in traditional taxonomy, *i.e*. exclusively on morphological features (Bicudo & Menezes 2017, Gontcharov 2008). The extensive morphological variation documented, even at population level, led to the descriptions of numerous infraspecific taxa, whose taxonomic validity remained largely unresolved (Kouwets 2008, Š'tastný *et al*. 2013). And due to this, it is possible that the real number of infraspecific taxa exceeds more than 5.000 species (Škaloud *et al*. 2012).

Recent molecular studies have been increasingly major changes in the taxonomy of desmids (McCourt *et al.* 2000, Gontcharov 2008, Gontcharov & Melkonian 2008, Hall *et al.* 2008, Neustupa *et al*. 2010, Škaloud *et al.* 2011, Nemjová *et al.* 2011, Š'tastný *et al*. 2013). These advances raised the question about the taxonomic value of many morphological characters, challenging the species concept in Desmidiaceae, and becoming questionable the largely number or the existence of many infraspecific taxa identified on the basis only of traditional taxonomy (Gontcharov & Melkonian 2008, Neustupa *et al*. 2010).

Phylogenetic relationship among genera and closely related species have been performed using a multigene analysis or multiple sequences '*per loci*', that combine various nuclear, mitochondrial or chloroplast markers resulted in an increased phylogenetic resolution and solved some inconsistencies among particular single gene phylogenies, providing a more complete investigation about them (Gontcharov & Melkonian 2004, 2008, Hall *et al*. 2008, Neustupa *et al*. 2010, Š'tastný *et al*. 2013).

Considering the genus *Micrasterias* as one of the most studied and well known inside genera of Desmidiaceae regarding the molecular phylogeny point of view. Recent studies that

used a combination of molecular, morphological, and geometric morphometric methods had revealed differentiation within closely related species or species complexes (Š'tastný *et al*. 2013). These studies had demonstrated that the species richness in *Micrasterias* is higher than previously thought and that several of the traditional taxa described solely by classical taxonomy may represent independent species and other ones shown to be artificial, and probably lacking any taxonomic value (Neustupa *et al*. 2010, 2011, Nemjová *et al.* 2011).

Micrasterias C.Agardh *ex* Ralfs is mainly composed of unicellular individuals, but pseudofilaments may be formed as well, e.g. *Micrasterias foliacea* Bailey *ex* Ralfs (Sormus 1980). Its individual representatives are formed by two identical and 2-radially symmetric semicells with a single polar lobe and several lateral lobes and lobules. The semicells are divided by a narrow isthmus that contains the centrally positioned nucleus (Neustupa *et al.* 2010, Neustupa & Š'tastný 2018).

According to the traditional morphological classification, presence of the first to fourth order lobes has been considered one of the most important characters to define taxa within the genus *Micrasterias* (Škaloud *et al.* 2011). The genus comprises 3-lobed individuals (with three lobes and two interlobular incisions) and 5-lobed individuals (with five lobes and four interlobular incisions) (Krieger 1939, Prescott *et al*. 1977), whose characteristics may be often related to the size, dimensions and the complexity of semicell (Sormus 1980).

Most of *Micrasterias* taxa were described in the 19th century, with many species and several hundreds of infraspecific taxa (Krieger 1939, Ružička 1981). Nowadays, according to Algaebase dataset, this genus comprises 221 species and 754 infraspecific names such as varieties and taxonomic forms (Guiry & Guiry 2020).

Previous phylogenetic studies showed that *Micrasterias* lineage comprises at least eight monophyletic clades that were assigned as A-H (Škaloud *et al*. 2011). This lineage includes taxa with morphology ranging from relatively simple oval semicell shape, without any marked incision, as is the case of *Micrasterias ralfsii* Škaloud *et al*. to extremely complex starlike shape (Neustupa 2016), besides other morphologically divergent species as *Micrasterias dickiei* Škaloud *et al*. (traditionaly named *Staurodesmus dickiei* (Ralfs) S.Lillieroth) and *Triploceras gracile* Bailey, the later having not yet been formally proposed as a member of the *Micrasterias* lineage (Hall *et al*. 2008, Gontcharov & Melkonian 2010, Škaloud *et al*. 2011).

Micrasterias arcuata is a 3-lobed species proposed by Bailey (1851) from material collected in the State of Florida, United States of America (Prescott *et al*. 1977). The species has basal lobes curved upward, concave apical margin, and polar lobe transversely projected (Bailey 1851, Prescott *et al.* 1977). It was already documented in the United States, Canada,

Cuba, West Africa, Asia and South America (Prescott *et al*. 1977, Sormus 1980, Fonseca *et al.* 2019), and in the former Union of Soviet Socialist Republics too (Kossinskaja 1960).

This species displays of a large amount of documented morphological variation (e.g. Nordstedt 1877, Borge 1899, Sormus 1980), that means that it is a complex taxon worthy of interest from both taxonomic and nomenclatural points of view. Beyond the typical variety, this species comprises nowadays 23 infraspecific taxa represented by varieties and taxonomic forms (Bailey 1851, Nordstedt 1877, West & West 1896, 1897, Borge 1899, Prescott & Scott 1943, 1952, Förster 1964, 1969, Guiry & Guiry 2020).

In Brazil, *M. arcuata* is frequently recorded in all Brazilian regions (Borge 1899, 1918, Förster 1964, 1969, Bittencourt-Oliveira & Mecenas 1994, Fonseca & Estrela 2015, Oliveira *et al.* 2017, Santos *et al.* 2018, Ramos *et al.* 2019). And seven varieties from all taxonomic entities recorded for this species, were proposed based on Brazilian material (Borge 1899, Förster 1964, 1969), representing the most taxa proposed for the species.

As in the other parts of the world, the great majority of the studies on *M. arcuata* made in Brazil focused, exclusively, on classical α taxonomy. A few studies were dedicated, at the same time, to its taxonomic revision and distribution (Sormus 1980), polymorphism and ecology (Gil-Gil & Bicudo 2000, Bicudo & Gil-Gil 2003), and a single one to the relationship between ecological characteristics of specific sites and the morphological features of *M. arcuata* populations using the geometric morphometric approach (Fonseca *et al.* 2019).

The present study aimed to evaluate the phylogenetic position of strains of *M. arcuata* species complex based on molecular data in order to test for the monophyly and phylogenetic differentiation of this species complex. In addition, morphological tools as geometric morphometric analyses, traditional morphological methods and Scanning Electron Microscopy (SEM) of cells are goingo to be used to analyze the morphology of the strains. Besides that, possible morphological peculiarities of the among phylogenetic lineages within this investigated complex of species were identified.

MATERIAL AND METHODS

Sampling, isolation and cultivation of strains

Phytoplankton samples were collected in November 2018 using a plankton net with a 20 μm mesh in a small pond covered to a large extent by *Sphagnum* located at the "*Reserva Experimental de Itirapina*" (47º00'52.12" W, 22º12'39" S), Itirapina municipality, São Paulo state, southeast Brazil.

Individual morphotypes traditionally assigned as *Micrasterias arcuata* species were isolated from natural samples by micropipeting. Cultures were cultivated in tubes with approximately 15 ml of MES-buffered DY IV liquid medium (pH ~ 5; Andersen *et al.* 2005) and they were maintained at the temperature of 18-24°C under constant illumination of 40 µmol photons.m^2.s^1 from 18 W cool fluorescent tubes (Philips TLD 18W/33, Royal Philips Electronics, Amsterdam, Netherlands) for approximately 8-10 weeks. Strains were deposited at the Culture Collection of Freshwater Microalgae at Universidade Federal de São Carlos (CCMA – UFSCar, WDCM 835) under the designations: *Pseudomicrasterias arcuata* CCMA-UFSCar 717, *Pseudomicrasterias perforata* CCMA-UFSCar 718 and *Pseudomicrasterias* sp. CCMA-UFSCar 719.

Traditional morphometric analysis

Seven morphological traits were measured from individual cell of each cultured strains of *M. arcuata* species complex. These qualitative morphological data were investigated using a light microscope (Zeiss Axioplan 2 optical microscope equipped with Zeiss Axiocam MRc digital camera and with AxioVision SE64 Rel 4.9 software) under 1000x and 400x magnification. And an inverted microscope (Zeiss Axio Observer D1 with a 2.5 optovar and image capture Axiocam MRc Rev. 3, Imaging Systems 4.7.2 and ZEN Zeiss software v. 2012 software), under 400x magnification.

The picture analyses tools softwares (ZEN Zeiss software v. 2012 and the AxioVision SE64 Rel 4.9 software), were used for the examination of the morphological attributes in each individual and to obtain their microphotographs. The morphological traits were maximum cell length (MCL) - distance of polar lobes at opposite semicells; average cell length (ACL) – distance of polar lobes midpoints at opposite semicells; maximum cell width (MCW) – distance between basal lobes of the same semicell; polar lobes distance (BPL) – distance between polar lobes of the same semicell; basal lobes distance (BLD) – distance between the tips of basal lobes at the opposite semicell. All these measurements were proposed in Bicudo & Gil-Gil (2003). In addition, we measured isthmus width (IW) and height of polar lobe (HPL) – distance between the basal and polar lobes. A schematic figure of these seven morphological attributes analyzed in this study was provided in figure 1A.

Geometric morphometric analysis

For each culture, semicells were investigated and the microphotographs were taken on cultured individuals of *M. arcuata* strains using the same procedure described above for the traditional morphometric analysis. To conduct the analyses, TpsUtil 1.61 software (Rohlf 2015) was used to transform the photomicrographs of specimens into a TPS files format. Then, the software TpsDig 2.32 (Rohlf 2005) was used to digitize landmarks and semi-landmarks on scaled TIFF images.

A total of 49 equidistant landmarks were depicted in the front view of the semicell of *M. arcuata* (Fig. 1B), according to the previous study concerning this species (Fonseca *et al.* 2019). One landmark was positioned on the bilateral symmetry axis of semicell, and the other 48 were bilaterally symmetrically positioned on each side (left and right as mirror images) of the semicell. Thirteen landmarks were considered fixed points, as follows: point number 1, 49 - margin of the isthmus; 7, 43 - basal lobes extremities; 13, 37 - base of basal lobes; 14, 36 - middle point of polar lobes height; 15, 35 - base of polar lobes lateral margins; 19, 31 - extremities of polar lobe lateral margins; 25 - polar lobe central incision (apical margin). The remaining 36 landmarks represented semi-landmarks and were positioned along the semicell outline to minimize Procrustes distances and any apparent shape difference (Zelditch *et al.* 2012).

Landmarks data were imported into RStudio v. 1.2 (Core Team 2019) and opened in the *Geomorph* R-package (Adams & Otárola-Castillo 2013). Data were aligned by Generalized Procrustes Analysis (GPA) using the "*gpagen*" function. And they were symmetrized by calculating the mean of the original coordinates and their reflected, relabeled copies by using the "*bilat.symmetry*" in the same package. The principal component analysis was generated by the "*plotTangentSpace*" function. To visualize the shape variation among individuals in the morphospace. Procrustes coordinates for the mean shape of each morphotype/strain were calculated by "*mshape*" function, and the Thin-Plate Spline deformation grids (TPS-grids) were created and plotted considering the first two PCA axes to allow visualization of the shape changes of morphotypes studied. Linear discriminant analysis (LDA) was conducted using all the non-zero PCA axes in the MASS R-Package (Venables & Ripley 2002) to evaluate the groups discrimination by reassignment probabilities (McLachlan 2004) that were analyzed by leave-one-out cross-validation. The non-parametric MANOVA (Anderson 2001) was also performed to test significant differentiation between morphotypes on the matrix of Euclidean distances in Past v. 2.17 (Hammer *et al.* 2001).

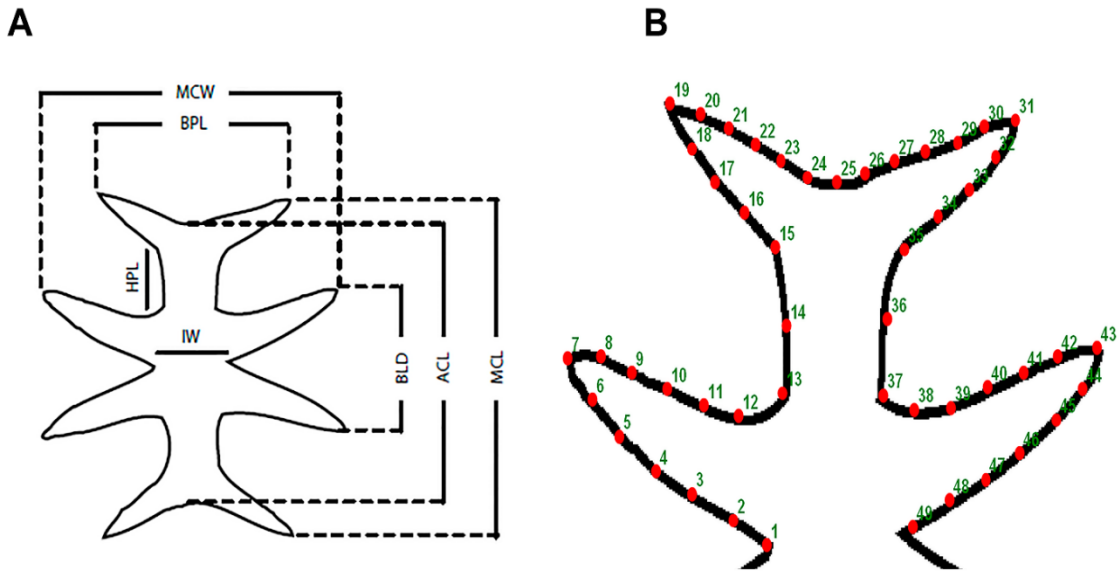

Figure 1. Morphological attributes of *M. arcuata* analyzed in traditional and geometric morphometric analyses. **A.** Morphological attributes analyzed in traditional analyses: Maximum cell length (MCL); Average cell length (ACL); Maximum cell width (MCW); Width of polar lobe or minimum cell width (BPL); Distance between basal lobes (BLD); Height of polar lobe (HPL); Isthmus width (IW); Linear measurements were proposed in Gil-Gil & Bicudo (2000), Bicudo & Gil-Gil (2003); Figure was modified in this study; **B.** Positions of the forty-nine equidistant landmarks depicted on front semicells outline in geometric morphometric analyses

Scanning Electron Microscope (SEM) analysis

For the scanning electron microscope (SEM) analysis, acetone-washed glass coverslips were heated, and coated three times with a poly-L-lysine solution (1:10 in deionized water) to ensure appropriate cell adhesion. A drop of the formaldehyde- fixed cell suspension was transferred into 30% acetone and dehydrated by using an acetone series. Subsequently, the cells were dried to a critical point by using liquid CO2. Finally, they were sputter coated with gold (Bal-Tec Sputter Coater SCD 050, Capovani Brothers Inc., Sconia, NY, USA) and examined using a JEOL 6380 LV (JEOL Ltd., Tokyo, Japan) scanning electron microscope (Š'tastný 2010, Š'tastný *et al*. 2013).

All photomicrographs were digitally manipulated and plates containing light microscopy and scanning electron microscopy images were mounted using Adobe Creative Cloud Photoshop software v. 2013.

DNA isolation, amplification and sequencing

Total genomic DNA was extracted from liquid culture of the *Micrasterias arcuata* strains with InstaGene matrix (Bio-Rad Laboratories, Hercules, CA, USA). The nuclear SSU rDNA and both chloroplast *rbc*L and *psa*A were amplified by PCR in 20 uL reaction volumes: 13.1 µl of sterile Mili-Q water, 2 µl of AmpliTaq Gold 360 Buffer 10x (Applied Biosystems, Life technologies, Carlsbad, CA, USA), 2.2 µl of MgCl2 (25 mM), 0.4 µl of dNTP mix (10 mM), 0.25 µl of each primer (25 nM), 0.6 µl of 360 GC enhancer, 0.2 µl of AmpliTaq Gold 360 DNA Polymerase, and 1 µl of DNA template (not quantified) using the amplification primers listed in table 1 and cycling conditions listed in table 2. The PCR products were purified and cleaned with AMPure (Beckman Coulter, Inc.) purification reagent and sequenced at Macrogen Inc. in Seoul, Korea.

Table 1. List of primers used for PCR amplification and sequencing.

Molecular marker	Primer	Primer sequence (5'-3')	Reference
SSU rDNA	18S-F	AACCTGGTTGATCCTGCCAGT	Katana *et al*. (2001)
SSU rDNA	18S-R	TGATCCTTCTGCAGGTTCACCTACG	Katana *et al*. (2001)
*psa*A	*psa*A 2100R	GGCAATTCCACCCAGAAGG	Hall *et al*. (2008)
*psa*A	*psa*A IF	TTCCTTTGCCTCATGAATTC	Hall *et al*. (2008)
*rbc*L	ZygF	TATGTCAACCACAAAC	Pichrtová *et al*. (2018)
*rbc*L	1385R	GGAAAGAAATTAAATTTGAATT	Manhart (1994) McCourt *et al*. (2000)

Table 2. PCR cycling conditions.

Region	Initial denaturation	Denaturation	Annealing	Extension	Cycles	Final denaturation)
SSU rDNA	94°C (4 min)	94°C (1 min)	52°C (1 min)	72°C (2 min)	35 x	72°C (10 min)
*rbc*L	95°C (10 min)	94°C (1 min)	48°C (1 min)	72°C (2 min)	35 x	72°C (10 min)
*psa*A	94°C (5 min)	94°C (1 min)	52.5°C (1 min)	72°C (1 min)	35 x	72°C (10 min)

Sequence analysis and phylogenetic tree construction

Individual chromatograms were read and edited using the SeqAssem program (Hepperle 2004). The newly three SSU rDNA, *psa*A and *rbc*L sequences obtained in this study and reference sequences of Desmidiales lineages published in previous studies (Hall *et al.* 2008, Gontcharov & Melkonian 2010) were retrieved from GenBank database. The multiple alignment was created and automatically edited using MAFFT 7.3 web interface version (Katoh *et al.* 2002) applying Q-INS-I algorithm strategy and trimmed using Trimal 1.2 (Capella-Gutierrez *et al.* 2009). A concatenated matrix of 4.675 pb (1.705 pb of 18S rDNA, 1.335 pb of *rbc*L and 1.635 pb of *psa*A) contained 95 taxa and was constructed for phylogenetic analysis. Four sequences of Peniaceae was selected as outgroup taxa. The GenBank accession numbers of all strains and sequences involved in the phylogenetic analyses are provided in Supplementary material 1.

The best evolutionary models for SSU rDNA and the 1st, 2nd and 3rd codon position of *rbc*L and *psa*A were determined in jModelTest2 based on the Bayesian Information Criterion (Darriba *et al.* 2012) as implemented in the CIPRES Science Gateway (Miller *et al.* 2010). This BIC-based model selected the General Time Reversible (GTR) substitution with Gamma distribution (G) and estimate of proportion of invariable sites (I) for SSU rDNA, 1st, 2nd and 3rd codon position of *psa*A and 1st and 3rd codon position of *rbc*L. The Hasegawa, Kishino and Yano (HKY) model + G + I was selected for the 2nd codon position of *rbc*L.

Phylogenies were estimated using maximum likelihood (ML) analysis and Bayesian inference (BI) analysis. Maximum likelihood analysis (ML) was performed in RAxML (Silvestro & Michalak 2012) using two independent runs and 1000 pseudoreplicates. For the concatenated dataset 18S SSU rDNA was analyzed as a single partition, whereas *rbc*L and *psa*A were partitioned by codon. All partitions were analyzed using a GTR + I + G model. Bayesian analysis was performed in MrBayes v.3.2.4 (Ronquist & Huelsenbeck 2003) on the CIPRES Science Gateway v.3.3 web portal (Miller *et al.* 2010). Two independent runs were carried out for 8 x 10 $^{-6}$ generations. Chains were sampled every 100th cycle and 25% of the records were discarded as burn-in. Convergence of the MCMC algorithm was assessed by (i) examination of the potential scale reduction factor (PSRF, average=1; maximum 1.012) and (ii) examination of the average standard deviation of split frequencies was lower than 0.01. The resulting tree were visualized using FigTree v. 1.4.3 (Rambaut 2016).

The sequence divergences between different lineages were calculated in MEGA 6.0 (Tamura *et al.* 2013) using p-distance estimation or number of base pair (bp) differences and the percentage was provided by the formula [1– (p-distance)]×100. The mean similarity was

estimated by the average similarity value of each sequence from all compared clades.

RESULTS

Morphology – traditional morphometric analysis

The box plot graphs (Fig. 2) obtained from the linear measurements of seven morphological attributes show clearly differences in the three *M. arcuata* like strains, especially, in their cell size and width.

Individuals of CCMA-UFSCar 717 presented the higher values of maximum cell width (MCW, fig. 2D) and the minimum cell width or width of polar lobe (BPL, fig. 2E), followed by CCMA-UFSCar 718 strain (Figure 2A-B). CCMA-UFSCar 717 presents the higher values of BLD (Fig. 2C) and HPL (Fig. 2G). Whereas CCMA-UFSCar 718 has the highest values of isthmus width (IW, fig. 2F), these were the main differences among these both strains.

Individuals belong CCMA-UFSCar 719 had, proportionally, shorter values of length and wide and consequently, presented higher values of length to wide ration (Fig. 2H). In addition, these individuals have the lowest values of BLD (Fig. 2C) and HPL (Fig. 2G).

Results obtained from NPMANOVA showed statistically significant differences among the strain CCMA-UFSCar 719 from all other both strains currently evaluated according to the traditional morphometric analysis ($F = 57.22$, $p < 0.0001$).

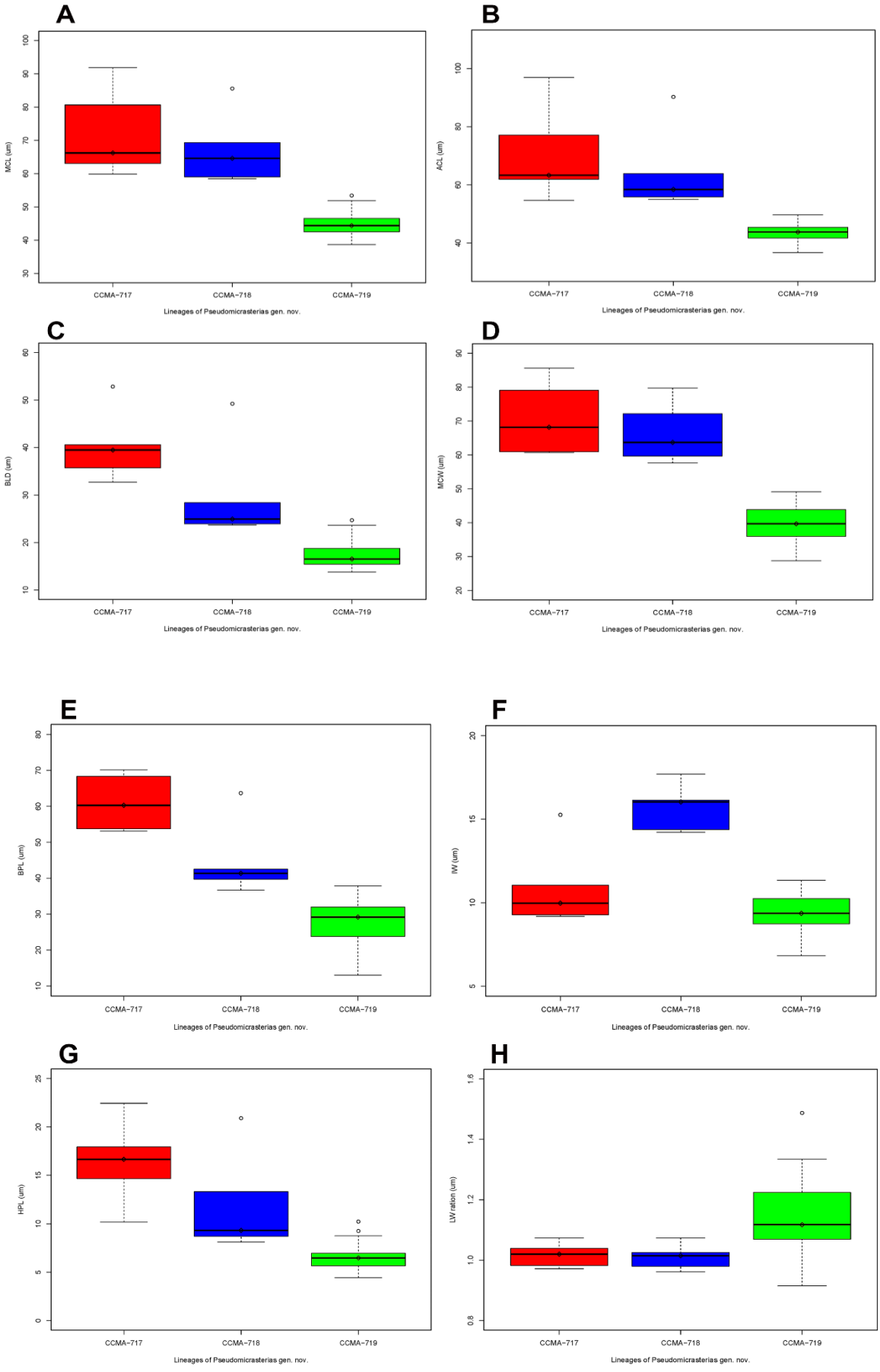
A
MCL (um)
CCMA-717
CCMA-718
CCMA-719
Lineages of Pseudomicrasterias gen. nov.
B
ACL (um)
CCMA-717
CCMA-718
CCMA-719
Lineages of Pseudomicrasterias gen. nov.
C
BLD (um)
CCMA-717
CCMA-718
CCMA-719
Lineages of Pseudomicrasterias gen. nov.
D
MCW (um)
CCMA-717
CCMA-718
CCMA-719
Lineages of Pseudomicrasterias gen. nov.
E
BPL (um)
CCMA-717
CCMA-718
CCMA-719
Lineages of Pseudomicrasterias gen. nov.
F
IW (um)
CCMA-717
CCMA-718
CCMA-719
Lineages of Pseudomicrasterias gen. nov.
G
HPL (um)
CCMA-717
CCMA-718
CCMA-719
Lineages of Pseudomicrasterias gen. nov.
H
LW ration (um)
CCMA-717
CCMA-718
CCMA-719
Lineages of Pseudomicrasterias gen. nov.

Figure 2. Box-plot of minimum, average and maximum cell obtained from seven Morphological attributes analyzed in traditional analyses: Maximum cell length (MCL); Average cell length (ACL); Maximum cell width (MCW); Width of polar lobe or minimum cell width (BPL); Distance between basal lobes (BLD); Height of polar lobe (HPL); Isthmus width (IW). Significant differences tested by NPMANOVA.

Morphology – geometric morphometric analyses

A total of 85 objects among the investigated three strains of *M. arcuata* (14 individuals from CCMA-UFSCar 717; 22 from CCMA-UFSCar 718 and 49 individuals from CCMA-UFSCar 719) were analyzed for geometric morphometric analysis. The principal component analysis of the symmetrized data divided the morphotypes into three groups that are illustrated in the figure 3. The first PC1 axis spanned 63.31% of the total variation and the second PC2 axis spanned 17.40%.

At the negative side of the first PC1 axis and in the negative side of PC2 axis were plotted the individuals belong the strain CCMA-UFSCar 717 (Figs. 3B-E). These individuals had relative longer polar lobe with its lateral extremities slightly to strongly curved downward. Apical margin straight to slightly concave, with both sides flattened at their base. Basal lobes were conical and slender, with its upper margin slightly straight at the base, and its extremities strongly curved upward (Figs. 3C, E). Some individuals presented longer polar lobe, with its extremities shorter and slightly curved downward, and apical margin convex. The basal lobes are conical and straight (Fig. 3B) or just curved downward (Fig. 3D).

Individuals of CCMA-UFSCar 718 were plotted at the positive extreme of PC1 axis to negative side of PC2 axis (Figs. 3H, J-K). These individuals were characterized by relatively shorter polar lobes, concave apical margin with both sides flattened at the base. Lateral extension of polar lobes is shorter, faintly straight to slightly curved upward (Fig. 3J), and in some cases, it is slightly curved downward (Figs. 3H, K). Basal lobes were conical, rounded, swollen and straight at the base.

Individuals of CCMA-UFSCar 719 (Figs. 3F-G, I, L-N) presented a wide morphological variability and they were plotted in the first, third and fourth quadrants of PCA. The shape of these individuals is characterized by compact cells with shorter polar lobes, and apex slightly notched (Figs. 3I, L) to slightly curved upward on its extremities, in this latter case, the apical margin is slightly concave (Figs. 3M-N). Some individuals showed conical basal lobes, swollen at the base and its extremities slightly straight (Figs. 3I, L-N) or slightly curved upward (Figs. 3F-G). Other individuals exhibited short polar lobe, apical margin concave with both sides

flattened at the base and lateral extremities of polar lobe slightly curved downward. Basal lobes were conical and straight at the base and its upper margin had the extremities slightly curved upward, and its bottom margin is slightly subsigmoid (Figs. 3F-G).

Considering the linear discriminant analysis (LDA), all the strains were separated from each other (Fig. 4). And according to the correct classification/ prediction, all of them had 100% of correct classification (Supplementary material 2).

Results obtained from NPMANOVA showed statistically significant differences among the three strains currently evaluated according to the geometric morphometric analysis ($F = 42.80$, $p < 0.0001$).

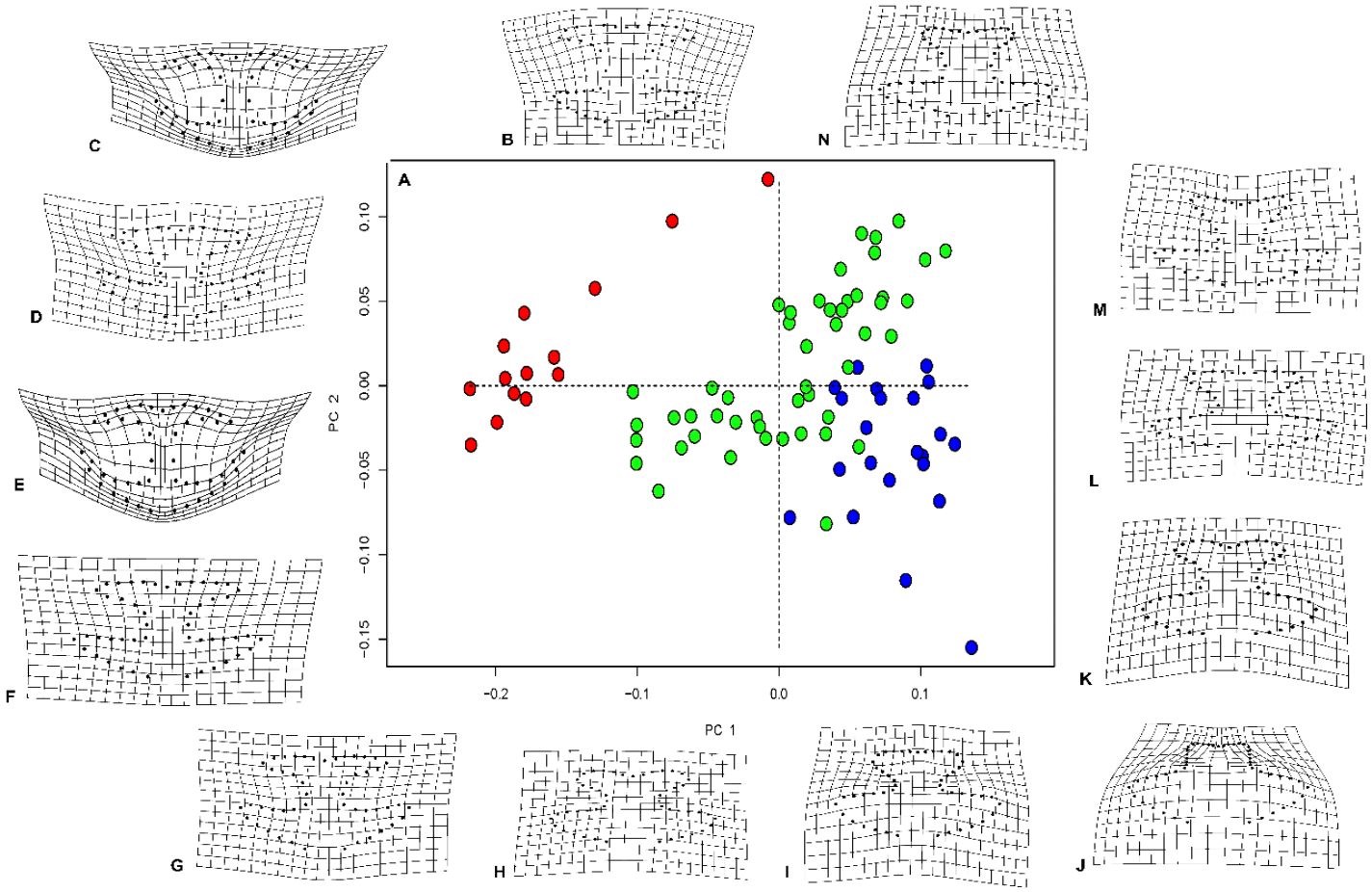

Figure 3. A. Principal component analysis showing the separation of *Micrasterias arcuata* strains across morphospace and its relationship with grid deformation or thin plate splines. PCA1 axis 63.31% and PCA2 axis = 17.40%. **B-E.** CCMA-UFSCar 717: red circles; **H, J-K.** CCMA-UFSCar 718: blue circles; **F-G, I, L-N.** CCMA-UFSCar 719: green circles.

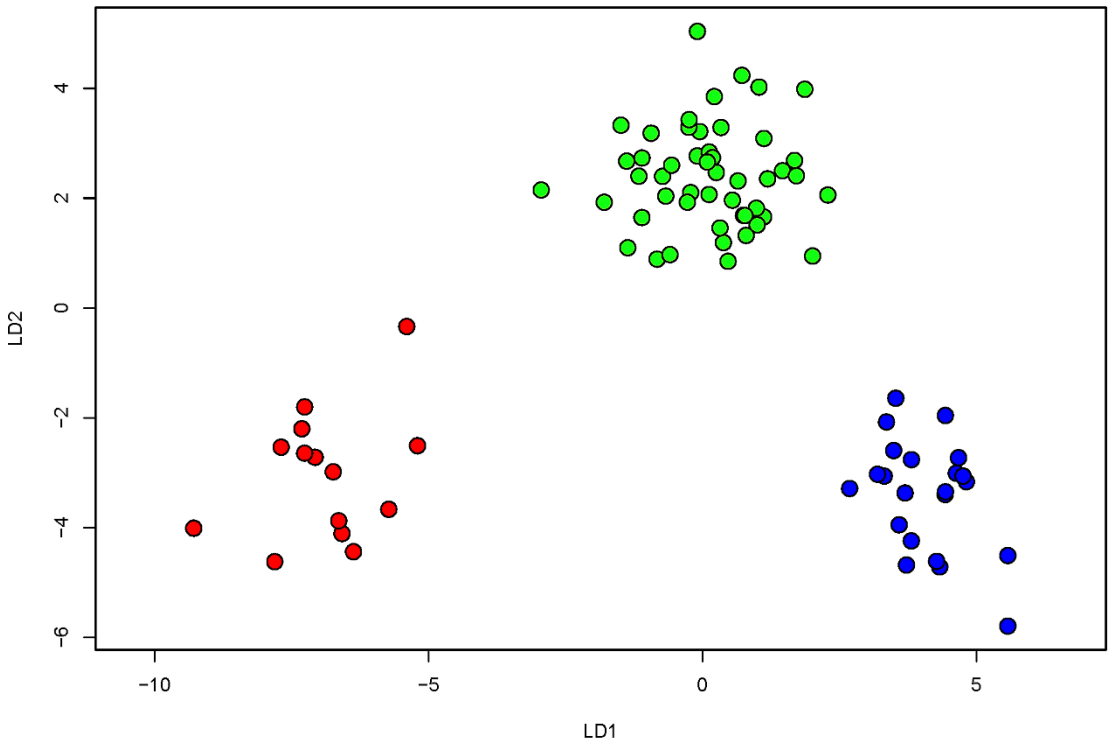

Figure 4. Linear discriminant analysis (LDA) of *Micrasterias arcuata* strains on geometric morphometric analysis. CCMA-UFSCar 717: red circles; CCMA-UFSCar 718: blue circles; CCMA-UFSCar 719.

Principal component analysis (PCA) was also performed in both dataset (85 individuals from three strains presently studied and 30 individuals from classical literature) to evaluate the relationship between them (Fig. 5). The first PC1 axis spanned 61% of the total variation and the second PC2 axis spanned 17.5%.

According to the correct classification/ prediction (Supplementary material 3), individuals from the strain CCMA-UFSCar 717 were assigned in its great majority with taxa traditionally identified as *Micrasterias arcuata* var. *arcuata* Bailey or *Micrasterias arcuata* var. *gracilis* West & West. Individuals from the strain CCMA-UFSCar 718 was associated with specimens identified as *Micrasterias arcuata* var. *perforata* Förster, *Micrasterias arcuata* var. *borgei* Förster, *Micrasterias arcuata* var. *robusta* and *Micrasterias arcuata* var. *robusta* f. *goyasensis* Förster. Whereas individuals from the strain CCMA-UFSCar 719 was related to *Micrasterias arcuata* var. *cornuta* Förster, *Micrasterias arcuata* var. *robusta* f. *goyazensis* Förster, *Micrasterias arcuata* var. *subcornuta* Förster, *Micrasterias arcuata* var. *subpinnatifida* f. *gracilis* Förster, *Micrasterias arcuata* var. *subpinnatifida* West & West form Borge, *Micrasterias arcuata* var. *subpinnatifida* f. Förster, *Micrasterias arcuata* Bailey.

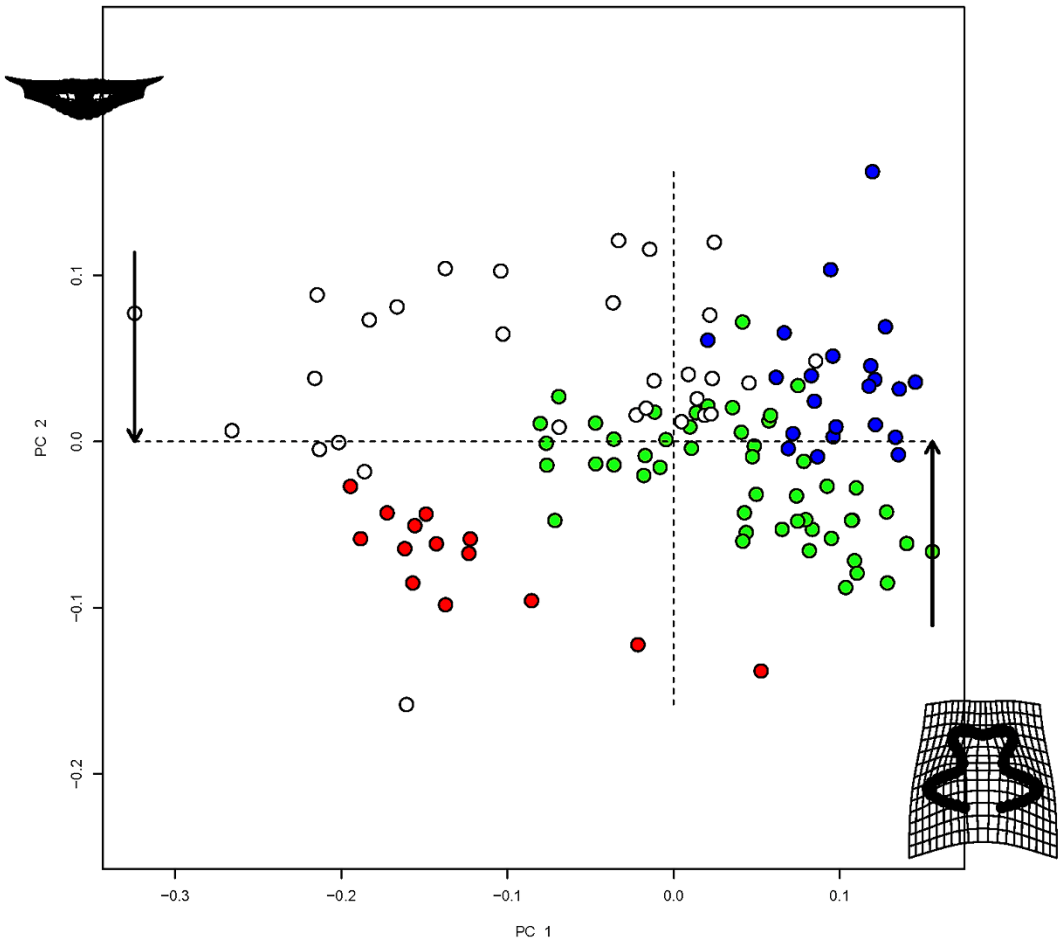

Figure 5. Principal component analysis showing the separation of *Micrasterias arcuata* strains across morphospace. PCA1 axis = 61% and PCA2 axis =17.5%. CCMA-UFSCar 717: red circles; CCMA-UFSCar 718: blue circles; CCMA-UFSCar 719: green circles. White circles represent the position of the iconotypes from the literature data.

Molecular data and phylogeny

The concatenated final alignment contained 4.675 nucleotides characters of 95 taxa and the analysis of the single gene SSU rDNA, *rbc*L and *psa*A (data not show) of the three *Micrasterias arcuata* like strains revealed that they were separated from all other *Micrasterias* lineage members (with p-distance values of 2.6% and 97.38 % in matrix of similarity for SSU rDNA, see Supplementary material 4 and Supplementary material 5) and with p-distance values of 7.0 % and 92.3% in matrix of similarity for *rbc*L, see Supplementary material 6 and Supplementary material 7). Otherwise, *M. arcuata* were closely related to *Euastrum* 2 lineage as a sister group with well-supported bootstrap values (54%), posterior probabilities (0.71) and p-distance values of 1.6% and 98.63% in in matrix of similarity for SSU rDNA and 6.0% of p-distance values

and 93.97 in matrix of similarity for *rbc*L. Besides that, the strains CCMA-UFSCar 717, CCMA-UFSCar 718 and CCMA-UFScar 719 were grouped within a distinct clade designated from now as *Pseudomicrasterias* gen. nov. (Fig. 6).

Pseudomicrasterias gen. nov. comprises two major clades well-supported by bootstrap values and/ or posterior probabilities (100/ 1.0, respectively) for the concatenated dataset of SSU rDNA, *rbc*L and *psa*A genes and according to the single gene analysis (data do not show). The first clade consisted of the single strain number CCMA-UFSCar 718, that showed a well-supported difference for the other clade with p-distance values of 0.4 % for SSU rDNA and a well-supported value of p-distance = 5.5-5.8% for *rbc*L (Supplementary material 8 and Supplementary material 9). The second clade was divided into two distinct subclades highly supported by bootstrap values and/ or posterior probabilities (100/ 1.0, respectively), and it comprised the other two strains, CCMA-UFSCar 717 and CCMA-UFSCar 719. These two sequences showed a well-supported but small difference (p-distance value) = 0.3 for SSU rDNA and a well-supported p-distance value of 1.3% for *rbc*L (Supplementary material 8 and Supplementary material 9).

DISCUSSION

Recent studies based on polyphasic approaches have evaluated the validity of the Desmidiaceae species concept, especially for species of the genus *Micrasterias* (Neustupa *et al*. 2010, 2011, 2014, Nemjová *et al*. 2011). In the present study, a multigene analysis was performed using the nuclear SSU rDNA and both chloroplast *psa*A and *rbc*L markers. And a total of three strains of subspecific taxa of *M. arcuata* species were well stablished by genetic analysis and morphological criteria.

Genetic analysis revealed that the three strains analyzed are separated from all species, presently analyzed, of *Micrasterias* lineage, and closely related to *Euastrum* 2 clade. These strains were grouped within a distinct clade designated here as *Pseudomicrasterias* gen. nov. (see taxonomical changes below) that comprises two well-supported major clades. The first clade comprises the strain CCMA-UFSCar 718 and the second one comprises the strains CCMA-UFSCar 717 and CCMA-UFSCar 719.

In agreement with the molecular and phylogenetic analysis, the morphometric analyses (traditional and geometric morphometric) showed a clear differentiation of all these three strains throughout the linear measurements, the shape of the semicell and by the SEM imagens.

The results of the geometric morphometric approach, in accordance with the Thin-Plate

Splines plots (or grids of shape deformation, figs. 3A-N) revealed that the polar lobes width, apical margin incision depth, and basal lobes curvature were the most important trends for morphological differentiation and delimitation between the strains. Whereas MCL, BLD, IW and HPL were important features as conventional criteria or traditional morphometric analysis (Fig. 2, table 3). These patterns obtained from the current data are in good agreement with some already recorded attempts in the classical literature referring to *M. arcuata* infraspecific taxa and their current descriptions (West & West 1897, Borge 1899, Förster 1964).

Individuals from the strain CCMA-UFSCar 718 were the most dissimilar of all them and had longer length and more robust cells. They were solitary in adult phase (Figs. 8A-E, K-L) or, sometimes, they were grouped while in cell development (Figs. 8F-J). In this latter case, they had the shape of the whole cell completely different from the adult or mature cells such as elliptical, oblong, but rarely circular.

The main morphological variability observed in these specimens were in the shape of the lateral projections of polar lobe, that can be slightly directed to upward or parallel to the basal lobes. Some individuals, while in growth, presented the lateral extremities of polar lobe are shorter (reduced), broadly conical or rounded, and the apex was straight. In this latter case, basal lobes could be rounded too. Besides that, they have cell wall strongly punctuated with tick pores (Figs. 11A-F).

Individuals of CCMA-UFSCar 718 were identified by traditional taxonomic criteria as *M. arcuata* var. *perforata* Kurt Förster & F.Eckert. According to the results of phylogenetic analysis performed in this study, it comprises a distinct and well supported (1.0 / 100 of posterior probabilities and bootstrap values, respectively) subclade within *Pseudomicasterias* genus. Thus, this taxon is named here as a new combination and it should be considered from now as: *Pseudomicrasterias perforata* (Förster & Eckert) comb. nov. C.B. Araújo (see taxonomical changes below).

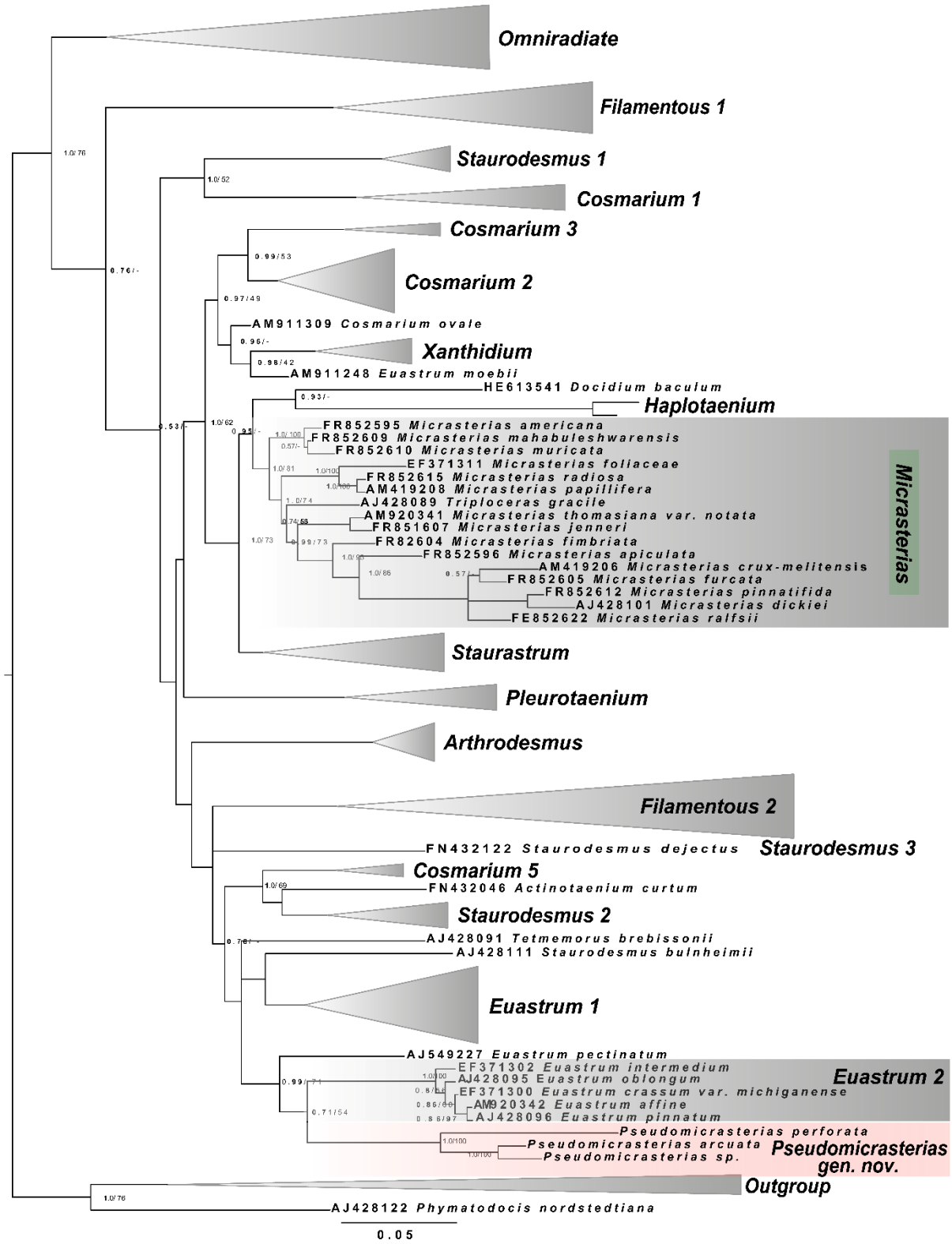

Figure 6. Phylogenetic tree reconstructed with the maximum-likelihood (ML) analysis of the concatenated data set of SSU rDNA, *rbc*L and *psa*A. Nineteen (19) major clades of Desmidiaceae clades sensu Gontcharov and Melkonian (2011) is indicated in the combined analysis. The tree was rooted with *Penium* spp. sequences as outgroup. Values at nodes indicate statistical support estimated by two methods – MrBayes posterior probability (left) and

maximum likelihood bootstrap (right). The posterior probabilities lower than 0.70 and bootstrap support levels below 50% are omitted. Scale bar – estimated number of substitutions per site.

In addition, some of the morphological variation recorded here is in accordance with the descriptions of the two forms of var. *perforata* proposed by Förster (1964). The f. *robustior* was collected in Serra das Almas, Bahia, Brazil, that was frequently recorded together with the typical variety. Whereas f. *magniperforata*, is a rare taxon recorded from material collected in Rio das Fêmeas, Goiás state, Brazil. The main difference among these two forms and the typical one is in the shape of the polar lobe. Therefore, the typical variety has the polar lobes apices straight whereas the f. *magniperforata* has the polar lobes curved upward, and f. *robustior* comprises smaller and compact individuals, with polar lobes short and robust. However, during the present study, all these morphological variations were observed simultaneously in the strain CCMA-UFSCar 718. This suggests that they may represent the same entity of var. *perforata*.

The relationship between the morphological data and the literature ones, and according to some remarks on classical taxonomy, revealed that, sometimes, specimens of var. *perforata* remind representative individuals of *M. arcuata* var. *robusta*. This similarity is attributed to the shape of their polar lobes and the swollen and robust basal and polar lobes. However, according to literature (Förster 1964), specimens of var. *perforata* display greater cell length and width, and cell wall strongly punctate than *M. arcuata* var. *robusta* being completely different entities.

According to the results obtained in phylogenetic analysis performed in this study, CCMA-UFSCar 717 is within a well-supported (1.0/ 100 values of posterior probabilities and bootstrap values, respectively) distinct subclade of *Pseudomicrasterias* genus. These individuals had long length and wide, and they were characterized by slender and compact cells with apical margin varying from faint concave to slight straight and lateral extension of polar lobes curved downward. Besides that, they exhibited basal lobes curved upward towards the polar lobes, and the sinus is widely open (figs. 7A-H, table 3).

These features agree with the circumscription of *M. arcuata* type species proposed by Bailey (Bailey 1851), as well as with the other specimens identified as *M. arcuata* var. *arcuata* (Förster 1964, 1969). Thus, the strain CCMA-UFSCar 717 were identified as *Micrasterias arcuata* var. *arcuata* Bailey and named here from now as a new combination: *Pseudomicrasterias arcuata* (Bailey) comb. nov. C.B Araújo (see taxonomical changes below).

This species varies morphologically during the cell development, especially, in the shape of its basal lobes and in the lateral extremities of polar lobe. Basal lobes can be reduced or

shorter in immature individuals, and sometimes they can be swollen and rounded in their extremities. The apex of the semicell were, sometimes, straight and rounded or just rounded (without lateral extension, figs. 7I-K). It was also observed individuals with polar lobe elongated, median constriction shallow (but it is visible) and the apex straight in the middle region and lateral extension of polar lobe subconical, divergent, reduced and slightly curved down (Figs. 7S-T). These individuals do not show basal lobes in development, and they can remember, morphologically, individuals that belong to the genus *Ichthyocercus* West & West.

According to the classical taxonomy, the original material examined by Bailey (1851) is broader than long and presents slender and upward curved basal lobes, which sometimes can resemble specimens of *M. arcuata* var. *gracilis* West & West. Despite of that, Thomasson (1971) questioned the existence of *M. arcuata* var. *gracilis* stating that it should be better considered a morphological expression of *M. arcuata* var. *arcuata*.

M. arcuata var. *gracilis* was formally proposed by West & West (1896), the authors referred to the material identified by Nordstedt (1877) as *M. arcuata* var. *arcuata*. However, *M. arcuata* var. *gracilis* has very slender cells, polar lobe subcylindrical and longer, apical margin very concave and flattened on both sides, and the extremities of polar lobe were very slender and curved downward. Besides that, basal lobes were longer and straight, and oriented or directed to upward (not curverd). These were the main differences observed among var. *arcuata* and var. *gracilis*.

Individuals from the strain CCMA-UFSCar 719 comprise the second well-supported subclade (1.0/ 100 of posterior probabilities and bootstrap values, rspectively) of *Pseudomicrasterias* genus together with *Pseudomicrasterias arcuata* species (or specimens of CCMA-UFSCar 717). These individuals presented a wide morphological variability during the cell development, especially, in the shape of the extremities of polar lobe and in the shape of basal lobes (Figs. 9A-T, table 3). The apex of semicell can be straight or rounded or just rounded (without lateral extension, figs. 9F, H, N-O), and the basal lobes were swollen and rounded or broadly conical. The lateral extension of polar lobe can be slightly curved to upward (in individuals in development or growth), slightly curved downward, or parallel to basal lobes (Figs. 9Q-T).

Considering the remarks on classical taxonomy, these individuals presented comparable cell morphology, cell shape and size with individuals relatively small, with shorter length and width, especially shorter polar lobe as described in *M. arcuata* var. *cornuta*, *M. arcuata* var. *subcornuta, M. arcuata* var. *subpinnatifida* and its some taxonomic forms, and as *Micrasterias arcuata* var. *robusta* f. *goyazensis* that has cells swollen and the extremities of basal lobes

slightly curved upward.

However accordingly to the linear discriminant analysis (LDA) and posterior probabilities analysis, specimens of this strain can be morphologically similar with individuals assigned as var. *robusta* proposed in Förster's material (Förster 1964, 1969) especially when those individuals were in development and presents immature cells. However, the main difference between individuals of CCMA-UFSCar 719 and specimens of var. *robusta* is that this latter one has basal lobes uniformly rounded, so they were open at an acute angle and straighter in the isthmus region, besides that it has the extremities of polar lobe arched upward.

Individuals of CCMA-UFSCar 719 could be morphologically similar with the figure 9 of *Micrasterias arcuata* var. *compacta* Förster, described in Förster's material (Förster 1969). In this case, individuals analyzed probably were in adult phase and have polar lobe slightly more elongated, and basal lobes swollen at their base, and its extremities slenderer and slightly curved upward. Besides that, these individuals can present the apical margin straighter to slightly concave in the middle region, with the extremities of polar lobe flattened in both sides.

Differently of the individuals with remarkable morphological differences in their size or shape, as it was observed in *Pseudomicrasterias arcuata* (CCMA-UFSCar 717) and *P. perforata* (CCMA-UFSCar 718), both erected to species level and proposed in this study as a new combination, individuals of CCMA-UFSCar 719 are morphologically extremely variable during their cellular cycle or growth, being similar with two taxonomic units described in literature. Although the morphology of the iconotypes, presently analyzed, reflect the real morphology of the cell and descriptions, they were collected just one more time on each locality without systematic sampling (Förster 1964, 1969) being impossible to know about their development in nature conditions, or even, if they represent the same or distinct taxonomics units. Due to this, individuals of CCMA-UFSCar 719 strain were identified in the present study as *Pseudomicrasterias* sp., see taxonomical descriptions below.

Sometimes *Pseudomicrasterias* sp. can resemble specimens assigned as the type species *P. arcuata*. However, the main difference presently observed in both species is in the height of polar lobe (HPL values) that in *Pseudomicrasterias* sp. is subcylindrical and shorter and in *P. arcuata* is subylindrical and extended. The other difference observed is in the distance between basal lobes (BLD values, table 3) of the more arched upward than *Pseudomicrasterias* sp..

Pseudomicrasterias gen. nov. and its species presented some morphological differences when compared with some other species of *Micrasterias* genus or lineage, as follows: cells 3-lobed, with lateral lobes attenuated and basal lobes parallel to arcuate; semicell is characterized by one polar and two lateral lobes, the latter ones were never divided into

lobules. In this study, the size of *Peseudomicrasterias* species were lower than 100 µm long and 90 µm wide. According to the literature data published of *M. arcuata*, the infraspecific taxa illustrated (*iconotypus*) had the size lower than 130 µm long.

Considering the cell wall ornamentation, species of *Pseudomicrasterias* gen. nov. have cell wall finely punctuated to strongly punctuated ones (Figs. 10, 11, 12 and table 3) and do not have spines or other type of ornamentation. Otherwise, specimens of *Micrasterias* lineage may present the cell wall very elaborated and ornamented, some species presented prominent spines in the apical margin of semicells, like in *Micrasterias fimbriata* Ralfs (Neustupa *et al*. 2011), or more than one spine or tooth at the extremities of basal and lateral lobes like in *Micrasterias pinnatifida* Ralfs, a 3-lobed species characterized by one polar and two lateral lobes (lateral extremities), never divided into lobules, that is morphologically very similar to *Pseudomicrasterias* species.

Considering particularly *Micrasterias pinnatifida*, this species is phylogenetically placed into clade A4, together with *Micrasterias furcata* C.Agardh *ex* Ralfs and *Micrasterias dickiei* within *Micrasterias* lineage (Škaloud *et al*. 2011). The main morphological differences between this species and species of *Pseudomicrasterias* gen. nov. (or the other ones designated as *M. arcuata* species complex) is in the apical margin, that is straight to slightly convex, in the the height of the polar lobe, the intumescence of the lateral lobes, and especially, by the presence of 2 spines or tooth at the end of polar and basal lobes in *M. pinnatifida* (Ralfs 1848).

Dixon (1859) proposed the genus *Tetrachastrum* and formally transferred *Micrasterias ocitans* Ralfs (*Tetrachastrum ocitans* R.V.Dixon) and *M. pinnatifida* Ralfs (*Tetrachastrum pinnatifidum* R.V.Dixon). *Tetrachastrum* was divided into two subsections: (1) individuals with lobe tips entire, mucronate or acute and (2) individuals with lobe tips bidentate (Pritchard 1861).

Taxa of *Micrasterias arcuata* (*M. arcuata* Bailey and *M. espansa* Bailey) were classified by Pritchard (1861), despite of not formally, into subsection one of *Tetrachastum*. And Krieger (1939) considered *Tetrachastrum arcuatum* R.V.Dixon and *Tetrachastrum expansum* synonyms of *M. arcuata* and *Micrasterias expansa* Bailey respectively.

According to Dixon (1859) and Pritchard (1861), there are some differences between the genera *Tetrachastrum*, *Micrasterias* and *Euastrum*. *Tetrachastrum* was only composed by 3-lobed segments and the other two genera by 3-5-lobed segments. The main difference, however, is in the typical cell division process, especially between *Tetrachastrum* and *Micrasterias*, that the direction of the individual semicells separation line is parallel and radial, respectively. Nowadays, *Tetrachastrum* is considered a heterotypic synonym for *Micrasterias* and all taxa classified into it are presently within the last genus or lineage (Guiry 2013).

Considering the closely related phylogenetic position of *Pseudomicrasterias* clade with some *Euastrum* taxa (*Euastrum* 2 clade, e.g. *Euastrum affine*, *Euastrum oblongum*) recorded in this study, all these both comprising 3-lobed species. The main differences among specimens of *Pseudomicrasterias* and *Euastrum* 2 clade was that this latter one has pyramidal semicells in outline with rather deep incurvations separating apical, lateral and basal lobes. The basal lobes were incurved too and showed rounded lateral lobes entire or sinuate emarginated (Pritchard 1861) and has an apical incurvation in the middle region is in the form of a deep, closed invagination (Prescott *et al.* 1977).

Studies focusing on the family Desmidiaceae and its species complexes (Neustupa *et al*. 2010, Nemjová *et al*. 2011, Š'tastný *et al*. 2013) have been showed that the phylogenetic relations of some of these species are in congruence with the morphological taxonomy, and due to that, in some cases, the phylogenetic species lineages corresponded exactly to the more accurately identification of traditional subspecific taxa (Nemjová *et al*. 2011).

The results of the present study revealed a pattern similar to those studies above mentioned and showed that the genetic diversity existing in *M. arcuata*-like strains, in addition to confirm their heterogeneity. As it was observed in those mentioned studies, some traditional morphological species (including the species complex) could be recognizable purely based on morphological analyses, by using morphometric methods, SEM images or even by traditional morphometry (Neustupa *et al*. 2010, 2014, Nemjová *et al*. 2011, Š'tastný *et al*. 2013).

The results concerning the phylogeny of the family Desmidiaceae have been indicated that the number of clades may exceed the number of genera currently recognized for the family (Gontcharov & Melkonian 2010). Nowadays, from the analysis of 291 *rbc*L sequences representing 23 traditional desmid genera recovered 34 clades/ lineages/ branches in this family. However, from that large dataset only 22 clades are well-supported, and eight single branch sequences remained unassigned (Gontcharov & Melkonian 2010).

In the present study, 92 sequences were downloaded to encompass the phylogenetic analysis here performed. Thus, a total of 20 well defined clades/ lineages/ branches, in this case, considering outgroup and a new clade corresponding the new genus (*Pseudomicrasterias* gen. nov.) proposed here were showed, and seven single branch sequences. Precisely in the case of *Pseudomicrasterias* gen. nov. short branches were observed, particularly in the subclade comprised by *P. arcuata* and *P. forsterii*, that present taxa with rather uniform morphotypes. Similarly, this fact was observed, for example, in *Arthodesmus* clade and *Euastrum* 2 clade (Gontcharov & Melkonian 2010).

Although previous studies were focused on resolving phylogenetic relationship above the

family level or were confined to a particular genus or a group of putatively related genera (Gontcharov & Melkonian 2010), recent studies have been focusing on resolving phylogenetic relationship in species complexes or closely related species. These latter ones have been showed important attempts for the knowledge of several infraspecific taxa, and several traditional varieties or taxonomic forms (Neustupa *et al*. 2010, Nemjová *et al*. 2011, Š'tastný *et al*. 2013). Even the traditional taxonomy or the know about phenotypic characters apparently do not reflect the phylogenetic relationships among species in Desmidiaceae (Gontcharov & Melkonian 2008, 2010).

On the other hand, little is recognizable about the phenotypic characters or the influence of environmental variables in the development (in nature and in experimental conditions) of some species of Desmidiaceae. For experimental conditions, desmids may exhibit high phenotypic variation affected by factors like changing environmental conditions in temperature (Neustupa *et al.* 2008) and pH (Černá & Neustupa 2009). As for some species have been portrayed a clearly geographic predisposition, where in some cases, strains and the species diversity investigated were closely related to their geographic distribution (Neustupa *et al*. 2010, 2011, Nemjová *et al*. 2011).

Few isolates (just three strains) were presently analyzed from one bog located in São Paulo State, Brazil. There are no strains or isolates of *M. arcuata* specimens in public culture collections around the world, and there are no DNA sequences of this species available. Considering the geographical distribution of *M. arcuata* around the world and its documented morphological variation, it is possible that exist a geographic preconception for this species and a greater genetic diversity in morphologically similar taxa too.

Present study demonstrated the usefulness of SSU rDNA, *rbc*L and *psa*A markers for resolution of the infraspecific diversity within *M. arcuata* species complex. Furthermore, that the use of traditional morphometric analysis and geometric morphometric methods together may be a powerful tool for a taxonomic revision for this species complex. Thus, the three strains of *M. arcuata* taxa presently analyzed are perfectly apart according to their morphological features and represent different taxonomic entities from the molecular and phylogenetic point of view and due to this, the proposition of *Pseudomicrasterias* gen. nov. is very adequate.

Moreover, the real diversity of *M. arcuata* species complex probably be much greater than is presently known. Especially in the case of the other taxa described in literature that are morphologically closer and show similar shape and measurements overlapping that, and at this moment, have unclear taxonomic value.

Consequently, biological delimitation to enhance the species concept in some species

complex is extremely necessary and may be carried with the combination of different approaches, including ecological or experimental studies too. Exceptionally, in the case of the family Desmidiaceae, where these investigations may consequently help to reveal which of those traditional taxa will indeed mean taxonomic meaningful units (independent taxa) or conversely will lack taxonomic value, or just represent an ecomorphas.

Genera and species descriptions

Class Zygnematophyceae

Order Desmidiales

Family Desmidiaceae

***Pseudomicrasterias* C.B.Araújo gen. nov.**

Single, bilaterally symmetrical cells. Semicells 3-lobed; polar lobe T-shaped, base subcylindrical, expanded into lateral projections on each side; apical margin concave or slightly retuse; basal lobes conical, divergent, straight or curved upward; median constriction deep, sinus open, acute; chloroplast axial following the shape of the semicell, several to many pyrenoids. Semicell fusiform in vertical view. Both basal and polar lobes ending in a conical tooth.

TYPE SPECIES*: Pseudomicrasterias arcuata* (Bailey) C.B.Araújo *comb. nov.*

ETYMOLOGY: From Greek *Pseudo* (false) and *Micrasterias* genus of the family Desmidiaceae, where the species, including the type-species, of this new genus were previously described.

***Pseudomicrasterias arcuata* (Bailey) C.B.Araújo *comb. nov.* (Figs. 7A-T and figs. 10A-F)**

BASIONYM: *Micrasterias arcuata* var. *arcuata* Bailey, 1851, Smithsonian Contributions to Knowledge: 37.

SYNONYM: *Tetrachastrum arcuatum* Dixon, 1859, The Natural History Review and Quarterly Journal of Science: 725.

HOLOTYPE: *Micrasterias arcuata* var. *arcuata* Bailey 1851: 37, pl. 1, fig. 6.

TYPE LOCALITY: 6 miles from Pilatka Lake, Florida State, USA.

GENBANK ACCESS NUMBER: MW678848 from SSU rDNA, MW686753 for *rbc*L and MW686756 for *psa*A. Phylotype differs from *P. perforata* and *Pseudomicrasterias* sp. regarding 18S rDNA, *rbc*L and *psa*A phylogenies.

Description based on light microscopy. Vegetative cell as long as broad; median constriction deep, sinus acute to widely open; polar lobe subcylindrical; lateral projections slightly arcuate downward; apical margin flat, often somewhat elevated on either side of the middle, sometimes retuse; basal lobes conical, slender to very slender, sometimes straight at the base, arcuate upward towards the lateral projection of the polar lobe; both basal lobes and the polar lobe projections ending in a conical, small tooth. Axial chloroplast following the shape of the semicell, with several pyrenoids. Vertical view narrow and fusiform. Zygospore not observed, but other sexual reproduction stages such as germling cells and pre-vegetative cells were observed. Asexual reproduction commonly observed.

Description based on Electron Microscopy. According to SEM, this species has cell wall with fine pores.

REMARKS: A formaldehyde fixed sample of strain CCMA-UFSCar 717 is deposited in the Herbário Científico do Estado "Maria Eneyda P. Kauffmann Fidalgo" (SP), at the Instituto de Botânica, São Paulo, São Paulo state, Brazil, under access number SP400043. Authentic strain CCMA-UFSCar 717 material is maintained at the Culture Collection of Freshwater Microalgae, Universidade Federal de São Carlos (CCMA-UFSCar), São Carlos, São Paulo state, Brazil.

***Pseudomicrasterias perforata* (Förster) C.B.Araújo *comb. nov.* (Figs. 8A-L and figs. 11A-F)**

BASIONYM: *Micrasterias arcuata* Bailey var. *perforata* Förster, 1964, Hydrobiologia: 373.

SYNONYM: *Micrasterias arcuata* var. *perforata* f. *magniperforata* (Förster). Förster, 1964: 373. *Micrasterias arcuata* var. *perforata* f. *robustior* (Förster). Förster, 1964: 373- 374.

HOLOTYPE: *Micrasterias arcuata* var. *perforata* Förster, 1964: 373, pl. 13, fig. 15.

TYPE LOCALITY: Serra das Almas (limit between Bahia state and Minas Gerais state, Brazil).

GENBANK ACCESS NUMBER: MW678849 for SSU rDNA and MW686754. Phylotype differs from *P. arcuata* and *Pseudomicrasterias* sp. regarding 18S rDNA and *rbc*L phylogenies.

ETYMOLOGY: The species epithet is based on morphological feature of the cell wall "strongly punctate" with lines of some very conspicuous pores.

Description based on light microscopy. Vegetative cells as long as broad; median constriction deep, sinus acute, sublinear to open; polar lobe subcylindrical, relatively short and robust; lateral projections of polar lobe subconical, sometimes slightly directed upwards, sometimes parallel to the basal lobes; apical margin concave in the mid region; basal lobes broadly conical, lateral projections extending horizontally, rare slightly curved upward; both

polar and basal lobes ending in a short, robust tooth. Axial chloroplast following the shape of the semicell, several pyrenoids. Vertical view fusiform. Although zygospore was not observed, some sexual reproduction stages were observed such as its germination phase with releasing of a germination vesicle containing two sister gones, and pre-vegetative cells.

Description based on Electron Microscopy. According to SEM, the species cell wall is strongly punctate, with thick pores. There is a series of 10 thick pores above the isthmus.
REMARKS: A formaldehyde fixed sample of strain CCMA-UFSCar 718 is deposited in the Herbário Científico do Estado "Maria Eneyda P. Kauffmann Fidalgo" (SP), at the Instituto de Botânica, São Paulo, São Paulo state, Brazil, under access number SP400044. Material of the authentic strain CCMA-UFSCar 718 is maintained at the Culture Collection of Freshwater Microalgae, Universidade Federal de São Carlos (CCMA-UFSCar), São Carlos, São Paulo state, Brazil.

***Pseudomicrasterias* sp. (Figs. 9A-T and figs. 12A-F)**

Description based on light microscopy. Vegetative cells longer than broad; median constriction deep, sinus acute, open; polar lobe short subcylindrical; lateral projections of polar lobe subconical, retuse to slightly curved downward; apical margin flat, often somewhat elevated on either side of the middle; basal lobes conical, swollen, straight at the base; upper margin of basal lobes slightly curved upward, bottom margin slightly subsigmoid; both basal and polar lobes ending in a conical, short, robust tooth. Axial chloroplast following the shape of the semicell, with several pyrenoids. Vertical view oblong to fusiform. Although zygospore was not observed, some sexual reproduction stages were detected such as pre-vegetative cells.
Description based on Electron Microscopy. According to SEM, the specimens have cell wall with fine pores.

GENBANK ACCESS NUMBER: MW678850 for SSU rDNA and MW686755 for *rbc*L. Phylotype differs from *P. arcuata* and *P. perforata* regarding 18S rDNA and *rbc*L phylogenies.
REMARKS: We chose figures 27 and 28 of present study as iconotypes of *Pseudomicrasterias* sp. Further studies are necessary to conclude the species circumscription and know the relationship of this material with other infraspecific taxa of *M. arcuata*. A formaldehyde fixed sample of strain CCMA-UFSCar 719 is deposited in the Herbário Científico do Estado "Maria Eneyda P. Kauffmann Fidalgo" (SP), at the Instituto de Botânica, São Paulo, São Paulo, Brazil, under access number SP400045. Material of the authentic strain CCMA-UFSCar 719 is

maintained at the Culture Collection of Freshwater Microalgae, Universidade Federal de São Carlos (CCMA-UFSCar), São Carlos, São Paulo state, Brazil.

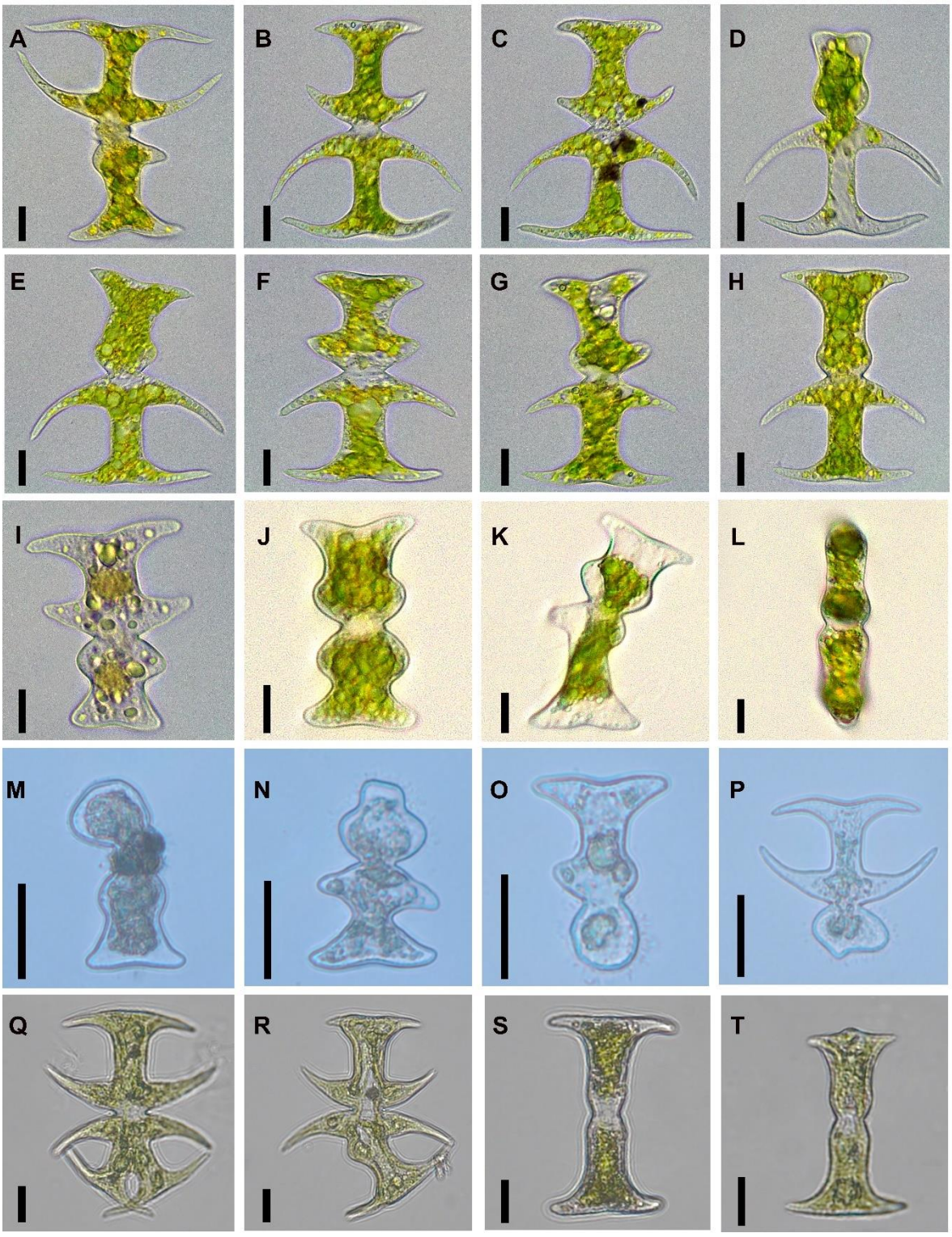

Figure 7. A-T. Light microscopy (LM) pictures of *Pseudomicrasterias arcuata*, CCMA-UFSCar 717 strain. **A-E, P.** Mature semicells. **E-I.** Immature semicells. **J-K, M-O.** Individuals

in development. **L.** Apical view. **Q-T.** Cells in development (teratological cells). **A-I:** scale bars = 10 µm. **M-P:** scale bars = 50 µm, 2.5 optovar. **Q-T:** scale bars = 50 µm.

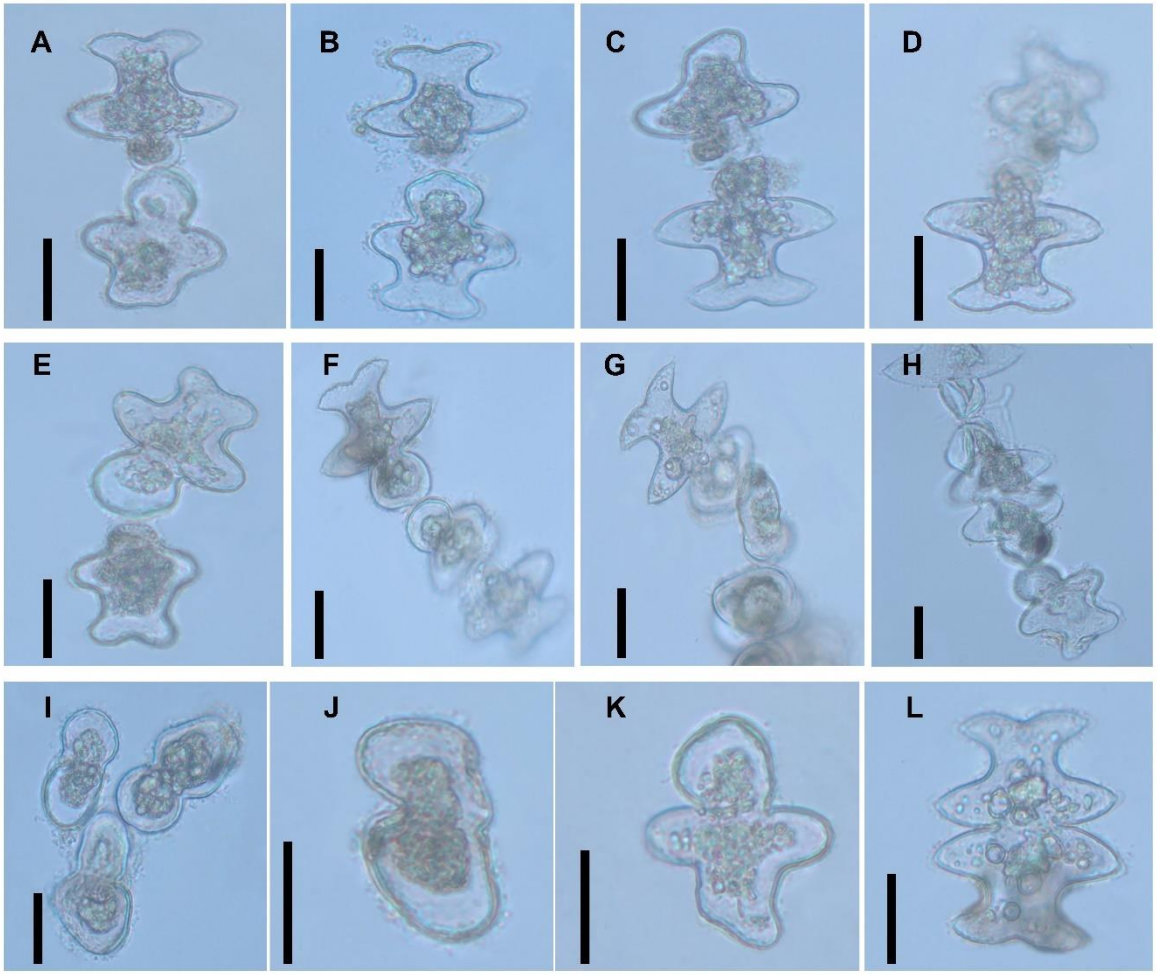

Figure 8. A-L. Light microscopy (LM) pictures of *Pseudomicrasterias perforata*, CCMA-UFSCar 718 strain. **A-E.** Semicells starting cellular Division process (asexual process). **F-H.** Cells in reproduction and development. **I-K.** Cells growing. **L.** Individual mature (adult). Scale bars = 50 µm, 2.5 optovar.

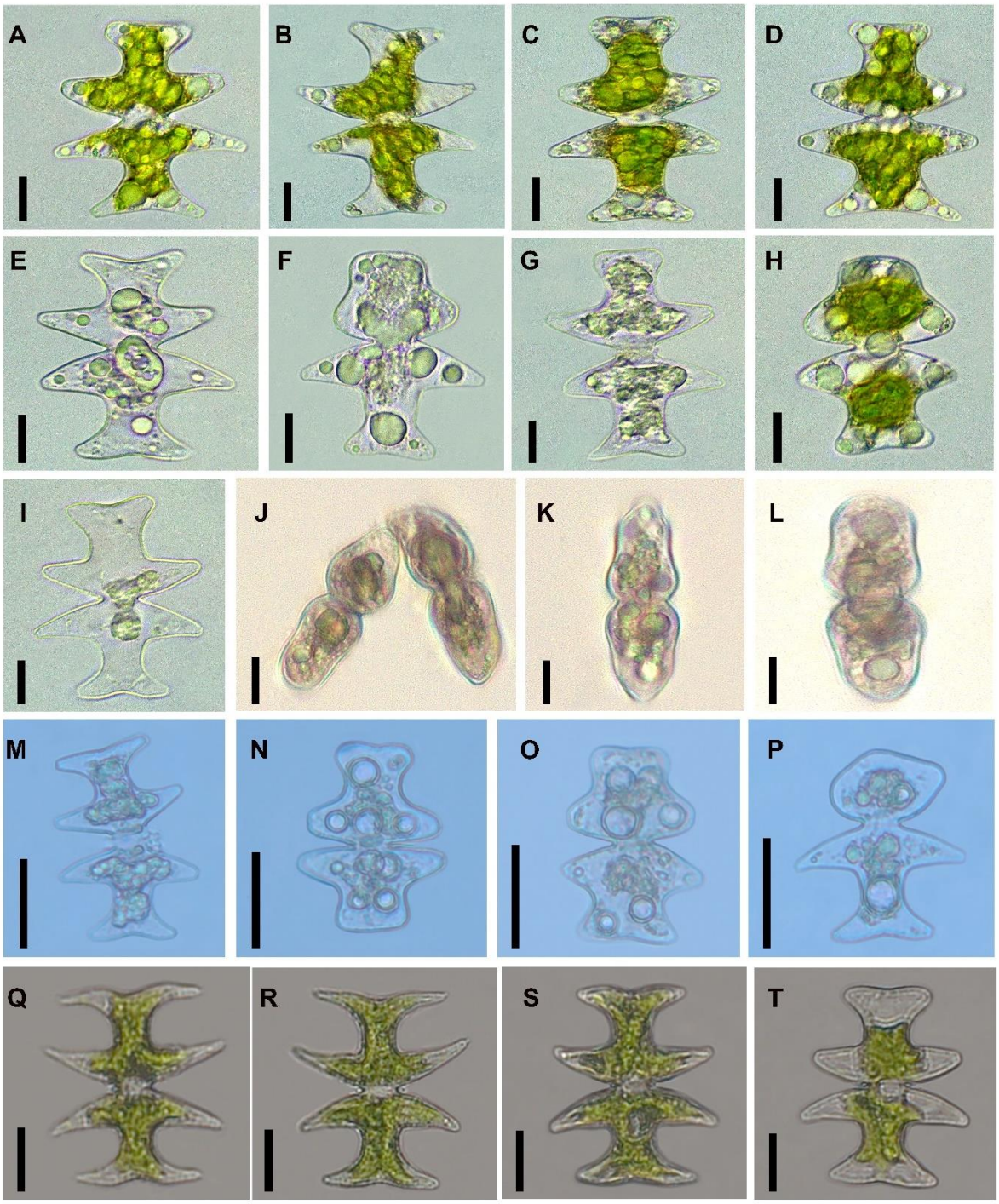

Figure 9. A-T. Light microscopy (LM) pictures of *Pseudomicrasterias* sp., CCMA-UFSCar 719 strain. **A-E, G, I, T.** Immature semicells. **F, H, P.** Cells growing. **J-L.** Apical view. **G-M**. Semicells starting cellular Division process (asexual process). **Q-R.** Individuals adult. **A-L:** scale bars = 10 µm. **M-P:** scale bars = 50 µm, 2.5 optovar. **Q-T:** scale bars = 50 µm.

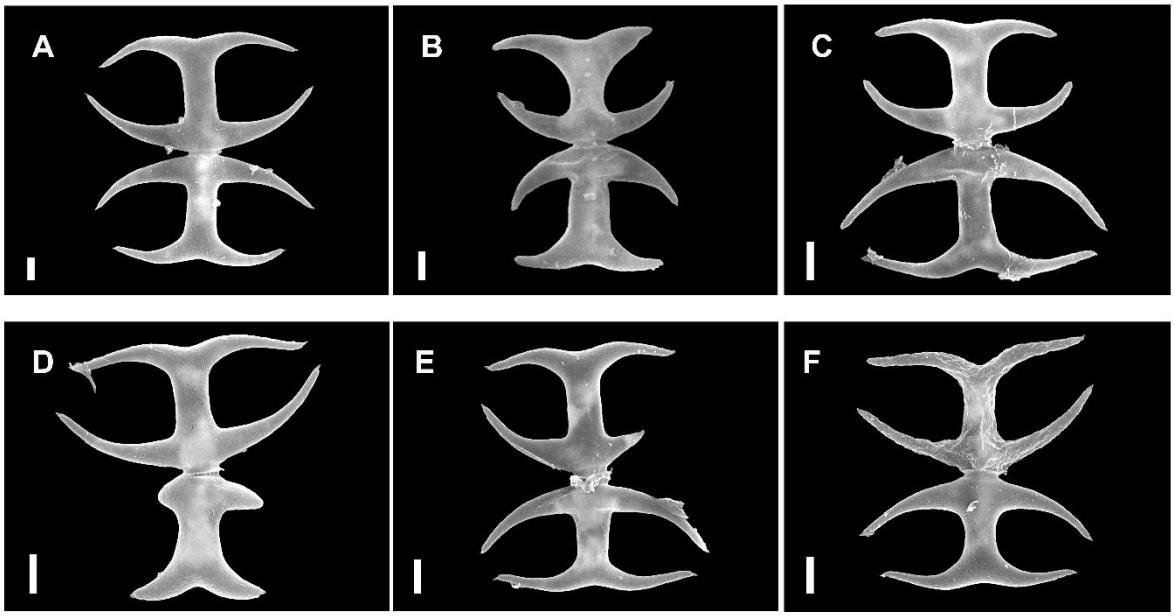

Figure 10. A-F. Scanning electron microscopy (SEM) pictures of investigated *Pseudomicrasterias arcuata*, CCMA-UFSCar 717 strain. Scale bars = 10 µm.

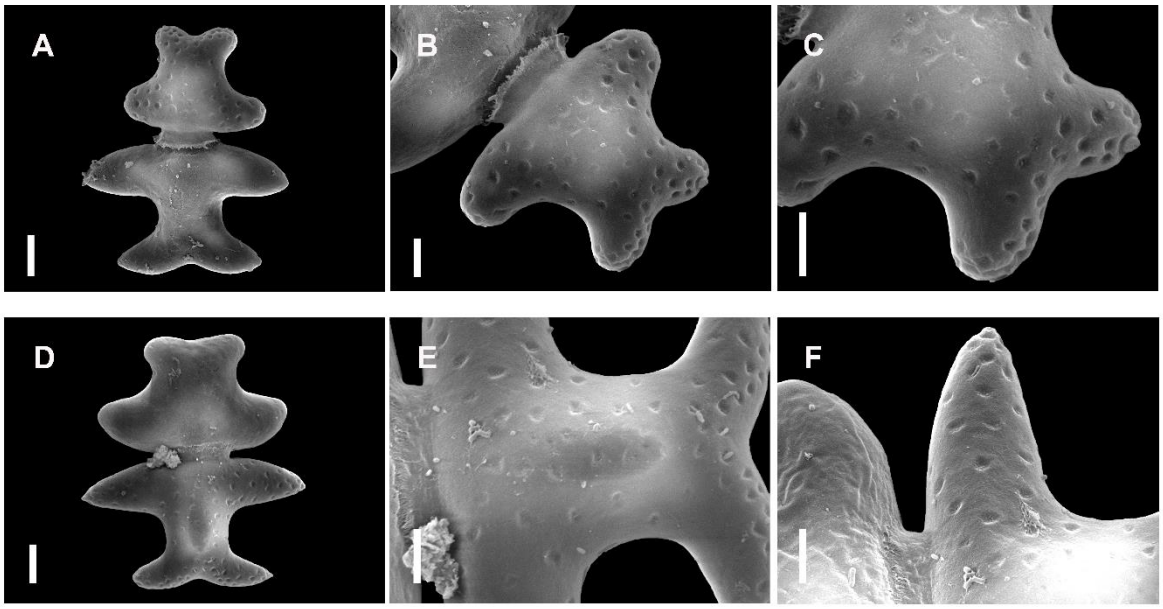

Figure 11. A-F. Scanning electron microscopy (SEM) pictures of investigated *Pseudomicrasterias perforata*, CCMA-UFSCar 718 strain. **A, D:** scale bars = 10 µm. B-**C, D-F:** scale bars = 5 µm.

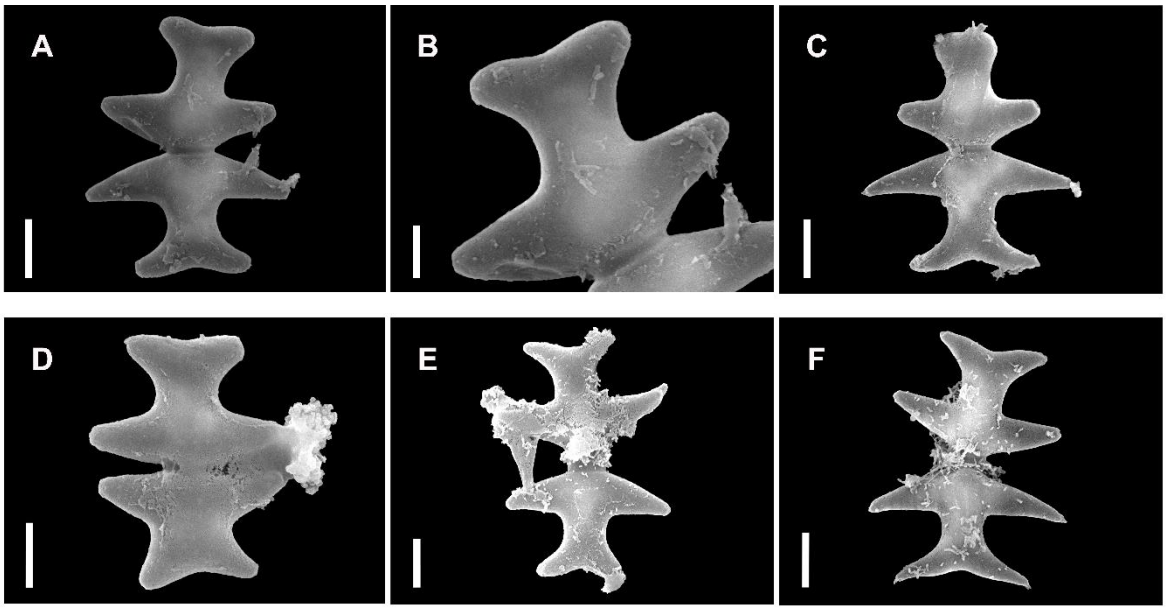

Figure 11. A-F. Scanning electron microscopy (SEM) pictures of investigated *Pseudomicrasterias* sp., CCMA-UFSCar 719 strain. **A, C-F:** scale bars = 10 µm. **B:** scale bars = 5 µm.

Table 3. Comparison among *Pseudomicrasterias* species based on traditional morphometric analysis (linear measurements) and analyses of the shape of semicell by geometric morphometric analyses and SEM images.

Feature	*P. arcuata*	*P. perforata*	*Pseudomicrasterias* sp.
MCL (µm)	72(-60-92)	67(-58-86)	45(-41-53)
ACL (µm)	71(-55-97)	65(-55-90)	44(-39-50)
BLD (µm)	40(-33-53)	30(-24-49)	18(-14-25)
MCW (µm)	71(-61-86)	67(-58-80)	41(-33-49)
BPL (µm)	61(-53-70)	45(-37-64)	30(-21-38)
IW (µm)	11(-9-15)	16(-14-18)	10(-8-11)
HPL (µm)	16(-10-22)	12(-8-21)	7(-4-10)
L:W ratio	1.0	1.0	1.1
Basal lobes	Slender to very slender, arcuate upward	Swollen, straight to slightly arcuate upward	Swollen, slightly arcuate upwards
Polar lobe	Slender, arcuate downward	Swollen, shorter, straight to slightly arcuate upward	Stout, shorter, straight, oriented upward (sometimes) to slightly arcuate downward
Apical margin	Straight to slightly concave, elevated on either side	Straight to strongly concave, elevated on either side	Straight to slightly concave, elevated on either side
Cell wall punctation	Finely punctate	Strongly punctate, thick pores	Finely punctate
Teeth	Short and thin	Short and robust	Short and thin

*Mean (min-max):

SUPPLEMENTARY DATA

(1). List of all 95 taxa used in the phylogenetic analyses. The GenBank accession numbers for the SSU rDNA, *rbc*L and *psa*A genes are also provided.

(2). Classification of the *Micrasterias arcuata* like strains according to the correct classification/prediction in linear discriminant analysis (LDA) based on geometric morphometric analysis.

(3). Classification of the *Micrasterias arcuata* like strains according to the correct classification/prediction in linear discriminant analysis (LDA) of geometric morphometric analysis and literature data.

(4). Pairwise distance matrix (uncorrected p-distances, %) between groups (clades) for 18S

rDNA of studied strains and their close relatives conducted with a total of 1.705 positions.

(5). 18S rDNA similarity matrix of between groups (clades) studied conducted using the p-distance model with a total of 1.705 positions.

(6). Pairwise distance matrix (uncorrected p-distances, %) between groups (clades) for *rbc*L of studied strains and their close relatives conducted with a total of 1.335 positions.

(7). *rbc*L similarity matrix of between groups (clades) studied conducted using the p-distance model with a total of 1.335 positions.

(8). Pairwise distance matrix (uncorrected p-distances, %) between *Pseudomicrasterias* subclade (clades) for 18S SSU rDNA gene.

(9). Pairwise distance matrix (uncorrected p-distances, %) between *Pseudomicrasterias* subclade (clades) for *rbc*L gene.

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Supplementary material 1. List of all 95 taxa used in the phylogenetic analyses. The GenBank accession numbers for the SSU rDNA, *rbc*L and *psa*A genes are also provided. Strain number abbreviations: **ACOI** – Coimbra Collection of Algae, Portugal; **CAUP** - Culture Collection of Algae, Charles University in Prague; **CCMA**- Coleção de culturas de Microalgas de Água Doce – Universidade Federal de São Carlos; **CCAP** – Culture Collection of Algae e Protozoa; **MZCH** - The Microalgae and Zygnematophyceae Collection Hamburg, previously SAG (Culture Collection of Algae, Georg-August University Göttingen); **NIES** - Microbial Culture Collection, National Institute for Enviromental Studies, Onogawa; **SAG** - Culture Collection of Algae, Georg-August University Göttingen; **SVCK** - Sammlung von Conjugaten-Kulturen, University Hamburg; **UTEX** - Culture Collection of Algae at University of Texas, USA. For own isolates, the initials of the isolator were given JH: Jonh Hall.

Taxon name	**Culture number**	**GenBank Accession number**		
		SSU	***rbc*L**	***psa*A**
Actinotaenium curtum (Brébisson ex Ralfs) Teiling ex Růžička & Pouza	SVCK 163		FN432046	-
Cosmarium contractum O.Kirchner	SVCK396	AJ428112	AJ553937	-
Cosmarium cf. *contractum* O.Kirchner	NIES452	AM920365	AM911283	-
Staurodesmus convergens (Ehrenberg ex Ralfs) S.Lillieroth	M1886	AJ428102	AM911344	-
Staurodesmus convergens (Ehrenberg ex Ralfs) S.Lillieroth	UTEX 2508	-	EF371281	EF371175
Actinotaenium cruciferum (De Bary) Teiling	M 2025	AM920332	AM911235	-
Penium cylindrus Brébisson ex Ralfs	ACOI 780	AJ553930	AJ553959	EF371220
Penium spirostriolatum J.Barker	SVCK189	AJ553928	AJ553961	-
Penium spirostriolatum J.Barker	SVCK 205	-	EF371324	EF371224
Cosmarium bioculatum Brébisson ex Ralfs	CCAP612/17	AM920354	AM911265	-
Cosmarium melanosporum W.Archer & J.Roy	JH0011	-	EF371289	EF371183
Cosmarium tinctum Ralfs	M2301	AM920355	AM911278	-
Actinotaenium cucurbita (Brébisson ex Ralfs) Teiling	M1199	AJ428099	AM911236	-
Cosmarium botrytis Meneghini ex Ralfs	SVCK274	AM920378	AM911295	-
Cosmarium botrytis Meneghini ex Ralfs	UTEX 301	-	EF371288	EF371182
Cosmarium crenatum Ralfs ex Ralfs	M2164	AM920370	AM911268	-
Cosmarium cyclicum P.Lundell	M1208	AM920388	AM911304	-
*Cosmarium phaseolu*s Brébisson ex Ralfs	M2302	AM920382	AM911299	-
Euastrum verrucosum Ehrenberg ex	JH 0068	-	EF371301	EF371195

Ralfs				
Cosmarium pseudoconnatum Nordstedt	JH 0264	-	EF371280	EF371174
Cosmarium punctulatum Brébisson	SVCK 570	AM920373	AM911289	-
Cosmarium ovale Ralfs ex Ralfs	SVCK342		AM911309	-
Euastrum moebii (Borge) A.M.Scott & Prescott	SVCK358		AM911248	-
Cosmarium granatum Brébisson ex Ralfs	M2127	AM920364	AM911282	-
Cosmarium laeve Rabenhorst	SVCK 35	AM920363	AM911280	-
Docidium baculum Brébisson ex Ralfs	C6	-	HE613541	-
Docidium undulatum (strain identified as *Haplotaenium* sp.)	NIES 285	-	FN432109	-
Euastrum affine Ralfs ex Ralfs	SVCK185	AM920342	AM911240	-
Euastrum crassum Ralfs	JH0018	-	EF371300	EF371194
Euastrum intermedium Cleve	JH0159	-	EF371302	EF371196
Euastrum oblongum Ralfs	ASW 07018	AJ428095	AM911239	-
Euastrum pinnatum Ralfs	SVCK 175	AJ428096		
Cosmarium dilatatum Lütkemüller	SVCK 463	AJ829665	FN432061	-
Euastrum bidentatum Nägeli	ACOI 282	AM920345	FN432103	-
Euastrum binale Ehrenberg ex Ralfs	ACOI 488	AM920347	AM911245	-
Euastrum biverrucosum Gontcharov & M.M.Watanabe	SVCK464	AM920346	AM911244	-
Euastrum divaricatum P.Lundell	SVCK 156	AM920344	AM911242	-
Euastrum subalpinum Messikommer	ACOI 855	AM920348	AM911246	-
Euastrum trigebberum West & G.S.West	ACOI 1144	AM920343	AM911241	-
Euastrum pectinatum Brébisson ex Ralfs	SVCK 203	AJ549227	FN432106	-
Phymatodocis nordstedtiana Wolle	SVCK327	AJ428122	AJ553962	-
Phymatodocis nordstedtiana Wolle	JH 0164	-	EF371325	EF371226
Cosmocladium perissum J.Roy & Bisset	UTEX LB 2447	AF408234	AF203494	-
Cosmocladium saxonicum De Bary	ACOI 95	-	EF371292	EF371186
Spondylosium planum (Wolle) West & G.S.West	SAG 41.81	AJ428123	AM911260	-
Spondylosium tetragonum West & G.S.West	JH0281	-	EF371336	EF371239
Teilingia granulata (J.Roy & Bisset) Bourrelly	JH 0140	-	EF371351	EF371256
Bambusina borreri (Ralfs) Cleve	JH0199	-	EF371283	EF371177
Desmidium baileyi (Ralfs) Nordstedt	JH 0228	-	EF371299	EF371193
Heimansia pusilla (L.Hilse) Coesel	SVCK 428	AJ428125	EF371291	EF371185
Hyalotheca dissiliens Brébisson ex Ralfs	SAG 384-2	-	AF203499	EF371202
Hyalotheca mucosa Ralfs	JH 0063	-	EF371306	EF371204
Spondylosium pulchellum (W.Archer)	SVCK 365	AJ428130	AM911261	EF371245
Haplotaenium minutum (Ralfs) Bando	SVCK302	AJ428090	AJ553947	EF371227
Micrasterias americana Ehrenberg ex Ralfs	SVCK 290	FR852595	-	FR852626
Micrasterias americana Ehrenberg ex	MZCH 139	-	KX676197	-

Ralfs				
Micrasterias apiculata Meneghini ex Ralfs	SVCK 247	FR852596	-	FR852628
Micrasterias apiculata Meneghini ex Ralfs	JH 0200	-	HQ380539	-
Micrasterias crux-melitensis Ralfs	CAUP K602	AM419206	-	FR852633
Micrasterias crux-melitensis Ralfs	MZCH 431	-	KX676206	-
Micrasterias dickiei (Ralfs) Skaloud et al.,	SVCK38	AJ428101	FN432123	-
Micrasterias dickiei (Ralfs) Skaloud et al.,	ASW 07056	-	-	FR852661
Micrasterias fimbriata Ralfs	M1188	AM910450	AM911257	-
Micrasterias fimbriata Ralfs	CAUP K608	FR852604	-	FR852637
Micrasterias foliacea Bailey ex Ralfs	NIES 297	-	EF371311	EF371209
Micrasterias furcata C. Agardh ex Ralfs	CAUP 609	FR852605	-	FR852639
Micrasterias jenneri Ralfs	SVCK 298	FR852607	-	FR852641
Micrasterias jenneri Ralfs	MZCH 298	-	KX676228	-
Micrasterias mahabuleshwarensis J. Hobson	SVCK 324	FR852609	-	FR852643
Micrasterias mahabuleshwarensis J. Hobson	JH 0481	-	HQ380532	-
Micrasterias muricata Bailey ex Ralfs	SAG 157.80	FR852610	-	FR852644
Micrasterias muricata Bailey ex Ralfs	JH 0119	-	HQ380533	-
Micrasterias papillifera Brébisson ex Ralfs	CAUP K603	AM419208	-	FR852646
Micrasterias papillifera Brébisson ex Ralfs	MZCH71	-	KX676243	-
Micrasterias pinnatifida Ralfs	SVCK 411	FR852612	-	FR852647
Micrasterias pinnatifida Ralfs	JH0509	-	HQ380546	-
Micrasterias radiosa Ralfs	SCVK 303	FR852615	-	FR852650
Micrasterias radiosa Ralfs	JH0372	-	HQ380531	-
Micrasterias ralfsii (Brébisson ex Ralfs) Skaloud et al.,	SVCK300	FR852622	AM911324	FR852660
Micrasterias thomasiana W. Archer	M2253	AM920341	AM911256	-
Triploceras gracile Bailey	SVCK173	AJ428089	AM911258	-
Triploceras gracile Bailey	SAG 24.82	-	EF371354	EF371259
Actinotaenium curcurbitinum (Bisset) Teiling	ACOI 901	-	EF371279	EF371172
Actinotaenium phymatosporum (Nordstedt) Kouwets & Coesel	M 1368	AJ428088	AM911233	-
Actinotaenium silvae-nigrae (Rabanus) Kouwets & Coesel	SVCK 295	AM920333	EF371323	EF371223
Cosmarium bisphaericum Printz	SVCK436	AM920338	AM911266	-
Cosmarium broomei Thwaites ex Ralfs	M2075	AM920340	AM911269	-
*Spondylosium panduriform*e (Heimerl) Teiling	SAG 52.88	AJ428124	AJ553969	-
Pleurotaenium baculoides (J.Roy & Bisset)	JH0008	-	EF371327	EF371228
Pleurotaenium constrictum (Bailey)	JH 0135	-	EF371328	EF371229

H.C.Wood				
Pleurotaenium ehrembergii var. *columellare* Irenee-Marie	JH 0331	-	EF371329	EF371230
Staurastrum arctiscon (Ehrenberg ex Ralfs) P.Lundell	JH0014	-	EF371343	EF371248
Staurastrum gladiosum W.B.Turner	JH 0132	-	EF371347	EF371252
Staurastrum punctulatum Brébisson	SAG 679-1	AF115442	FN432117	-
Staurastrum pseudosuecicum Prescott & A.M.Scott	JH 0010	-	EF371342	EF371247
Staurodesmus extensus (Borge) Teiling	ACOI956	AM920358	AM911337	-
Staurodesmus extensus (Borge) Teiling	JH0386	-	EF371317	EF371217
Staurodesmus glaber (Ralfs) Teiling	ACOI954	AM920356	AM911334	-
Staurodesmus spencerianus (Nordstedt) Teiling	ACOI735	AM920357	AM911335	-
Cosmarium impressulum Elfving	SVCK58	AM920362	AM911279	-
Cosmarium meneghinii Brébisson ex Ralfs	SVCK59	AM920366	AM911284	-
Staurodesmus bienianus (Rabenhorst) Florin	M1130	AJ829659	AM911341	-
Staurodesmus dejectus (Brébisson) teiling	CCAP 681/1	-	FN432122	-
Tetmemorus brebissonii Ralfs	SVCK 214	AJ428091	FN432133	-
Tetmemorus brebissonii Ralfs	SVCK 409	-	EF371352	EF371257
Xanthidium antilopaeum var. *minneapoliense* Wolle	SVCK 147	AM920353	AM911264	-
Xanthidium cristatum var. *ornatum* (W.B.Turner) E.A.J.De Wildeman	SVCK 426	AM920352	AM911263	-
Xanthidium tumidum (Ralfs) Stastny, Skaloud & Neustupa	SVCK 85	AJ428108	AJ553972	-
Staurodesmus bulnheimii (Raciborski) Round & A.J.Brook	SVCK 84	AJ428111	FN432118	-
Pseudomicrasterias arcuata	CCMA-UFSCar 717	MW678848	MW686753	MW686756
Pseudomicrasterias perforata	CCMA-UFSCar 718	MW678849	MW686754	-
Pseudomicrasterias sp.	CCMA-UFSCar 719	MW678850	MW686755	-

Supplementary material 2: Classification of *Pseudomicrasterias* strains according to the correct classification/prediction in linear discriminant analysis (LDA) based on geometric morphometric analyses.

ID	C1*	C2*	C4*
C1	1	1,13E-27	4,36E-17
C1	0,999998	4,34E-20	2,22E-06
C1	1	4,23E-25	7,21E-19
C1	1	8,5E-19	6,69E-11
C1	1	5,19E-38	7,09E-27
C1	1	7,18E-31	8,11E-24
C1	1	1E-28	1,67E-15
C1	1	6,59E-29	1,35E-16
C1	1	4,86E-24	5,23E-19
C1	1	4,79E-26	1,06E-16
C1	1	1,19E-30	1,54E-18
C1	1	4,24E-21	3,15E-15
C1	1	2E-25	1,67E-18
C1	1	1,37E-28	1,72E-17
C2	1,08E-28	1	1,16E-11
C2	4,45E-24	1	5,07E-08
C2	1,98E-28	1	8,15E-15
C2	1,18E-25	1	5,36E-09
C2	1,58E-34	1	1,12E-19
C2	2,45E-23	1	6,7E-09
C2	2,3E-20	1	2,21E-08
C2	3,78E-28	1	1,85E-14
C2	1,7E-30	1	9,72E-12
C2	2,24E-23	0,999998	1,74E-06
C2	6,9E-26	1	9,95E-13
C2	1,05E-28	1	1,51E-11
C2	4,16E-24	0,999989	1,1E-05
C2	8,96E-25	1	1,32E-11
C2	3,38E-25	1	2,48E-10
C2	1,77E-28	1	4,86E-08
C2	1,36E-29	1	4,96E-11
C2	9,63E-23	1	1,33E-08
C2	2,54E-34	1	1,95E-16
C2	9,63E-30	1	2,17E-10
C2	3,43E-30	1	2,22E-11
C2	1,54E-25	1	1,1E-13
C4	1E-19	3,49E-14	1
C4	4,98E-22	2,51E-15	1
C4	1,33E-19	2,99E-08	1
C4	1,35E-16	1,16E-11	1
C4	1,56E-18	3,87E-13	1
C4	1,49E-18	5,92E-09	1
C4	5,99E-17	1,24E-15	1
C4	7,17E-12	1,16E-09	1
C4	2,3E-19	6,12E-13	1
C4	6,28E-25	2,31E-14	1

C4	2,67E-19	1,42E-12	1
C4	9,94E-16	2,74E-13	1
C4	2,25E-11	6,22E-14	1
C4	2,89E-16	2,47E-08	1
C4	2,71E-19	1,03E-14	1
C4	4,17E-24	3,07E-07	1
C4	1,04E-16	1,6E-13	1
C4	1,23E-22	8,83E-10	1
C4	1,57E-17	5,32E-11	1
C4	1,76E-24	1,95E-15	1
C4	2,11E-08	1,77E-16	1
C4	3,47E-23	4,12E-09	1
C4	1,05E-16	4,11E-09	1
C4	1,85E-21	7,08E-10	1
C4	1,42E-19	7,04E-09	1
C4	8,88E-13	1,83E-09	1
C4	6,93E-19	8,85E-12	1
C4	6,64E-19	4,36E-08	1
C4	7,92E-19	1,52E-12	1
C4	9,49E-24	7,09E-10	1
C4	4,48E-21	1,06E-13	1
C4	1,28E-18	6,7E-09	1
C4	8,09E-13	4,79E-12	1
C4	6,8E-24	7,36E-19	1
C4	2,01E-14	4,83E-14	1
C4	1,2E-19	4,62E-15	1
C4	1,29E-15	5,99E-17	1
C4	1,03E-15	2,51E-07	1
C4	5,42E-23	7,59E-12	1
C4	9,96E-11	4,18E-11	1
C4	2,14E-14	4,07E-15	1
C4	4,62E-15	2,78E-12	1
C4	7,83E-18	5,98E-08	1
C4	5,32E-16	2,52E-11	1
C4	2,25E-27	8,02E-13	1
C4	1,4E-18	5,33E-10	1
C4	1,01E-19	1,04E-10	1
C4	2,22E-15	8,85E-15	1
C4	1,22E-20	6,5E-05	0,999935

***:**
C1: CCMA-UFSCar 717
C2: CCMA-UFSCar 718
C4: CCMA-UFSCar 719

Supplementary material 3: Classification of *Pseudomicrasterias* strains according to the correct classification/prediction in linear discriminant analysis (LDA) of geometric morphometric analyses and literature data.

Literature taxa	ID	CCMA-UFSCar 717	CCMA-UFSCar 718	CCMA-UFSCar 719
M. arcuata	Lit1	1	8,54276E-13	2,30681E-19
M. arcuata var. *borgei*	Lit10	4,99661E-22	0,999873334	0,000126666
M. arcuata var. *compacta*	Lit11a	0,910810715	6,59843E-05	0,0891233
M. arcuata var. *compacta*	Lit11b	0,754586825	4,73965E-08	0,245413128
M. arcuata var. *cornuta*	Lit12	2,383E-14	1,6154E-12	1
M. arcuata var. *perforata*	Lit14a	9,12859E-08	0,984928598	0,01507131
M. arcuata var. *perforata*	Lit14b	1,22443E-08	0,956294022	0,043705966
M. arcuata var. *perforata* f. *magniperforata*	Lit15	9,24502E-07	0,999999075	2,43028E-10
M. arcuata var. *perforata* f. *robustior*	Lit16	1,30803E-23	1	4,8955E-10
M. arcuata var. *perforata* f. *robustior* f. *minor*	Lit17a	3,10052E-27	1	7,59316E-17
M. arcuata var. *perforata* f. *robustior* f. *minor*	Lit17b	9,38187E-31	1	6,47937E-16
M. arcuata var. *perforata* f. *robustior* f. *minor*	Lit17c	1,15847E-27	1	2,53205E-16
M. arcuata var. *robusta* f. *goyazensis*	Lit18a	1,53925E-07	0,999999846	8,21359E-13
M. arcuata var. *robusta* f. *goyazensis*	Lit18b	0,069371026	0,004971609	0,925657365
M. arcuata var. *subcornuta*	Lit19	0,026619049	0,007601954	0,965778997
M. arcuata var. *subpinnatifida* f. *gracilis*	Lit20	1,40891E-17	3,6896E-16	1
M. arcuata var. subpinnatifida f. *eckertii*	Lit21	3,08756E-19	0,999994925	5,07477E-06
M. arcuata morpha minor Bailey	Lit22a	1	1,85416E-14	7,55049E-11
M. arcuata morpha minor Bailey	Lit22b	0,98182601	2,43962E-20	0,01817399
M. arcuata var. *robusta* f. *goyazensis*	Lit24a	0,999987726	1,21355E-05	1,38413E-07
M. arcuata var. *robusta* f. *goyazensis*	Lit24b	3,21546E-08	0,999999968	2,7241E-12
M. arcuata var. *robusta* Borge	Lit25a	1,64305E-13	0,999999797	2,02582E-07
M. arcuata var. *robusta* Borge	Lit25b	1,6243E-16	0,999686483	0,000313517
M. arcuata var. *subpinnatifida* West & West form Borge 1918	Lit26a	8,66999E-12	0,995670239	0,004329761
M. arcuata var. *subpinnatifida* West & West form Borge 1918	Lit26b	2,68192E-21	0,000979474	0,999020526
M. arcuata var. *subpinnatifida* f. Förster 1964	Lit27	3,38455E-20	3,29228E-08	0,999999967
M. arcuata Bailey 1851	Lit28	0,053132208	1,57446E-11	0,946867792
M. arcuata f. Förster 1964	Lit29	0,999955075	1,65348E-07	4,476E-05
M. arcuata var. *gracilis* f.1 Förster 1964	Lit30	1	6,81261E-37	1,44228E-18
M. arcuata var. *gracilis* f.2 Förster 1964	Lit31	1	8,26233E-17	1,19098E-13

Supplementary table 4. Pairwise *distance matrix* (uncorrected *p*-distances, %) between groups (clades) for 18S rDNA of studied strains and their close relatives conducted with a total of 1.705 positions.

	Clades	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*Pseudomicrasterias gen. nov.*																					
2	*Arthrodesmus*	0.017																				
3	*Outgroup*	0.024	0.022																			
4	*CO_1*	0.022	0.019	0.025																		
5	*CO_2*	0.020	0.020	0.022	0.023																	
6	*CO_3*	0.017	0.016	0.018	0.017	0.011																
7	*CO_5*	0.011	0.013	0.019	0.017	0.016	0.011															
8	*Euastrum_2*	0.016	0.018	0.023	0.022	0.020	0.015	0.013														
9	*Euastrum_1*	0.014	0.017	0.022	0.022	0.020	0.014	0.009	0.015													
10	*Euastrum_pectinatum*	0.021	0.022	0.030	0.023	0.025	0.022	0.019	0.021	0.023												
11	*Filamentous_1*	0.021	0.016	0.020	0.020	0.018	0.014	0.014	0.020	0.018	0.026											
12	*Filamentous_2*	0.017	0.019	0.023	0.023	0.021	0.014	0.012	0.016	0.016	0.024	0.019										
13	*Haplotaenium*	0.022	0.019	0.025	0.023	0.023	0.017	0.017	0.023	0.021	0.028	0.020	0.021									
14	*Micrasterias*	0.026	0.025	0.029	0.028	0.027	0.023	0.022	0.026	0.026	0.030	0.027	0.026	0.026								
15	*Omniradiate*	0.025	0.021	0.026	0.025	0.025	0.020	0.020	0.025	0.024	0.030	0.021	0.023	0.024	0.031							
16	*Staurastrum*	0.022	0.020	0.025	0.023	0.022	0.018	0.016	0.021	0.020	0.028	0.018	0.021	0.019	0.026	0.023						
17	*STD_1*	0.021	0.019	0.025	0.019	0.023	0.017	0.015	0.020	0.019	0.023	0.018	0.019	0.022	0.028	0.025	0.022					
18	*STD_2*	0.015	0.017	0.022	0.021	0.020	0.015	0.008	0.015	0.012	0.022	0.017	0.016	0.021	0.026	0.021	0.019	0.018				
19	*Tetmemorus_brebissoniii*	0.018	0.020	0.027	0.023	0.024	0.019	0.013	0.019	0.018	0.022	0.023	0.019	0.020	0.027	0.028	0.023	0.021	0.017			
20	*Xanthidium*	0.014	0.013	0.018	0.017	0.014	0.009	0.009	0.014	0.014	0.018	0.014	0.014	0.017	0.021	0.019	0.017	0.017	0.014	0.017		
21	*Staurodesmus_bulnheimii*	0.017	0.019	0.025	0.024	0.022	0.016	0.013	0.017	0.016	0.024	0.023	0.017	0.025	0.028	0.026	0.025	0.022	0.017	0.019	0.015	

Supplementary table 5. 18S rDNA similarity matrix of between groups (clades) studied conducted using the p-distance model with a total of 1.705 positions.

	Clades	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*Pseudomicrasterias gen. nov.*																					
2	*Arthrodesmus*	98,32																				
3	*Outgroup*	97,61	97,798																			
4	*CO_1*	97,77	98,073	97,465																		
5	*CO_2*	97,95	98,01	97,754	97,67																	
6	*CO_3*	98,31	98,395	98,241	98,33	98,86																
7	*CO_5*	98,91	98,655	98,112	98,27	98,41	98,93															
8	*Euastrum_2*	98,4	98,238	97,743	97,79	97,99	98,51	98,69														
9	*Euastrum_1*	98,63	98,278	97,75	97,82	98,01	98,56	99,06	98,472													
10	*Euastrum_pectinatum*	97,93	97,788	96,976	97,69	97,46	97,79	98,13	97,928	97,711												
11	*Filamentous_1*	97,94	98,398	97,964	98,01	98,15	98,63	98,6	98,002	98,198	97,359											
12	*Filamentous_2*	98,26	98,123	97,723	97,73	97,91	98,61	98,75	98,384	98,441	97,609	98,15										
13	*Haplotaenium*	97,78	98,075	97,541	97,67	97,7	98,27	98,27	97,666	97,917	97,239	97,95	97,94									
14	*Micrasterias*	97,38	97,453	97,112	97,24	97,29	97,72	97,78	97,364	97,42	96,974	97,33	97,42	97,401								
15	*Omniradiate*	97,46	97,887	97,409	97,46	97,55	98	98	97,481	97,622	97,027	97,85	97,66	97,649	96,95							
16	*Staurastrum*	97,78	97,99	97,521	97,66	97,79	98,21	98,42	97,869	98,026	97,239	98,22	97,94	98,086	97,42	97,66						
17	*STD_1*	97,95	98,14	97,538	98,11	97,74	98,25	98,46	97,989	98,114	97,686	98,22	98,05	97,789	97,17	97,54	97,8					
18	*STD_2*	98,48	98,283	97,81	97,93	98,01	98,53	99,16	98,458	98,764	97,773	98,28	98,39	97,852	97,39	97,88	98,12	98,21				
19	*Tetmemorus_brebissoniii*	98,19	97,95	97,313	97,7	97,61	98,09	98,7	98,052	98,241	97,849	97,67	98,09	97,969	97,33	97,23	97,72	97,87	98,31			
20	*Xanthidium*	98,59	98,676	98,173	98,33	98,56	99,1	99,05	98,569	98,643	98,196	98,6	98,57	98,255	97,94	98,1	98,29	98,31	98,63	98,34		
21	*Staurodesmus_bulnheimii*	98,25	98,071	97,465	97,64	97,76	98,39	98,7	98,293	98,431	97,606	97,7	98,3	97,544	97,21	97,38	97,54	97,81	98,35	98,09	98,52	

Supplementary table 6. Pairwise distance matrix (uncorrected p-distances, %) between groups (clades) for *rbc*L of studied strains and their close relatives conducted with a total of 1.335 positions.

	Sequence identification	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	*Actnotaenium_curtum*																										
2	*Arthrodesmus*	0,063																									
3	*Outgroup*	0,103	0,109																								
4	*CO_1*	0,077	0,084	0,113																							
5	*CO_2*	0,045	0,050	0,092	0,072																						
6	*CO_3*	0,053	0,059	0,102	0,083	0,040																					
7	*Cosmarium_ovale*	0,042	0,046	0,087	0,067	0,024	0,040																				
8	*Euastrum_moebii*	0,045	0,050	0,090	0,071	0,029	0,046	0,013																			
9	*CO_5*	0,049	0,064	0,100	0,084	0,052	0,066	0,048	0,055																		
10	*Haplotaenium*	0,070	0,077	0,106	0,097	0,062	0,074	0,054	0,054	0,081																	
11	*Euastrum_2*	0,056	0,062	0,094	0,085	0,053	0,061	0,042	0,044	0,065	0,067																
12	*Euastrum_1*	0,056	0,061	0,101	0,084	0,053	0,061	0,043	0,046	0,059	0,073	0,052															
13	*Euastrum_pectinatum*	0,044	0,053	0,097	0,082	0,040	0,055	0,030	0,035	0,053	0,067	0,033	0,041														
14	*Filamentous_1*	0,078	0,088	0,111	0,093	0,074	0,083	0,071	0,075	0,076	0,099	0,088	0,084	0,083													
15	*Filamentous_2*	0,089	0,097	0,118	0,111	0,083	0,089	0,080	0,080	0,087	0,103	0,091	0,093	0,088	0,103												
16	*Micrasterias*	0,050	0,050	0,095	0,078	0,040	0,056	0,029	0,031	0,059	0,056	0,045	0,051	0,040	0,077	0,085											
17	*Omniradiate*	0,066	0,077	0,096	0,090	0,061	0,072	0,053	0,059	0,069	0,084	0,070	0,069	0,064	0,088	0,100	0,063										

18	*Pleurotaenium*	0,067	0,072	0,106	0,085	0,058	0,069	0,047	0,053	0,064	0,079	0,071	0,070	0,063	0,086	0,097	0,063	0,074												
19	*Staurastrum*	0,050	0,057	0,095	0,078	0,043	0,056	0,034	0,040	0,057	0,063	0,054	0,056	0,044	0,073	0,086	0,037	0,062	0,064											
20	*STD_1*	0,065	0,071	0,109	0,087	0,061	0,074	0,053	0,059	0,064	0,091	0,079	0,073	0,065	0,085	0,096	0,069	0,080	0,072	0,067										
21	*STD_2*	0,044	0,058	0,106	0,087	0,048	0,061	0,046	0,050	0,044	0,077	0,060	0,060	0,048	0,081	0,091	0,055	0,072	0,067	0,055	0,067									
22	*STD_3*	0,055	0,068	0,105	0,080	0,055	0,065	0,050	0,059	0,068	0,080	0,067	0,070	0,059	0,079	0,093	0,059	0,075	0,072	0,063	0,079	0,066								
23	*Tetmemorus_brebissonii*	0,052	0,070	0,104	0,091	0,051	0,064	0,043	0,047	0,062	0,076	0,054	0,055	0,039	0,090	0,094	0,051	0,074	0,075	0,054	0,074	0,059	0,068							
24	*Xanthidium*	0,055	0,050	0,100	0,079	0,041	0,046	0,032	0,031	0,066	0,070	0,056	0,058	0,049	0,083	0,087	0,047	0,071	0,066	0,052	0,069	0,059	0,066	0,058						
25	*Staurodesmus_bulnheimii*	0,059	0,071	0,108	0,094	0,060	0,067	0,053	0,057	0,052	0,081	0,061	0,057	0,050	0,085	0,095	0,057	0,077	0,073	0,065	0,071	0,060	0,072	0,054	0,065					
26	*Pseudomicrasterias gen. nov.*	0,074	0,084	0,117	0,108	0,072	0,089	0,067	0,070	0,091	0,088	0,060	0,074	0,057	0,105	0,112	0,070	0,086	0,091	0,076	0,091	0,080	0,083	0,074	0,083	0,087				

Supplementary table 7. *rbc*L similarity matrix of between groups (clades) studied conducted using the p-distance model with a total of 1.335 positions.

	Sequence identification	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	*Actnotaenium_curtum*																										
2	*Arthrodesmus*	93,692																									
3	*Outgroup*	89,745	89,091																								
4	*CO_1*	92,281	91,588	88,68																							
5	*CO_2*	95,539	94,966	90,83	92,81																						
6	*CO_3*	94,692	94,113	89,83	91,73	95,97																					
7	*Cosmarium_ovale*	95,834	95,423	91,28	93,35	97,62	96,03																				
8	*Euastrum_moebii*	95,467	95,034	91,04	92,87	97,11	95,41	98,694																			
9	*CO_5*	95,085	93,572	89,99	91,56	94,84	93,41	95,194	94,55																		
10	*Haplotaenium*	92,997	92,293	89,43	90,26	93,76	92,57	94,599	94,66	91,96																	
11	*Euastrum_2*	94,396	93,789	90,56	91,47	94,66	93,93	95,798	95,67	93,47	93,301																
12	*Euastrum_1*	94,418	93,859	89,95	91,55	94,75	93,91	95,659	95,36	94,09	92,726	94,79															
13	*Euastrum_pectinatum*	95,612	94,666	90,26	91,84	95,97	94,52	97,041	96,51	94,72	93,322	96,77	95,917														
14	*Filamentous_1*	92,197	91,242	88,94	90,72	92,55	91,69	92,866	92,48	92,48	90,102	91,21	91,559	91,68													
15	*Filamentous_2*	91,142	90,312	88,24	88,86	91,74	91,08	91,975	91,99	91,29	89,738	90,85	90,735	91,24	89,73												
16	*Micrasterias*	95,001	94,954	90,54	92,24	95,96	94,48	97,106	96,86	94,09	94,396	95,51	94,851	96,04	92,33	91,493											
17	*Omniradiate*	93,404	92,268	90,41	90,99	93,88	92,76	94,733	94,12	93,12	91,607	93,01	93,097	93,57	91,19	89,991	93,719										
18	*Pleurotaenium*	93,254	92,798	89,38	91,55	94,25	93,14	95,271	94,75	93,63	92,083	92,92	92,986	93,72	91,44	90,252	93,725	92,64									

19	*Staurastrum*	94,96	94,29 1	90,5	92,2 1	95,7 2	94,4	96,65	95,9 9	94,2 8	93,73 6	94,6 4	94,42 2	95,5 8	92,7 4	91,35 1	96,30 6	93,8 2	93,5 8							
20	*STD_1*	93,50 1	92,91 8	89,1	91,2 7	93,8 8	92,5 6	94,67 7	94,1 3	93,5 5	90,86 8	92,1	92,68 8	93,5 2	91,4 9	90,44 7	93,14 8	92,0 4	92,8	93,27 8						
21	*STD_2*	95,62 7	94,22 3	89,4	91,2 9	95,2 2	93,9 2	95,43 3	94,9 7	95,6 4	92,30 4	93,9 8	93,97 2	95,2 3	91,8 7	90,93 5	94,45 1	92,8 4	93,3 3	94,49 4	93,3 4					
22	*STD_3*	94,50 1	93,21 3	89,5 4	92,0 4	94,5 2	93,5 1	94,96 7	94,1	93,1 5	92,01	93,3 4	93,00 5	94,0 9	92,1 3	90,73 1	94,06 4	92,4 5	92,8 5	93,66 2	92,1 4	93,4 1				
23	*Tetmemorus_brebissonii*	94,75 5	93,03 5	89,6 2	90,8 7	94,9 5	93,5 7	95,66 8	95,2 8	93,7 7	92,39 1	94,6	94,50 1	96,1 3	91,0 4	90,62 9	94,88 1	92,5 5	92,5 3	94,55 1	92,6 4	94,0 5	93,2 2			
24	*Xanthidium*	94,51 4	94,97 5	89,9 7	92,1 4	95,9 2	95,3 6	96,82 6	96,9 1	93,4	92,95 6	94,4	94,21 7	95,0 7	91,6 5	91,26 7	95,34 4	92,8 6	93,3 7	94,76 5	93,0 7	94,0 9	93,3 8	94,1 9		
25	*Staurodesmus_bulnheimii*	94,07 1	92,91 2	89,2 5	90,5 7	93,9 5	93,2 8	94,69 7	94,3 3	94,7 6	91,88 6	93,9	94,25	94,9 8	91,4 7	90,52 3	94,29 1	92,2 6	92,6 7	93,50 6	92,9 1	93,9 9	92,7 8	94,6 1	93,4 6	
26	*Pseudomicrasterias gen. nov.*	92,55 9	91,64	88,3	89,2	92,8 3	91,1 3	93,25 8	93,0 5	90,8 6	91,23 4	93,9 7	92,58 2	94,2 6	89,5 5	88,77 6	92,99 5	91,3 7	90,9 4	92,40 4	90,8 7	91,9 5	91,6 6	92,6 3	91,6 7	91,3 4

Supplementary material 8: Pairwise distance matrix (uncorrected p-distances, %) between *Pseudomicrasterias* subclade (clades) for 18S SSU rDNA gene.

Sequence identification	***Pseudomicrasterias arcuata***	***Pseudomicrasterias perforata***	***Pseudomicrasterias forsterii***
Pseudomicrasterias arcuata			
Pseudomicrasterias perforata	0,4		
***Pseudomicrasterias* sp.**	0,3	0,4	

Supplementary material 9: Pairwise distance matrix (uncorrected p-distances, %) between *Pseudomicrasterias* subclade (clades) for *rbc*L gene.

Sequence identification	***Pseudomicrasterias arcuata***	***Pseudomicrasterias perforata***	***Pseudomicrasterias forsterii***
Pseudomicrasterias arcuata			
Pseudomicrasterias perforata	5,3		
***Pseudomicrasterias* sp.**	1,3	5,8	

Considerações finais

Na presente tese foram apresentados importantes avanços quanto ao uso da taxonomia polifásica de nível ω em desmídias, especialmente para a espécie *Micrasterias arcuata* Bailey a qual apresenta uma ampla variabilidade morfológica e, consequentemente, uma taxonomia e nomenclatura extremamente problemática e entulhada de variedades e formas taxonômicas.

Foram analisados dados provenientes de amostras naturais e amostras provenientes de cultivos que, junto com os dados provenientes da literatura e com o uso da morfometria tradicional (representado por medidas estritamente lineares) associadas à morfometria geométrica (análise multivariada da forma da célula), constituíram ferramentas extremamente úteis capazes de separar morfotipos de *Micrasterias arcuata*.

O método de morfometria tradicional utilizado na presente pesquisa representado pelo uso de sete atributos morfológicos jamais utilizados para fins taxonômicos e de revisão deste complexo de espécie, mostrou-se bastante eficaz na delimitação de seus morfotipos. Isso mostra que, métodos como este, que abrangem uma maior caracterização celular, mesmo que usando medidas lineares, podem ser adotados e adequados para outras espécies de *Micrasterias* consideradas problemáticas, assim como para outros gêneros de desmídias ou até mesmo, para outros grupos de microalgas.

Considerando os questionamentos que nortearam este estudo:

1. Até que ponto a variabilidade morfológica documentada e amplamente registrada em *M. arcuata* é valida para separar espécies em variedades e formas taxonomicas?

A variabilidade morfológica documentada e amplamente registrada em *M. arcuata* se mostrou muito bem delimitada no que diz respeito aos morfotipos (variedades taxonômicas) investigadas neste estudo, os quais contituíram a grande maioria dos táxons, tais como as variedades de *M. arcuata* documentadas em literatura. A partir dos dados obtidos, inclusive os morfológicos aqui apresentados, concluiu-se que é válida e recomendável a separação de tais táxons infraespecíficos de *M. arcuata* em espécies.

De acordo com alguns autores, o status de forma taxonômica deveria ser abandonado por completo, pois ao considerar que não são entidades geneticamente “fixas”, sua separação

taxonômica não é justificável. Dessa forma, em casos como este, sugere-se a indicação de "ecomorfia" (Kowets 1984, 2008, Šťastný *et al*. 2013).

Segundo Kowets (1984), alguns táxons podem responder rapidamente e fortemente, a partir de sua próxima divisão celular, a qualquer mudança ambiental ocorrida no ambiente em que se encontram, produzindo células mal formadas, células anômalas ou células mais simplificadas em relação ao seu contorno e ornamentação. No entanto, com o retorno de condições consideradas "normais" para o desenvolvimento de cada espécie, tais formas desaparecem e são produzidas formas "normais", semelhantes à forma típica. Muitos estudos a nível populacional para o grupo das desmidías registraram a ocorrência de formas intermediárias de desenvolvimento celular, os quais muitas vezes foram propostos como variedades e novas formas taxonômicas.

Especialmente para *M. arcuata*, foi observado que existe e ocorre variabilidade morfológica a nível populacional em cada morfotipo proveniente da natureza ou presente nas culturas. Essas variações morfológicas ocorreram, principalmente, no comprimento máximo da célula, largura dos lobos polares e basais (o que é influenciado pela forma e arqueamento ou não de ambos e, na altura do lobo polar). Além disso, tais variações ocorreram ao longo de todas as amostras analisadas provenientes de doze meses de amostragem, no caso das poulações naturais. Sendo que em algumas populações/ morfotipos tal variabilidade foi mais acentuada e visivelmente registrada.

No caso das análises em especimens provenientes das culturas, foi evidenciado que a variabilidade morfológica está totalmente relacionada ao desenvolvimento desses indivíduos. Foi observado que, quando não há o desenvolvimento total dos lobos polares e/ ou basais (em muitos casos chamados de "formas imaturas"), muitos especimens se tornam parecidos quanto a sua forma, especialmente aqueles que são mais robustos ou compactos.

M. arcuata apresenta simetria bilateral assim como outras espécies de *Micrasterias* ou de outros gêneros de desmídias em geral. Dessa forma, o crescimento e caracterização celular é direcionado através de dois eixos de simetria. Um deles que divide as semicélulas em jovens e adultas, enquanto o outro eixo separa as semicélulas em lado esquerdo e direito (Savriama *et al*. 2010). Algumas diferenças podem ser observadas levando-se em consideração os componentes simétricos e assimétricos de um único indivíduo. Ou através da alometria, a qual identifica diferenças na forma celular, de acodo com o processo de crescimento (alometria ontogenética) ou através de variações em um único indivíduo (alometria estática, considerando os vários eixos de simetria), ou ainda, através da alometria evolucionária, a qual identifica mudanças quanto a forma de indivíduos entre espécies com diferentes posicionamentos

filogenéticos (Neustupa 2016).

Especialmente considerando a alometria estática, alguns estudos têm evidenciado que até 2/3 da grande variabilidade morfológica ocorrida em espécies de *Micrasterias* pode ser explicada por diferenças em semicélulas adultas e jovens, ou seja, através de processos morfogenéticos da divisão celular assexuada, fato este obervado nas culturas de *M. arcuata*.

Considerando que semicélulas jovens são menores que semicélulas adultas, já foi evidenciado, também, através de estudos experimentais, que mudanças nos fatores ambientais podem influenciar de forma consistente nos processos morfogenéticos causando marcadas alterações nas duas semicélulas de um mesmo indivíduo (Meindl 1993, Neustupa *et al*. 2008, Neustupa & Šťastný 2018). E no que diz respeito aos lados esquerdo e direito (mesmo que ambos não sejam identificados em células de *Micrasterias*), tem sido evidenciada uma leve assimetria em relação ao desenvolvimento de ambos (desenvolvimento não sincronizado), sinalizando que mesmo em indivíduos considerados simétricos, especialmente no caso de *M. arcuata*, cada um apresenta um sinal específico de desenvolvimento, especialmente sob determinada condição ambiental, o que resulta em grande variabilidade morfológica, seja no ambiente ou em condições experimentais (Savriama *et al*. 2010, Neustupa 2016, Neustupa & Šťastný 2018).

Dessa forma, considerando os dados morfológicos e filogenéticos ora apresentados, a literatura acerca da influência de váriaveis ambientais no desenvolvimento e, consequentemente, na morfologia de espécies de desmídias, sugere-se que os status de variedade e forma taxonômica sejam revistos, assim como a manutenção de ambos em muitos táxons, como os de *Micrasterias arcuata*.

2. A classificação morfológica de *M. arcuata* representa sua classificação filogenética?

Interessantemente, todos os morfotipos de *M. arcuata* analisados em condições naturais ou de cultivo que foram diferenciados através de medidas lineares tradicionais e através da morfometria geométrica apresentaram posicionamento diferente (clados distintos/ espécies distintas) ao longo da árvore filogenética de Desmidiaceae.

Fato como este foram observados, também, por Neustupa *et al*. 2010, 2011, Nemjová *et al*. 2011, Skaloud *et al*. 2012, Šťastný *et al*. 2013), pois em todos estes estudos, diferenças na morfologia celular resultaram em diferenças filogenéticas. Ressaltando que em muitos casos, espécies filogeneticamente distintas podem ser evidenciadas por diferentes métodos, inclusive, criteriosas análises morfológicas.

3. Qual o posicionamento filogenético de *M. arcuata* em relaçao ao gênero

Micrasterias, assim como gêneros e espécies próximas?

O resultado das análises moleculares e filogenéticas mostrou-se muito interessante não só por revelar o distanciamento filogenético de *M. arcuata* em relação à linhagem de *Micrasterias* e à proximidade com táxons tradicionalmente identificados como *Euastrum*. Mas principalmente, por revelar uma grande diversidade genética desconhecida dentro de uma espécie ou complexo de espécie. Foram presentemente utilizadas apenas três cepas de *M. arcuata*, utilizando três marcadores moleculares, mostrando que ainda há muito o que investigar, explorar e descobrir, neste sentido.

Considerando os resultados apresentados na presente tese, provenientes das análises filogenéticas, morfológicas (morfometria tradicional e geométrica) e, especialmente, em relação às análises dos dados provenientes da literatura clássica de *Micrasterias arcuata*, algumas importantes observações foram pautadas e até algumas modificações em alguns táxons infraespecíficos são sugeridas:

1) *Micrasterias arcuata* compreende um novo gênero da família Desmidiaceae, o qual será formalmente proposto no futuro.

2) Três morfotipos de *M. arcuata* foram delimitados filogeneticamente e morfologicamente, das quais um é considerado como espécie nova: *Micrasterias arcuata* var. *arcuata*, *(Pseudomicrasterias arcuata*), *Micrasterias arcuata* var. *perforata* (*Pseudomicrasterias perforata*) e *Pseudomicrasterias* sp..

3) Embora seu posicionamento filogenético permaneça indefinido, *Micrasterias arcuata* var. *expansa* apresentou-se morfologicamente muito bem delimitada (Capítulo 1 da presente tese), a partir dos resultados obtidos nas análises de morfometria geométrica e tradicional em populações naturais, assim como nas análises em microscópio eletrônico de varredura. Além disso, sua delimitação morfológica permaneceu clara em dados obtidos a partir da análise de populações provenientes de culturas (dados não apresentados na presente tese). Dessa forma, sugere-se que *Micrasterias arcuata* var. *expansa* seja elevada ao nível de espécie, o que será formalmente proposto no futuro.

Algumas considerações taxonômicas são sugeridas a partir da análise detalhada dos dados provenientes da literatura clássica de *Micrasterias arcuata*:

4) Embora *Micrasterias arcuata* var. *arcuata* (*Pseudomicrasterias arcuata*) e *Micrasterias arcuata* var. *gracilis* apresentam semelhanças quanrto à sua morfologia, estudos futuros são necessários para concluir o real valor taxonômico de *M. arcuata* var. *gracilis*.

5) Suegere-se que *Micrasterias arcuata* var. *robusta* f. *recurvata* Prescott & Scott, proposta por Prescott & Scott (1952) e analisada neste trabalho a partir do iconotipo e das descrições presentes em Förster (1969), seja sinonimizada em *Micrasterias arcuata* var. *expansa* ou em seu respectivo táxon em nível específico a ser proposto;

6) Sugere-se que *Micrasterias arcuata* var. *minuta* Förster, proposta como uma nova variedade por Förster (1964) seja sinonimizada em *Micrasterias arcuata* var. *expansa* ou em seu respectivo táxon em nível específico a ser proposto;

7) Sugere-se que *Micrasterias arcuata* var. *perforata* f. *magniperforata* Förster proposta por Förster (1964) seja sinonimizada em *Micrasterias arcuata* var. *perforata* Förster. ou em seu respectivo táxon em nível específico a ser proposto;

8) Sugere-se que as formas grandes e pequenas de *Micrasterias arcuata* var. *perforata* f. *robustior* Förster, porposta por Förster (1964) seja sinonimizada em *Micrasterias arcuata* var. *perforata* Förster ou em seu respectivo táxon em nível específico a ser proposto;

Outras observações referentes à taxonomia clássica de *Micrasterias arcuata* devem ser levadas em consideração:

9) *Micrasterias arcuata* var. *eckertii* Förster publicada em Förster (1981b: 245) corresponde a *Micrasterias arcuata* var. *subpinnatifida* f. *eckertii* Förster publicada por Förster (1964: 375).

10) *Micrasterias arcuata* var. *subparallela* Förster publicada em Förster (1981b: 245) corresponde a *Micrasterias arcuata* var. *subpinnatifida* f. *gracilis* Förster publicada em Förster (1964: 376).

11) *Micrasterias arcuata* var. *robusta* f. *semilunata* Förster & Eckert é citada na descrição de *Micrasterias arcuata* var *robusta* f. *goyazensis* Förster em Förster (1964: 374, 375) como referência a um material coletado pelo referido autor na região Norte do Brasil. No entanto, além desta citação, não existe nenhuma outra informação em literatura referente à forma *semilunata*.

12) *Micrasterias arcuata* var. *expansa* f. *intermedia* Norsdstedt e *Micrasterias arcuata* f. *intermedia* Nordstedt também são consideradas citações. Ambas foram documentadas em Nordstedt (1877) e buscavam caracterizar quanto a forma de *Micrasterias arcuata* var. *expansa* Nordstedt e *Micrasterias arcuata* var. *arcuata* Bailey, respectivamente. Não apresentando, portanto, nenhum valor taxonômico.

Resultados referentes a estudos polifásicos com ênfase em análises filogenéticas e

moleculares em táxons de *Micrasterias arcuata* foram apresentados pela primeira vez na presente pesquisa. Dessa forma, este estudo é pioneiro em escala mundial, pois além de ampliar o conhecimento e esclarecer as possíveis implicações taxonômicas e nomenclaturais existentes em populações de *M. arcuata*, visou, principalmente, suprir a carência de informação sobre o polimorfismo e a plasticidade fenotípica na referida espécie e em suas categorias taxonômicas infraspecíficas.

Apesar dos avanços na utilização da taxonomia polifásica, o uso da taxonomia clássica de nível α, baseada em medidas tradicionais, ainda é usada mundialmente e constitui ferramenta imprescindível na identificação, delineamento e caracterização de táxons de desmídias, especialmente no Brasil, onde o uso da biologia molecular, filogenia, estudos ecofisiológicos ou ecológicos e até mesmo, o uso da morfometria geométrica como ferramenta auxiliar à morfometria tradicional, são praticamente inexistentes para este grupo de microalgas.

No entanto, é extremamente necessário sistematizar coletas, analisar e identificar populações e não indíviduos, e acima de tudo, ter cautela ao fazer revisões taxonômicas, propor novos táxons, sejam eles espécies, variedades e / ou formas taxonômicas, para que os mesmos não correspondam a meras variações da forma típica, como formas intermediárias ou "ecomorfias", ou até mesmo, não apresentar nenhum status ou valor taxonômico. Sendo que a proposição de formas e variedades deve ser totalmente desencorajadas para o grupo das desmídias.

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