UNIVERSIDADE FEDERAL DO PARANÁ

RAQUEL CRISTINA MARRA

DISTRIBUIÇÃO, EVOLUÇÃO MORFOFISIOLÓGICA E TESTES DE TOXICIDADE (PFOA) DE DIATOMÁCEAS

CURITIBA

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DISTRIBUIÇÃO, EVOLUÇÃO MORFOFISIOLÓGICA E TESTES DE TOXICIDADE (PFOA) DE DIATOMÁCEAS

Tese apresentada ao curso de Pós-Graduação Genética, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutora em Genética.

Orientadora: Profª. Drª. Vanessa Merlo Kava
Coorientadora: Profª. Drª. Thelma Alvim Veiga Ludwig

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ATA DE SESSÃO PÚBLICA DE DEFESA DE DOUTORADO PARA A OBTENÇÃO DO GRAU DE DOUTOR EM GENÉTICA

No dia trinta de abril de dois mil e vinte às 14:00 horas, na sala Anfiteatro 01, Departamento de Genética, foram instaladas as atividades pertinentes ao rito de defesa de tese da doutoranda **RAQUEL CRISTINA MARRA**, intitulada**: DISTRIBUIÇÃO, EVOLUÇÃO MORFOFISIOLÓGICA E TESTES DE TOXICIDADE (PFOA) DE DIATOMÁCEAS.**, sob orientação da Profa. Dra. VANESSA MERLO KAVA. A Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação em GENÉTICA da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: VANESSA MERLO KAVA (UNIVERSIDADE FEDERAL DO PARANÁ), DAIANI CRISTINA SAVI (PONTIFíCIA UNIVERSIDADE CATóLICA DE JOINVILLE), LYGIA VITORIA GALLI TERASAWA (UNIVERSIDADE FEDERAL DO PARANÁ), DIEGO DE OLIVEIRA CORREA (UNIVERSIDADE FEDERAL DO PARANÁ). A presidência iniciou os ritos definidos pelo Colegiado do Programa e, após exarados os pareceres dos membros do comitê examinador e da respectiva contra argumentação, ocorreu a leitura do parecer final da banca examinadora, que decidiu pela APROVAÇÃO. Este resultado deverá ser homologado pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais definidos pelo programa. A outorga de título de doutor está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, VANESSA MERLO KAVA, lavrei a presente ata, que vai assinada por mim e pelos demais membros da Comissão Examinadora.

CURITIBA, 30 de Abril de 2020.

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VANESSA MERLO KAVA
Presidente da Banca Examinadora

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em GENÉTICA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **RAQUEL CRISTINA MARRA** intitulada: **DISTRIBUIÇÃO, EVOLUÇÃO MORFOFISIOLÓGICA E TESTES DE TOXICIDADE (PFOA) DE DIATOMÁCEAS.**, sob orientação da Profa. Dra. VANESSA MERLO KAVA, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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RESUMO

Diatomáceas são microalgas que compõem a base da cadeia trófica e possuem parede celular composta por sílica a qual está diretamente relacionada com a divisão celular e identificação do táxon (taxonomia). Estas microalgas apresentam grande diversidade de espécies e são encontradas em diversos habitats, porém a identificação de espécies para diversos táxons das diatomáceas ainda não segue padrões universais aceitos para a comumidade científica. Identificações equivocadas podem comprometer diferentes estudos, como a análise de bioindicadores, na distribuição de espécies e na diversidade. O uso de ferramentas genéticas em conjunto com dados morfológicos poderia auxiliar nesta questão, porém ainda não há consenso sobre qual ou quais regiões do genoma de diatomáceas deveriam ser utilizadas. Os objetivos deste trabalho foram: 1) no primeiro capítulo, apontar o status sobre a identificação, distribuição e situação atual das espécies do gênero *Brachysira.* 2) No segundo capítulo, avaliar o efeito do ácido perfluorooctanóico (PFOA) no crescimento algal e na resposta fisiológica de defesa em diatomáceas como um possível bioindicador. 3) No terceiro capítulo, analisar a distribuição das espécies do gênero *Gomphonema,* um dos gêneros com maior número de espécies em diatomáceas, e ainda comparar a distribuição de caracteres morfológicos acoplada em uma árvore filogenética obtida a partir de sequências de DNA disponíveis em banco de dados. Para o gênero *Brachysira* foram observadas algumas incongruências na identificação das espécies, sendo que estudos moleculares para este gênero ainda são preliminares. A distribuição da diversidade se concentrou na Nova Caledônia assim como esforço amostral. Na Europa e no Brasil (Sul e Sudeste) também foi possível identificar um esforço amostral. Diatomácea teve seu crescimento afetado por PFOA (Acido Perfluorooctanóico) de forma indireta, porém o conteudo de Malondialdeído (MDA) apresentou uma relação positiva com a concentração de PFOA, mostrando que a célula na presença desse ácido ativa sua auto defesa. A análise da distribuição de caracteres morfológicos para o gênero *Gomphonema*, indicou que a presenca de estigma e forma da margem valvar foram os melhores caracteres para separar os grupos. A diversidade de espécies se concentrou apenas em um local no Canadá, provavelmente devido a estudos extensivos. Na Índia foram observados *hotspots* de diversidade, já descritos para outros organismos como plantas e animais. Em conclusão para estudos que envolvam diversidade morfológica das espécies para diatomáceas é necessário a adoção de outras ferramentas, tal como dados genéticos, para uma identificação mais acurada e que evite resultados equivocados, como por exemplo, em estudos com bioindicação. O uso de diatomáceas como bioindicador acarreta respostas fisiológicas desses organismos que ainda não são bem compreendidas e que precisam ser minuciosamente estudadas, principalmente devido ao seu genoma complexo, que deu origem a diferentes rotas metabólicas.

Palavras-chave: diatomáceas, distribuição, diversidade, molecular, caracteres morfológicos, evolução,

ABSTRACT

Diatoms are microalgae that form part of the base of the food chain and have a cell wall composed of silica which is directly related to cell division and taxon identification (taxonomy). These microalgae have a great diversity of species that are found in different habitats, however the identification of species for different taxa of diatoms is not an easy task. And the mistaken identifications can tend to results from several areas of study, as in the analysis of bioindication and distribution of cosmopolitan, endemic and diversity species. The use of genetic tools in conjunction with morphological data could help in this matter, however due to the high complexity of the diatoms genome, an efficient genetic tool has not yet been defined. The objective of this work was: In the first chapter, to point out the status on the identification, distribution of species of the genus *Brachysira* and current situation on applications of genetic data for the genus. In the second chapter, the effect of perfluorooctanoic acid on algal growth and on the physiological defense response in the species of diatoms was evaluated as a possible bioindicator. And in the third chapter, the distribution of species of one of the largest genus in number of species, the genus *Gomphonema*, was analyzed and the evolution of morphological characters was analyzed with material available in an online database for this genus. For the genus *Brachysira*, some inconsistencies were observed in the identification of species, the distribution of diversity was concentrated in New Caledonia as well as a sampling effort that, in addition to New Caledonia, was also concentrated in Europe and Brazil (South and Southeast), as expected status for genetic molecular studies for this genus is still in the beginning. Diatoms had its growth affected by PFOA (Perfluorooctanoic Acid) indirectly, showing no positive relationship (PFOA:growth rate), however the content of Malondialdehyde (MDA) showed a positive relationship with the concentration of PFOA, showing that the cell in the presence of PFOA activates your self defense. The analysis of the evolution of characters for the genus *Gomphonema*, showed that the presence of stigma and shape of the valve margin were the best characters to separate the groups and the diversity of species was concentrated in only one location in Canada, probably due to extensive studies in this area, and in India, indicating a diversity hotspots already known to other organisms, such as plants and animals. In conclusion, for studies involving morphological diversity of species for diatoms, it is necessary to adopt other tools (such as genetic data) for a more accurate identification and to avoid erroneous results, both for pure identification (taxonomy) and for studies that use species identification to obtain results, for example in bioindication. The use of diatoms as a bioindicator leads to physiological responses of this organism that are still not well understood and that still needs to be thoroughly studied, mainly due to its complex genome that gave rise to different metabolic routes.

Palavras-chave: diatoms, distribution, diversity, molecular, evolution, morphological characters.

SUMÁRIO

1. INTRODUÇÃO

Diatomáceas são as microalgas mais abundantes que estão presentes em quase todo ambiente aquático desde de águas continentais, oceanos, (MANN; DROOP 1996; MANN, 1999; BALDAUF, 2008) e até mesmo em ambientes subaéreos úmidos (CAMBURN 1982; LOWE et al., 2014) apresentam um relevante papel ecológico participando da base da cadeia alimentar aquática (ARMBRUST, 2009), sendo o principal representante dos produtores primários para a cadeia trófica contribuindo em aproximadamente 40% da produção primária marinha (MANN, 1999; FALKOWSKI et al., 2004). A principal característica das diatomáceas é a presença de uma parede celular formada por sílica, chamada frústula, que tem importante papel na reprodução/divisão celular e na identificação das espécies (KOOISTRA et al., 2007), com ornamentações e formas que variam de espécie para espécie, sendo uma ferramenta importante para os taxonomistas nas identificações (ROUND; CRAWFORD; MANN, 1990).

Estas microalgas também apresentam características fisiológicas/ moleculares específicas, como por exemplo, diferenciação no cloroplasto e a presença de clorofila *c*, resultante da endossimbiose secundária que também ocasionou troca de material genético (BOWLER et al., 2008) transferência horizontal de genes (BAULDALF 2008; HOCKIN et al., 2012; RAYMOND; KIM, 2012). O cloroplasto é resultado de fagocitose de um organismo já fotossintetizante, filogeneticamente próximo as algas vermelhas que originou até 4 membranas em torno desta organela (BAULDAF, 2008) e que manteve a clorofila *c*. Com esse consequente mosaicismo de evolução a diatomáceas apresentam genes para diferentes novas rotas metabólicas (CURTIS et al., 2012) provenientes de trocas de sinais de genes, entre o núcleo e a organela (WILHELM et al., 2006) os resultados são mudanças fisiológicas e bioquímicas significativas neste grupo. Essa permuta de genes apresenta um importante papel evolucionário na diferenciação entre os grupos das microalgas (CHAN et al., 2011; KEELING, 2010).

Entretanto, poucas espécies de diatomáceas tiveram seu genoma sequenciado, *Phaeodactylum tricornutum, Thalassiosira oceanica* e *Thalassiosira pseudonana* (BOWLER et al., 2008). Para diatomáceas de água doce ainda não há nenhuma espécie com o todo o genoma sequenciado. O não sequenciamento e o mosaicismo de genes que as diatomáceas apresentam, traz uma dificuldade maior no aprofundamento dos estudos

moleculares genéticos, como na filogenia, e na determinação de único barcode para identificação de espécies.

A utilização de barcode para auxílio na identificação de espécies já é comum em outros grupos, tais como o uso do marcador ITS, utilizado em fungos (TAYLOR et al., 2008) e em plantas vasculares (SHIBA et al., 2006). Apesar de já haver alguns marcadores que são utilizados para identificação de espécies, a definição de um barcode único para as diatomáceas é essencial, já que dependendo do gênero/espécie, alguns marcadores são menos eficientes que outros na determinação da espécie. Além disso, um barcode definido auxiliaria numa correta identificação de espécies, como nas espécies crípticas, e contribuiria em diversas áreas de estudos, tais com na taxonomia, bioindicação e distribuição das espécies.

Há peculiaridades entre gêneros/espécies, como restrição a algum ambiente com determinada condição ou apresentar um amplo espectro de tolerância a diversos ambientes com diversas condições abióticas e limnológicas (por exemplo *Gomphonema parvulum*) (POULICKOVÁ et al., 2017). Além dessas características, a grande diversidade de espécies e um curto ciclo de vida faz com que as diatomáceas sejam consideradas excelentes bioindicadores de ambientes aquáticos (ROUND; CRAWFORD; MANN, 1990; KITNER; POLÍCKOVÁ 2003; MEDEIROS et al., 2017; POULICKOVÁ et al., 2017). Porém, estudos que utilizam a identificação de espécies como ferramenta "chave" para obter resultados, precisam utilizar outras ferramentas além da morfologia (ROSE; COX, 2014), evitando identificações imprecisas e conclusões equivocadas.

Devido sua complexidade genética (GOULD et al., 2006; MOUSTAFA et al., 2009) e morfológica comparado a outras microalgas, ainda há muitos estudos a serem feitos com diatomáceas. Dessa forma, nesse trabalho o primeiro capítulo pretende apontar o status sobre a identificação, distribuição das espécies do gênero *Brachysira* e situação atual sobre aplicações de dados genéticos para o gênero. No segundo capítulo, foi avaliado o efeito do ácido perfluorooctanóico no crescimento algal e na resposta fisiológica de defesa nas diatomáceas como um possível bioindicador. E no terceiro capítulo foi analisada a distribuição das espécies de um dos maiores gênero em número de espécie, o gênero *Gomphonema* e foi analisado evolução de caracteres morfológicos com material disponível em banco de dados online.

2. OBJETIVOS

Objetivo Geral

- Conhecer aspectos da distribuição e status dos gêneros *Brachysira*, distruibição da diversidade morfológica e desenvolvimento de caracteres morfológicos para o gênero *Gomphonema* e bioindicação da diatomácea a um poluente ambiental pouco estudado com diatomáceas.

Objetivo específico

- Identificar formas de distribuições do gênero *Brachysira* e *Gomphonema*

- Estabelecer o status sobre estudos moleculares para o gênero *Brachysira* e *Gomphonema*

- Apontar formas para uma melhor identificação das espécies de *Brachysira*

- Estabelecer evolução de caracteres para o gênero *Gomphonema* visando verificar relação de ancestralidade em relação a morfologia das espécies do gênero.

- Verificar mecanismos de defesa expressas pelas diatomáceas em respostas fisiológicas quando exposto ao ácido perfluorooctanóico.

3. REVISÃO BIBLIOGRÁFICA

3.1 DISTRIBUIÇÃO DAS ESPÉCIES DE DIATOMÁCEAS

Para estudos que envolvem distribuição de espécies é necessária uma correta identificação dos táxons, para que não haja resultados tendenciosos. No entanto, existem práticas taxonômicas não padronizadas entre pesquisadores, além disso a taxas de diversificação de espécies pode apresentar diferenças entre os habitats (NAKOV et al 2018), como por exemplo, no complexo *Gomphonema gracile* comumente encontrada nos ambientes aquáticos de águas continentais (REICHARDT, 2015) que apresentam espécies crípticas/semicripticas o que dificulta a identificação destes táxons.

A análise molecular é uma ferramenta importante para auxiliar na diferenciação de morfoespécies/ complexos (ABARCA et al., 2019) como por exemplo, na identificação de espécies que antes eram considerados generalista e/ou cosmopolitas (VANORMELINGEN et al., 2008). O contrário também é válido, juntando morfotipos de uma única espécie que podem apresentar diferenças morfológicas durante seu

desenvolvimento resultante em identificações de diferentes espécies do mesmo organismo (ROSE; COX, 2014), o mesmo pode ser aplicado em espécies que são tipicamente mais isoladas, podendo apresentar variabilidade morfológica dentro de uma espécie como por exemplo, espécimes de ambientes de água doce, (VANOEMELINGEN et al., 2007).

Para entender sobre distribuição de espécies de diatomáceas, além de dominar a taxonomia para o grupo, é necessário compreender sobre a forma de dispersão, o endemismo e a diversidade de espécies. Em diatomáceas a dispersão é principalmente passiva (VANORMELINGEN et al., 2008), causada sobretudo pela água, pelos animais, pelo vento e pelos humanos (KRISTIANSEN, 1996), sendo este último o maior causador de introdução de espécies exóticas (FOISSNER, 2006).

Segundo Vanormelingen et al. (2008), estudos baseados em morfologia refinada, trazem a prevalência de padrões de distribuição restrita entre espécies de diatomáceas, ou seja, endemismo por espécies, porém dentro de um nível taxonômico mais alto (gênero), considerando que os gêneros de diatomáceas são cosmopolitas. Porém um estudo mais recente, contradiz essa afirmação ao trazer gêneros endêmicos (vivos e fósseis), encontrado em boa parte na Ásia (KOCIOLEK, 2019). Entretanto, ainda há lacunas para melhor compreender o endemismo de gênero já que a utilização da filogenia não é rotineiro para as diatomáceas e além disso essas microalgas apresentam grande número de gêneros e os dados disponíveis e incluídos em análises filogenéticas são insuficientes (KOCIOLEK, 2019).

Além do endemismo, ainda há questões não esclarecidas na diversidade de espécies, uma vez que é pressuposto que a diversidade de espécies de micro-organismos aquáticos é subestimada (LOGARES 2006). Para diatomáceas essa baixa diversidade pode ser resultado de diversas identificações baseadas somente nas floras europeias em diferentes locais no mundo durante muito tempo que podem resultar em identificações equivocadas influenciando na distribuição e diversidade de espécies (VANOEMELINGEN *et al*. 2008). Entretanto, nessa última década para o gênero *Gomphonema* há muitas espécies novas endêmicas sendo descritas (KARTHICK et al., 2011; KARTHICH et al., 2015; KOCIOLEK et al., 2016). Além disso, comunidades de água doce apresentam frequentemente maior riqueza de espécies do que comunidades marinhas, consequentemente devido a alterações do ambiente e a uma elevada taxa de mudanças de espécies dentro das comunidades. (NAKOV et al., 2018).

De forma geral a diversidade morfológica para diatomácea é muito ampla e há muitas variáveis que podem influenciar na morfologia dessa microalga que ainda não são claramente definidas. Para tanto a identificação das espécies com utilização de dados taxonômicos consistentes ainda apresenta diversos obstáculos os quais métodos moleculares podem auxiliar. A padronização e efetiva identificação das espécies pode influenciar a forma como vemos a distribuição da diversidade de espécies como no endemismo e espécies cosmopolitas, auxiliando em diversos estudos de diferentes áreas.

3.2 O USO DAS DIATOMÁCEAS COMO BIOINDICADORAS

A ampla distribuição e diversidade de espécies em diferentes ambientes no mundo tornaram as diatomáceas uma ferramenta importante nos estudos com bioindicação de ambientes aquáticos. A substituição de alguns táxons por espécies mais tolerantes de diatomáceas em uma assembleia impactada é o ponto central em pesquisas sobre bioindicação (POLICKOVÁ et al., 2017), podendo esta assembleia impactada ser comparada com outras de um ambiente de referência, não impactado (FALASCO et al., 2019). Outra forma em que podemos verificar alterações ambientais atuais ou paleolimnológicas é através de alterações morfológicas, uma vez que em condições estressantes, como o herbivorismo, variáveis abióticas e limnológicas, podem ocorrer alterações na morfologia da frústula sendo esta plasticidade uma estratégia adaptativa da diatomácea para o habitat que se encontra (EVANS FOULDS; CARR, 1976; ZHU et al., 2015). A mesma relação pode ser aplicada em pesquisas paleolimnológicas como nos estudos de Spanbauer et al. (2018) no qual os autores analisaram amostras de 400 kyr (400.000 years ago) e relacionaram as alterações morfológicas de uma diatomácea cêntrica, com alterações na hidrodinâmica local. De forma resumida, as diatomáceas utilizadas como bioindicadores para ambientes aquáticos contemporâneos e paleolimnológicos trazem informações valiosas sobre variáveis abióticas e limnológicas do ambiente em questão (MEDEIROS et al., 2017; NGUYEN et al., 2012; RUOCCO et al., 2016).

Alguns estudos utilizando diferentes metodologias para identificação de espécies de diatomáceas na bioindicação, como ferramentas genéticas ainda apresentam algumas dificuldades. Por exemplo, utilização de *metabarcoding* para identificação e formulação de um inventário taxonômico, que é utilizado para calcular o índice de qualidade apresenta pela água, resultados que quando comparado com a análise morfológica

(taxonomia), evidencia discrepância na presença de táxons. Isto pode ocorrer na presença DNA livre, de frústulas de células mortas e diversidade de espécies crípticas e pode comprometer os resultados do estudo, principalmente quando baseado em apenas uma dessas metodologias (metabarcoding ou taxonomia) (VALENTIN et al., 2019).

Outros trabalhos mostraram que a diversidade morfológica pode não ser resultado de espécies diferentes. Segundo Rose e Cox (2014) a variabilidade nos caracteres morfológicos durante o ciclo de vida pode gerar fenótipos diferentes a partir de um mesmo genótipo. E diversos fatores podem influenciar na plasticidade morfológica, como substâncias mutagênicas, tais como alguns elementos químicos encontrados no ambiente aquático (TIAM et al., 2019; WOOD et al., 2017). Eles não só podem causar alterações na sequência de nucleotídeos da molécula de DNA (LI, 2018) como também acarretar mudanças morfológicas.

Além dessas mudanças, as diatomáceas também respondem com alterações fisiológicas quando estão expostas ao efeito de poluentes (GONÇALVES et al., 2018; MOISET et al., 2015). Essas alterações resultados da influência desses poluentes na expressão gênica das diatomáceas (MOISSET et al., 2015; TIAM 2012). De forma geral essas microalgas apresentam diferentes respostas quando expostas a ambientes alterados, como a poluição aquática.

Porém, apesar de vários estudos relacionarem bioindicação e resposta do organismo à exposição a poluentes (morfológica, fisiológica e genética) (ÇELEKLI et al., 2018; GONÇALVES et al. 2018; WOOD et al., 2016), ainda existem alguns poluentes, como o PFOA (ácido perfluorooctanóico), comumente encontrado em águas doces e marinhas, que apresentam poucos estudos utilizando diatomáceas com uma ferramenta de bioindicação.

3.3 ESTUDOS MOLECULARES COM DIATOMÁCEAS

Existe uma grande complexidade no genoma das diatomáceas, possivelmente devido à transferência horizontal de genes (ARMBRUST et al., 2004; BOWLER et al., 2008; RAYMOND; KIM, 2012), apesar dessa “quimera genômica”, ter conduzido à vantagens fisiológicas (BOWLER et al., 2010) essa complexidade traz dificuldades em estudos moleculares. Embora já existam alguns estudos sobre o assunto (ABARCA et al., 2019; ALVERSON et al., 2006; MEDLIN, 2018; MEDLIN et al., 2014; SIMS et al.,

2006; RUCK et al. 2016; NAKOV et al. 2018), ainda existe muito desconhecimento na área da genética com essas microalgas.

Entre essas dificuldades, temos os estudos para definição de um *barcode* do grupo (HAMSHER et al., 2011; ZIMMERMANN et al., 2011; MONIZ; KARCZMARSKA, 2009, 2010). Um barcode auxiliaria na identificação das espécies e contribuiria nos resultados de diversas áreas de estudos, tais como análise de dispersão de espécies ou estudos de identificação de espécies críptica (MANN et al., 2010).

Alguns marcadores (ribossômico, mitocondrial, cloroplastidial) já são indicados como promissores para desempenhar o papel de *barcode* para as diatomáceas e já são utilizados na identificação de algumas espécies. Os marcadores mais estudados são, *18S, LSU, Cox1*, *rbcL* e *ITS* (EVANS et al., 2008; HAMSHER e al., 2011, MANN et al., 2010, MONIZ KARCZMARSKA, 2009).

Para os marcadores para as sequências ribossômicas (rDNA) há a facilidade de análise comparativa devido ao crescimento de sequências de rDNA disponíveis em bancos de dados, como por exemplo o 18S que é o marcador mais comumente utilizado em estudos filogenéticos de diatomáceas com o maior banco de dados disponível quando comparado aos outros marcadores para algumas diatomáceas (ABARCA et al., 2014; MEDLIN. 2014; MEDLIN 2016). Além disso, o rDNA possui regiões hipervariáveis que fornecem um grande número de caracteres filogeneticamente informativos observado também que as subunidades ribossômicas diferentes podem resolver níveis muito diferentes de relações filogenéticas. Segundo Alverson (2008), *18S* auxilia nas relações de nível superior e *LSU*, assim como o *ITS*, resolvem relações de espécies.

As sequências Cox1 e rbcL possuem algumas vantagens quando comparadas ao rDNA, como por exemplo, pouca ou nenhuma variação intragenômica, além de serem fáceis de alinhar e comparar. Além disso, Cox1 e rbcL apresenta várias cópias em suas organelas, apresentando amplificação tão simples quanto o rDNA (MANN et al., 2010). Porém segundo Moniz a Kaczmarska (2009), para o gene *Cox1*, a universalidade e a qualidade da sequência são inferiores às de outros marcadores, assim como também observado por Mann et al. (2010). E em outros estudos, observou-se que para diferenciar de algumas espécies crípticas como no complexo *Sellaphora*, o *rbcL* foi mais conservado que o *Cox1*, mas com igual poder de distinção de espécies (EVANS et al., 2008; HAMSHER et al., 2011). Porém, segundo Alverson (2008), o DNA

mitocondrial, tal como Cox1, oferece caracteres filogenéticos que são informativos em vários níveis, incluindo o das espécies, mas ainda não foram totalmente explorados.

Além da variação na eficiência da determinação das espécies há outras dificuldades encontradas para a utilização dos marcadores, como a qualidade das sequências disponíveis em banco de dados mostra-se ainda é muito heterogênea (por exemplo, Genbank) (EVANS et al., 2007) e a proporção de espécies de diatomáceas por sequência disponível de genes ainda é muito pequena (MANN et al., 2010).

No entanto, cada marcador mostra características distintas que contribuem para sua eficácia como *barcode* para identificação de espécies de diatomáceas (MONIZ; KARZMARSKA, 2009). E por isso ainda não foi determinada uma única sequência como barcode que seria tão eficiente e eficaz quanto o ITS é para os fungos (TAYLOR et al., 2008).

O uso de dados moleculares como o barcode auxiliaria a taxonomia, sendo aplicados para auxiliar na identificação precisa de espécies (CASTELEYN et al., 2008, VOUILLOUD et al., 2013, KAHLERT et al., 2019), ajudando a distinguir morfótipos aparentemente cosmopolitas (POULÍBKOVÁ; MANN 2006, VORMORMINGEN et al., 2007; CASTELEYN et al., 2008; VOUILLOUD et al., 2013) e definir separações de espécies (semi)crípticas (EVANS et al 2007; MONIZ et al 2009). Em estudos ecológicos, podem auxiliar na determinação endemismo e na diversidade das espécies (AN et al., 2017; KAHLERT et al. 2019). Isto também é válido para estudos sobre a evolução de espécies às quais os dados moleculares são fundamentais nos dias de hoje (MEDLIN. 2014; MEDLIN; SIMS et al. 2006, RUCK et al. 2016).

4. Submetido (07/03/2020): Diatom Reserch; status “Under Revision”.

STATUS OF GENUS *BRACHYSIRA* (BACILLARIOPHYTA) AND ITS GLOBAL DISTRIBUTION

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Abstract

Brachysira is a widely distributed and species-rich genus. We analyzed the current status of 121 taxonomically accepted species names based on 757 available registers. We reviewed the environmental and habit preferences of species in this genus, the geographical distribution of both living and fossil species, and the sampling effort and diversity distribution in Brazil. Some inconsistencies with regard to some species names were detected, such as invalid names cited in some articles and misconceptions in taxon identification. Our results showed that *Brachysira* has a predominantly periphytic habit (363) and prefers lentic waters (327). Regarding its geographic distribution, the Australian region presented the greatest diversity of species and number of endemisms. In Brazil, there were 13 restricted species and the sample effort analysis reflected the distribution of reference centers in diatom taxonomy. Records of fossil species were only found in the western hemisphere. Our literature review showed that the number of molecular studies of *Brachysira* is still very scarce. Therefore, we emphasize that the combination of molecular and morphological data, will help accurate taxonomy and conduct to more reliable results in various research areas, such as biogeography, paleolimnology and evolution.

Keywords: Cosmopolitan, diversity, endemism, systematic review

1. Introduction

The genus *Brachysira* Kützing is a cosmopolitan and considered one of the most common diatom genera worldwide (Kociolek 2018). In 1836, Kützing described the genus as monospecific, based on the type species *Brachysira aponina* Kützing. However, some years later, this author transferred *Brachysira aponina* to *Navicula*

Bory. Round & Mann (1981) restored *Brachysira* by transferring *Anomoeoneis exilis* (Kützing) Cleve, *A. serians* (Brébisson) Cleve and *A. zellensis* (Grunow) Cleve to this genus. These authors analyzed a sample from the bay of Santorini (Aegean Sea) and found certain affinity with the material of the genus *Caloneis* Cleve and *Navicula* Bory, but light and scanning electron microscopy indicated it did not belong to these genus and it was closely related to *A. exilis*, *A. serians* and *A. zellensis*. However, they found no affinities with *A. sphaerophora* Pfitzer, the type species of the genus *Anomoeoneis*. As a result, they determined that besides the Santorini material, *A. exilis*, *A. serians*, and *A. zellensis* were similar to *Navicula aponina* Kützing.

Currently, there are 121 taxonomically accepted *Brachysira* species, most of them from freshwater (Guiry & Guiry 2019). Many studies have contributed to increasing the number of known species (Metzeltin & Lange-Bertalot 1998, Wolfe & Kling 2001, Shayler & Siver 2004), including fossil species (Lange-Bertalot & Moser 1994, Metzeltin & Lange-Bertalot 1998, Metzeltin & Lange-Bertalot, 2007). Lange-Bertalot & Moser (1994) contributed significantly to this genus with the analysis of 67 species and description of 34 new taxa (32 species, one subspecies, and one new variety). Half of these species were found in New Caledonia, contributing to broaden the geographic distribution of this genus.

Recently, Kociolek (2018) classified *Brachysira* as cosmopolitan, considering its distribution in all continents including Antarctica. However, some species in this genus are thought to be endemic (Round 1995, Metzeltin & Lange-Bertalot 2007), such as *B. acutirhomboides* Lange-Bertalot & Gerd Moser, *B. angusta* Lange-Bertalot & Gerd Moser, *B. bacillifera* Lange-Bertalot & Gerd Moser and *B. blancheana* Lange-Bertalot & Gerd Moser, which are restricted to New Caledonia (Lange-Bertalot & Moser 1994).

Species in this genus have a broad tolerance to different ecosystem conditions, which makes them useful as aquatic bioindicators. *Brachysira vitrea* (Grunow) R. Ross, *B. brebissonii* R. Ross, *B. arctoborealis* A.P. Wolfe & H.J. Kling, and *B. serians* (Brébisson) Round & D.G. Mann are commonly found in waters with low pH (Foged 1978, Comperè 1986, Cassie & Cooper 1989, Round 1990, John 1993, Caballero-Miranda 1996, Cantonati 1998, Wolfe & Kling 2001).

Brachysira is a morphologically diverse naviculoid genus that exhibits a remarkable morphological diversity (Figs 1–117), being its key features the presence of valve striae formed by transapically elongated areolae, clearly separated by one or more longitudinal hyaline undulations. The raphe is straight and filiform, with simple, straight, proximal and distal raphe endings. There are often two siliceous ribs bordering the raphe and at the junction between the valve face and mantle (Round & Mann 1981, Krammer & Lange-Bertalot 1986, Wolfe & Kling 2001). Most key features for species identification are morphological, such as the valve outline and morphometry, shape of the proximal and distal raphe endings, striae density, and the presence or absence of some features especially in the valve face (Krammer & Lange-Bertalot 1986, Lange-Bertalot & Moser 1994, Kennedy & Allott 2007).

The use of electron microscopy allows a more precise taxonomic identification, which contributes to several areas of study, such as paleolimnology and ecology, besides taxonomic studies (Wolfe & Kling 2001, Vijver 2014). However, in modern diatom taxonomy, the use of DNA molecular data is mandatory and complementary to morphological data for species identification (Casteleyn et al. 2008, Abarca et al. 2014, Kahlert et al. 2019) but molecular studies are still very scarce. *rbcL*, *SSU*, and *LSU* sequences are most commonly used for molecular identification of diatom species (MacGillivary & Kaczmarska 2011, Luddington et al. 2012, Ruck et al. 2016).

In the case of *Brachysira*, only six *SSU* and *rbcL* sequences of six specimens (two of them unidentified) are currently available at GenBank and INRA-R-Syst Diatom databases. This last-mentioned database and other microalgae-specific online databases, such as Phycobank, are in constant development or at their early stages, showing little available molecular data and thus, challenging the study of most diatoms.

Our main aim was to review the status of species names, habit and environmental preferences of the genus *Brachysira*. We also reviewed the geographic distribution of living and fossil species in published studies available, and analyzed the sampling effort and diversity distribution in Brazil, indicating the most cited species and the current status of molecular studies of this genus.

2. Material and methods

The term "*Brachysira*" and other names of genera that have already been synonymous or basionyms of *Brachysira*, alone or simultaneously with other terms, were used as keywords for the publication search (Table 1). (Table 1, near here)

We selected 757 records (Fig. 118) (Table S1) retrieved from the following databases: Science Direct, Google Scholar, Scientific Electronic Library Online - Scielo, Guiry & Guiry 2019 - AlgaeBase, Wiley Online Library, Symbiont.ansp.org, with a temporal cut-off between 2018 and 1956. (Fig. 118, near here)

We constructed a table with all screened publications, using the following variables: Journal; Year of publication; Authors; Theme; Image (presence/absence); Molecular data (presence/absence); Habit; Environment; Country; Locality; Coordinates; Limnological data (presence/absence); and Species (Table S1). Based on the species list, we constructed two tables, one with the current status of species names, the type species (when available), synonym/basionyms (Table 2) and other

morphological data comparable between species (Figs. 1–117) (Table 3). Invalid names were not included in this study.

Habit types were identified as: phytoplankton, periphyton (episammon, epiphyton, epipelon, epizoon, epiliton), *mixed* (periphyton and phytoplankton in the same article, without discerning in what kind of habit the species was found) and subaerial (only once citated). We classified the environment as: lentic; lotic; mixed (with characteristics of lentic and lotic) and *other* (rock and sandstone cliffs).

The species distributed in all continents were classified as cosmopolitan, whereas the species with frequency ≤ 32 and ≥ ten (10), which were present on almost all continents, were classified as widely distributed.

Fossil, endemic, cosmopolitan/widely distributed species and sampling effort were calculated using QGIS, version 3.0 (Quantum GIS Development Team; http://qgis.osgeo.org). The species diversity was estimated using the R environment (R Core team 2010), with the "monographaR" package (Reginato, 2016). This package generates a shapefile that was subsequently analyzed in the QGIS program.

3. Results and discussion

3.1. *Identification/ Taxonomy Highlights*

During the screening process, we detected some inconsistencies related to some species names (Table 2). *Brachysira insolita* Lange-Bertalot & Gerd Moser (synonyms: *Anomoeoneis gomphonemoides = B. gomphonemoides*), *B. palustris* (Maillard) Gerd Moser, A. Steindorf & H. Lange-Bertalot, *B. pseudoexilis* H. Lange-Bertalot & Gerd Moser, and *B. rhombica* (Guiry & Guiry 2019), which all have invalid names, were not included in Table 2. Although *Brachysira antirhomboides* Lange-Bertalot & Gerd Moser is considered a valid name in Algaebase (www.algaebase.org), there are no other

references of this name available. We also found that researchers have great difficulty in assigning species to this genus, e.g. *Anomoeoneis costata* (Kützing) Hustedt was incorrectly identified as *Brachysira costata* (see figure in Al-Handal et al. 2014). This evidences that although this genus was described a long time ago, there are still some difficulties for the correct identification of specimens. Furthermore, the description of new species is still a difficult task. For example, Kirs (1995) studied the genus *Brachysira* from the British Isles and proposed two new species, *B. pseudoprocera* and *B. tenuis*. These species were described based on valve size, number of longitudinal striae and apex shape but never validated. According to this author, *B. pseudoprocera* diferred from the closely related *B. procera* Lange-Bertalot & Gerd Moser by the presence of '*one distinct longitudinal hyaline space and slightly coarser striae*' and the presence of a population '*which seems to have a constant cell size*'. This also highlights the importance of establishing standardized features for describing new species and including other informative characters, such as the presence/absence of papillae, spines and ribs and differences in the valve shape. (Table 2–3, near here)

One of the main problems for species identification is the lack of detailed and updated images of some species, such as *B. exilis* (Kützing) Round & D.G. Mann and *B. formidulosa* (Cholnoky) Lange-Bertalot & Gerd Moser (Figs 27–30). In addition, images of type species are generally not readable due to the lack of adequate tools at the time they were described. In order to overcome these problems, it is important to continue using informative and detailed images, such as Scanning Electron Microscopy (SEM) images that would help species delineation (Round et al. 1990, Alverson 2008). In addition to the use of SEM images, the application of molecular techniques in systematic studies is essential for the identification of species and differentiation

between cryptic species (i.e. species morphologically and/or genetically identical) (Alverson 2008). (Figs 1–117, near here)

3.2 *Habit and environmental preferences*

Benthic diatoms are present in almost all types of stable substrates, such as rocks (epilithon), sand (epipsammon), aquatic animals (epizoic), sediments (epipelon) and on aquatic plants (epiphyton) (Lowe & Laliberte 1996, Townsend & Gell 2005), being classified as periphytic. Many taxa are present in more than one substrate type, however, some of them proved to have preference for only one type of habit (Lim et al. 2001).

Two-hundred and eighty of the total publications we screened (757), did not classify the species according to the type of habit (periphyton/phytoplankton). As shown in Table 4, 160 of these records corresponded to review articles; 94 to taxonomic records from 1981 to 2007, 21 to floristic studies and only five (05) to other research areas (e.g. Bioindicators and Ecology). Of the 477 that cited the habit type, 235 were floristic, 133 ecological, 47 review articles, 42 about Bioindicators and 20 on other themes (*Other*) (Table 4). Of these articles, 363 records corresponded to periphyton, 69 to *mixed* substrates, 44 to phytoplankton, and only one of these records corresponded to the sub-aerial habit type (*A. serians* var. *brachysira* = *B. brebissonii* from sandstone cliffs, Camburn, 1982) (Table 5). Some studies showed species restricted to the phytoplankton, whereas others can be found both adhered to substrates and in the phytoplankton (Bartozek et al. 2017). However, we believe that most phytoplanktonic species might have been detached from the substrate by the water current.

Our study showed that most species were periphytic, corroborating most previous records of *Brachysira* species in diatom communities (Patrick & Reimer 1966, Lange-

Bertalot & Moser 1994, Camburn & Charles 2000, Gaiser & Johansen 2000, Shailer & Siver 2004). Besides the habit type, it is also important to better understand the environmental preference of species, since abiotic and limnological factors can affect the microalgal communities (Benito et al. 2018, Oliveira & Bicudo 2018). (Tables 4–5, near here)

As shown in Table 6, 174 of the screened publications (22.99%) did not report the type of environment from which the specimens were collected, being 141 revision records, 26 taxonomic studies, and seven (07) floristic inventories. We found 583 records citing the environment of each species (Tables 6–7), which corresponded to lentic (327), lotic (172), *mixed* (82), and *other types of environment* (02). We expected the greatest number of taxa in lentic environments, although they were found in both lotic and lentic environments (Kahlert & Gottschalk 2014). Most studies indicated that *Brachysira* is commonly found in lentic and acidic environments, such as lakes and ponds (Patrick & Reimer 1966, Lange-Bertalot & Moser 1994, Battarbee et al. 1997, Camburn & Charles 2000, Gaiser & Johansen 2000, Kovács et al. 2006, Kennedy & Allott 2017). (Table 6–7, near here)

3.3 *Geographic distribution*

3.3.1 Endemic, cosmopolitan and widely distributed taxa

According to Taylor (1996) and Vyverman et al. (2007), the Australian region, where New Caledonia is located, has a high degree of endemism. Lange-Bertalot & Moser (1994) found 25 species of *Brachysira* in New Caledonia, and only one species, *B. brebissonii*, was cited at that time as cosmopolitan.

Vanormelingen et al. (2007) and Pla-Rabés et al. (2016) observed that diatom communities have a limited dispersion, which is regulated by the same processes

applied to other organisms, but with different intensity, resulting in endemism in isolated areas.

In the Neotropical region, endemism is concentrated in northern South America, with seven endemic species in Colombia, four in Brazil and three in Guyana (Amazon region) (Fig. 119). Lange-Bertalot & Moser (1994) described eight new species of *Brachysira,* contributing significantly to the diversity of this genus in South America.

Four species of the 13 endemic species found in Brazil are restricted to the Amazon region, whereas the remnants are distributed throughout the country (Fig. 119). The high number of endemisms in this region may be due to the preservation of the place, which is little explored with few studies, mainly taxonomic compared to the rest of the country. (Fig 119, near here)

To identify endemic species, taxonomic and morphological analyses of fine grains are very valuable (Vanormelingen et al. 2007). Molecular data is essential to corroborate morphological identification (Vouilloud et al. 2013), avoiding errors due to the impact of various factors on the cell wall structure and consequently, on the morphology (Kilham & Kilham 1975, Abarca et al. 2014).

Poulíková & Mann (2006) demonstrated that environmental factors can foster morphological changes in diatoms. These authors found that morphologically different species from very distant environments were able to reproduce, and consequently belong to a single cosmopolitan species.

Although this genus is considered cosmopolitan, *B. styriaca* (Grunow) R. Ross and *B. zellensis* (Grunow) Round & D.G. Mann have not been registered yet in Brazil. Lange-Bertalot & Moser (1994) published a note on the distribution of these species, highlighting the need for revision of the identifications of *B. styriaca* outside Nordic-

Alpine conditions. *Brachysira zellensis* is also distributed in temperate and cold regions of the northern hemisphere.

In the Neotropical region, Brazil has the greatest number of cosmopolitan species of *Brachysira*, especially in the South-Southeastern region (Figs 120–121), probably due to the distribution of diatom-specialist centers. These centers are located in the states of Paraná (UFPR), São Paulo (IBT-SP), and Rio Grande do Sul (UFRGS), with 85%, 36%, and 13% of all Brazilian citations, respectively (Fig. 121). (Fig 120–121 , near here)

It is interesting to note that most studies ecological studies involving species distribution showed that cosmopolitan or widely distributed species are dominant or abundant (Gaiser & Johansen 2000, Kilroy et al. 2006, Kapetanovic & Hafner 2007, Kim et al. 2007, Lee et al. 2013). Some species, such as *B. wygaschii* Lange-Bertalot, are widely distributed but have not been recorded yet for Brazil (Fig. 122). *Brachysira procera* has only one reference available up to date, which is most likely due to its endangered species status (Lange-Bertalot 1996, Gesierich & Kofler 2010) (Fig. 123). In the case of *B. styriaca* (Lange-Bertalot 1996, Cantonati et al. 2009) and *B. zellensis* (Lange-Bertalot 1996, Gesierich & Kofler, 2010) both are considered cosmopolitan in the present study. (Fig 122–123, near here)

In general, it is essential to identify correctly both endemic and cosmopolitan species, which requires that researchers use several tools to obtain a correct and reliable result (Casteleyn et al. 2008, Vouilloud et al. 2013). Therefore, the use of molecular tools could help understanding diatom taxonomy and biogeography, especially when comparing worldwide distributed populations (Vouilloud et al. 2013).

Fossil species

The cell walls of diatoms are formed by silica and therefore, are easily preserved as fossils for thousands or millions of years. The earliest records of diatom fossils are from sponge reef deposits of the Early Jurassic (~ 180 million years ago (Mya)) (Rothpletz 1896). Other diatom records were confirmed from the Cambrian (Vologdin 1962, Gapeev 1992, 1995, Allison & Hilgest 1986), although Sieminska (2015) considered that they have already existed in freshwater environments of the Eon Proterozoic.

Diatom fossils have great economical importance, and also diatomite mines have a well-known application in various areas (Gürü et al. 2008, Chu et al. 2010, Ergun 2010, Engh & Staff, 2014). Diatom fossils are also used as a tool for indicating the presence of oil and gas pockets, age dating of oil reserves, as well as being themselves essential sources of oil and gas (Krebs, 1999). This was due to the expansion in the occurrence of marine diatoms from the late Eocene throughout the Miocene, which was accompanied by carbon deposits that represents more than 10% of the world's conventional oil and gas discovered so far (Klemme & Ulmishek 1991, Cermeño 2016). As there is often a spatial coincidence of silica and fossil fuels, diatoms have a key role in the formation of oil reserves and recent studies are already using this information together with biomarkers such as 24-norcholestane or C28-C29 steranes in sediments and source rocks (Cermeño 2016, Beinoiston 2017).

Besides their economic importance, the increase in knowledge of new fossil diatoms is also key for understanding the evolution and diversification of species. Furthermore, fossil information is essential for understanding species evolution (Sims et al. 2006), and together with molecular phylogeny, can unravel the evolutionary history of diatoms (Koistra et al. 2003, Medlin & Kaczmarska 2004, Sims et al. 2006, Nakov et al. 2018a) together with species change in fossil communities.

The pattern of evolution of some modern genera agrees reasonably with some extinct diatoms (Sims et al. 2006). Diatom fossils showed marine radiation at the Cenozoic era, with increased diversity at the late Eocene (~ 38–31 Mya) and a subsequent decrease followed by an increase at the Miocene (23-5.3 Mya).

Diatom fossils are also used in Palaeolymnology to obtain information from species fluctuations/changes in the communities at a given geological time and to infer the characteristics of ancient environments (Bradbury 1971, Reid et al. 1995) with regard to water quality (e.g. pH, salinity, etc), humidity, and presence of anthropogenic stress (Bradbury 1971, Sato & Kumano 1986, Reavie & Cai 2019).

Due to the importance of diatom fossils for both academic and economic studies, we first need to better understand the diversity of species, as well as where and in which geological time they occurred.

In our study, fossils species to only 7.43% of all species recovered and were represented by *B. delicatissima* D.Metzeltin & Lange-Bertalot, *B. elliptica* D.Metzeltin & Lange-Bertalot, *B. fossilis* (Reimer) Lange-Bertalot & Gerd Moser, *B. hannae* (Reimer) Lange-Bertalot & Gerd Moser, *B. hedyklingiae* D.Metzeltin & Lange-Bertalot, *B. insulsa* D.Metzeltin & Lange-Bertalot, *B. macroserians* D.Metzeltin & Lange-Bertalot, *B. potapovae* D.Metzeltin & Lange-Bertalot, and *B. wolfe* D.Metzeltin & Lange-Bertalot. These fossil species have hitherto been documented almost exclusively in Iconographya Diatomologica and Bibliotheca Diatomologica (Table 8).

The distribution of all fossil species of this genus has been restricted to the Americas (North, Central, and South America). According to Metzeltin and Lange-Bertalot (2007), *B. delicatissima*, *B. potapovae* and *B. wolfei* are found only inrestricted to Florida, Santa Rosa County (USA) but some years later, Montoya-Moreno et al.

(2013) recorded *B. delicatissima* in Colombia. In the Neotropical region, fossil species are restricted to Brazil, Colombia, and Guyana (Fig. 124) (Table 8). (Table 8, near here)

Fossil species of this genus have already been found in sediments dating more than 22,000 years ago (Haberyan 2017). *Brachysira vitrea* was found 9,000 years ago between the late Pleistocene and Holocene, indicating an acidic environment (Bradbury 1986) and also in 50 Canadian arctic lakes of the preindustrial period (Rühland et al. 2003). In addition, they were found in almost all studies involving temporal and aquatic environmental transitions such as changes from acidic to alkaline conditions (Paleolymnology: Bradbury 1986, Rühland et al. 2003, Quillen et al. 2013, Li et al. 2018). However, most records are of fossil species that are contemporary, such as *B. neoacuata* Lange-Bertalot (Quillen et al. 2013), *B. serians*, and *B. vitrea* (Bradbury, 1986). (Fig 124, near here)

3.3.2 Sampling Effort

Our research showed 165 records for Brazil (Fig. 125) (Table 9), mostly from the South and Southeastern regions (Rio Grande do Sul, Paraná, and São Paulo), which corresponded to 81.2% of the Brazilian records (Fig. 126) (Table 10). This is due to a large number of diatom studies conducted in these regions (Moreira & Moreira-Filho et al. 1982, Torgan et al. 1999, Tremarin et al. 2009, Bertolli et al. 2010, Marra et al. 2016, Oliveira & Bicudo 2018, Pellegrini & Ferragut 2018). (Table 9, near here) (Fig 125–126, near here)

France showed 43 records of *Brachysira,* however, these records include New Caledonia and Guadaloupe Island, which geopolitically belong to France but are located in the southwest Pacific Ocean and Caribbean sea, respectively. Most of these records attributed to France (95.35%) are from New Caledonia, according to Bibliotheca Diatomologica. (Table 10, near here)

Our results showed that *Brachysira* is a cosmopolitan genus distributed worldwide, as observed by Kociolek (2018). However, we should consider that older studies did not have access to most tools we use today, such as scanner electron microscopy and sequencing, so the species identification could be not as refined as it is today. In addition, many taxonomic studies in different regions of the world used European floras as reference (Hustedt 1927-1966, Krammer & Lange-Bertalot 1986-1991) (Kociolek & Spaulding 2000, Vanormelingen et al. 2007), adjusting the local flora to European standards (Taylor 1996). In this case, we must take into account that our study was also based on old records (1956) and may present some identification of species that today would be considered equivocal.

3.3.3 Diversity of species

The species diversity of *Brachysira* was as expected, with most species (17) distributed in New Caledonia, Australian region (Fig. 127), as a result of the review of Lange-Bertalot & Moser (1994), followed by South America with 01 to 15 species, the United States with 01 to 14 and Europe, with 1 to 12 species (Fig. 128). Other studies of diatom diversity corroborated that the Australian region has an isolated flora with a high level of endemism and species diversity (Taylor 1996, Vyverman et al. 2007). (Fig 127, near here)

In diatom diversity, historical processes (i.e., colonization and extinction, dispersion and migration) constrain global patterns in regional and local diatom diversity (Vyvermann et al. 2007). Isolation together with some particular physical and chemical characteristics seem to favor divethe high diversity (Kennedy & Allott 2017), since Lange-Bertalot & Moser (1994) found that in New Caledonia, the soil is ultra-basic, rich in heavy metals and poor in phosphorus, nitrogen, potassium, and calcium.

In South America (Fig. 128), the greatest species diversity was found in Colombia (Montoya-Moreno & Aguirre 2012, 2013, Vouilloud et al. 2013). In Brazil, although the sampling effort was high (Table 10), the species diversity was low (one to seven species), being the highest in the state of Paraná (seven species).

The species diversity in the USA was similar to South America, whereas the European diversity can be due to the numerous taxonomic studies performed in this region (Fig. 128). (Fig 128, near here)

According to Vanormelingen et al. (2007), species diversity has traditionally been underestimated due to very subtle morphological differences between some species. Another common problem in diatom taxonomy, which hampers species identification is that often more than one name is attributed to a single species (Kociolek & Spaulding 2000). Again, molecular studies (such as phylogeny) are essential to better understand diatom diversity patterns (Nakov et al. 2018b) and to identify species correctly (Vouilloud et al. 2013).

4 Conclusion

Current status and future perspectives of Brachysira

We found some incongruities or uncertainties with regard to the correct species name. Therefore, since most types lack available images for consultation, a detailed analysis of all types of *Brachysira* species is required. This will bring a more robust basis for the species identification and description of new species. Likewise, future studies need to provide more careful feature documentation for describing new species.

We should also consider that the data analysed in the present study could have inaccurate identifications since most studies analysed were from 1956 to 2018, did not have molecular information, and only some of them have images (Table S1).

The correct species identification is also relevant to analyse distribution patterns. Understanding diatom distribution is very important, and is almost always integrated with other study areas (Kociolek & Spaulding 2000). Considering this, our study provides a rough picture of the distribution of *Brachysira*, identifying hotspots of diversity and endemism, as well as most cited locations, particularly in Brazil.

Our results also showed that New Caledonia has a great number of endemisms, high diversity, and sampling effort, with a distinct diatom flora, corroborating the data of Vyverman et al. (2007) for the Australian region (Taylor 1996).

In South America, Colombia presented the greatest number of endemisms and species diversity, which will probably increase in the future because there are still few studies of *Brachysira* in this country. In the case of Brazil, there is a large number of citations (i.e. sampling effort) with little endemism and species diversity, probably due to the presence of diatom-specialized study centers located in the South and Southeastern regions of the country.

With regard to diatom fossils, we believe that further studies are needed to identify fossil species in other regions, since most fossil diatoms are so far restricted to Americas.

In addition, molecular techniques are considered a main tool in almost all areas of study (e.g. in studies on species evolution and phylogeny; Koistra et al. 2003, Medlin & Kaczmarska 2004, Sims et al. 2006, Ruck et al. 2016, Nakov et al. 2018a) and an auxiliary tool to morphology, contributing to the correct species identification (Casteleyn et al. 2008, Vouilloud et al. 2013, Kahlert et al. 2019) and to differentiation between apparently cosmopolitan morphotypes (Poulíbková & Mann 2006, Vormormingen et al. 2007, Casteleyn et al. 2008, Vouilloud et al. 2013). In the case of

ecological studies, molecular information might help to determine endemic species (Kahlert et al. 2019).

Diatoms usually show great complexity, probably due to horizontal gene transfer derived from secondary endosymbiosis (Armbrust et al. 2004, Bowler et al. 2008, Raymond & Kim, 2012) which resulted in a "genomic chimera" that brought them physiological advantages to dominate the sea (Bowler et al. 2010). Molecular studies are still scarce, probably due to this genomic complexity (Alverson et al. 2006, Amato et al. 2007, Behnke et al. 2004, Koistra et al. 2003, Medlin & Kaczmarska 2004, Sims et al. 2006, Ruck et al. 2016, Nakov et al. 2018a).

Currently, most molecular studies are focused diatom barcoding. The use of DNA barcodes would help microscopic identification, especially in certain cases in which extinct and modern species cannot be physically distinguished, and in analyses of species dispersal and cryptic species (Mann et al. 2010). Some markers (ribosomal, chloroplastidial, mitochondrial) already are indicated as promising to play the barcode role.

rDNA has hypervariable regions that provide a large number of phylogenetically informative characters. Different ribosomal subunits are used to resolve phylogenetic relationships at different levels. The small rDNA subunit (SSU or 18S) is useful for analysing higher-level relationships, whereas the large subunit (LSU or 28S) D1 - D3 and internal transcribed spacer regions (ITS) are employed to resolve relationships between species (Alverson 2008). Due to the increasing number of rDNA sequences available in public databases, the use of rDNA as diatom barcodes will allow comparative analysis. However, COI and rbcL sequences have some advantages compared to rDNA, such as little or no intragenomic variation, and the presence of multiple copies within their organelles. Furthermore, their amplification is as

straightforward as rDNA sequences, and they are easy to align and compare (Mann et al. 2010). According to Alverson (2008), mitochondrial DNA, such as COI, although not yet fully explored for diatoms, provides informative phylogenetic characters at various levels, including at and below the species level. rbcL sequences proved to be more conserved than COI for differentiation of species of the *Sellaphora* complex but showed equal power than COI for species distinction (Evans et al. 2007, Hamsher et al. 2011). In addition, COI showed some problems of universality and failed to differentiate between some diatom strains (Mann et al 2010).

The quality of diatom sequences of these and other markers and vouchers deposited in public databates, such as Genbank, is still very heterogeneous (Mann & Evans, 2007). Furthermore, the number of diatom species sequenced is yet small (Mann et al. 2010).

Our research showed no previous molecular studies published on the genus *Brachysira*. However, there are only six sequences of this genus currently available as unpublished data in public databases, two (02) of which belong to unidentified *Brachysira* spp (gen: SSU and rbcL), *Brachysira exilis* (Kützing) Round & D.G. Mann (gen: SSU), *B. microcephala* (Grunow) Compère, *B. neoexilis* Lange-Bertalot (gen: rbcL), and *B. vitrea* (Grunow) R.Ross (gen: rbcL) (Genbank 2019; Rimet et al. 2015 - INRA-R-Syst Diatom). The scarce number of sequences evidenced that molecular studies of diatoms are still at an early stage compared to other diatom genera (e.g., *Nitzschia* spp.). In addition, the quantity and quality of some sequences available at databases are very heterogeneous (Genbank 2019; Rimet et al. 2015- INRA-R-Syst Diatom).

Molecular data can be considered an essential tool in reconstructing evolutionary phylogenies of species, as mentioned above for fossil species (Koistra et al. 2003,

Medlin & Kaczmarska 2004, Sims et al. 2006, Ruck et al. 2016, Nakov et al. 2018a), and also in ecological studies, to evaluate the environmental quality (Keck et al. 2016) by linking different environmental factors to taxonomic information (Kahlert et al. 2019). In the future, the use of diatom barcoding will not replace the use of microscopy, because many aspects of community structure and function cannot be determined otherwise (Mann et al. 2010).

In conclusion, our study shows the importance of including molecular data in different areas of diatom research, which will, in turn, provide information of great reliability on species distribution and taxonomy. The use of molecular data would avoid some errors in species identification, such as those observed herein for *Brachysira*, enable the differentiation between cryptic species and resolve doubts about cosmopolitan/endemic species. This review provided an overview of the current status of the genus *Brachysira*, showing the current knowledge about its species in different study areas and the need for studies that aggregate more than one research area. However, there is still a need for a broader study involving the reanalysis of types and cryptic species and the application of diatom barcoding for species identification.

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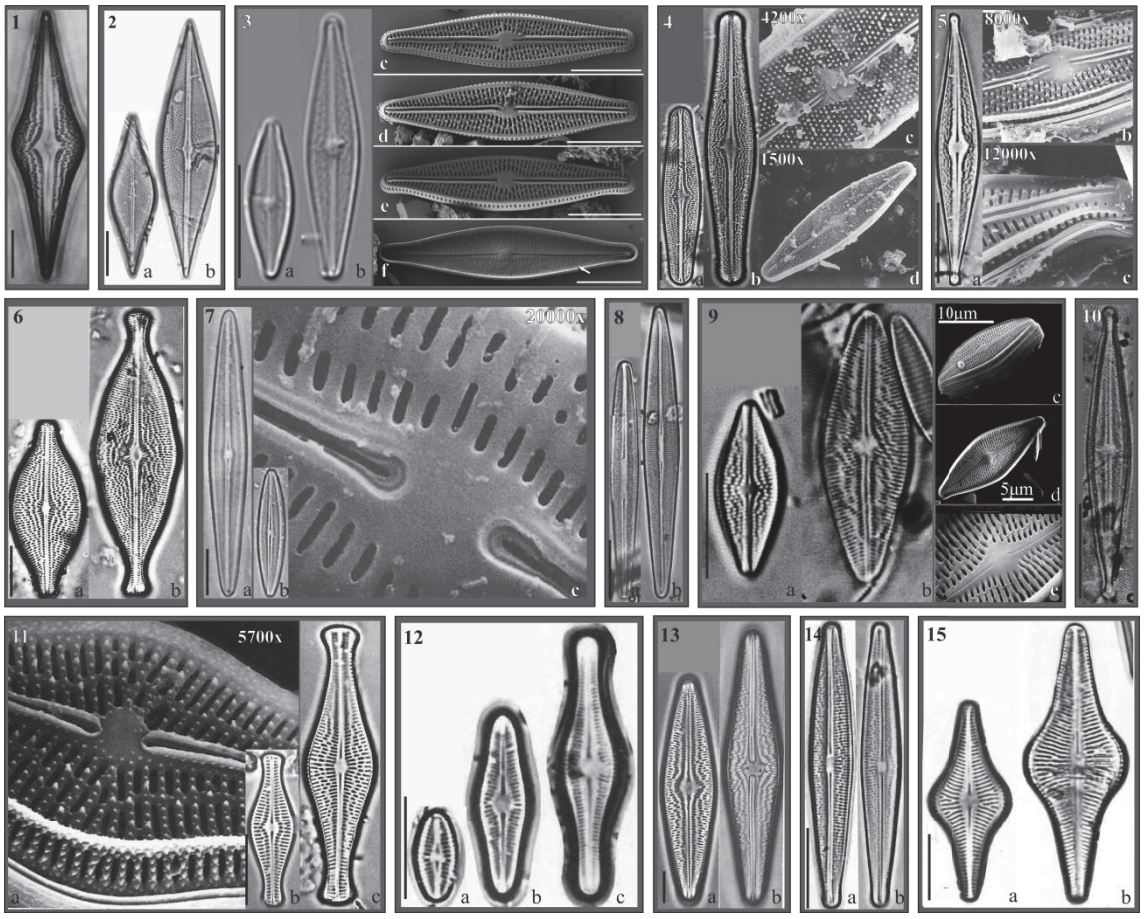
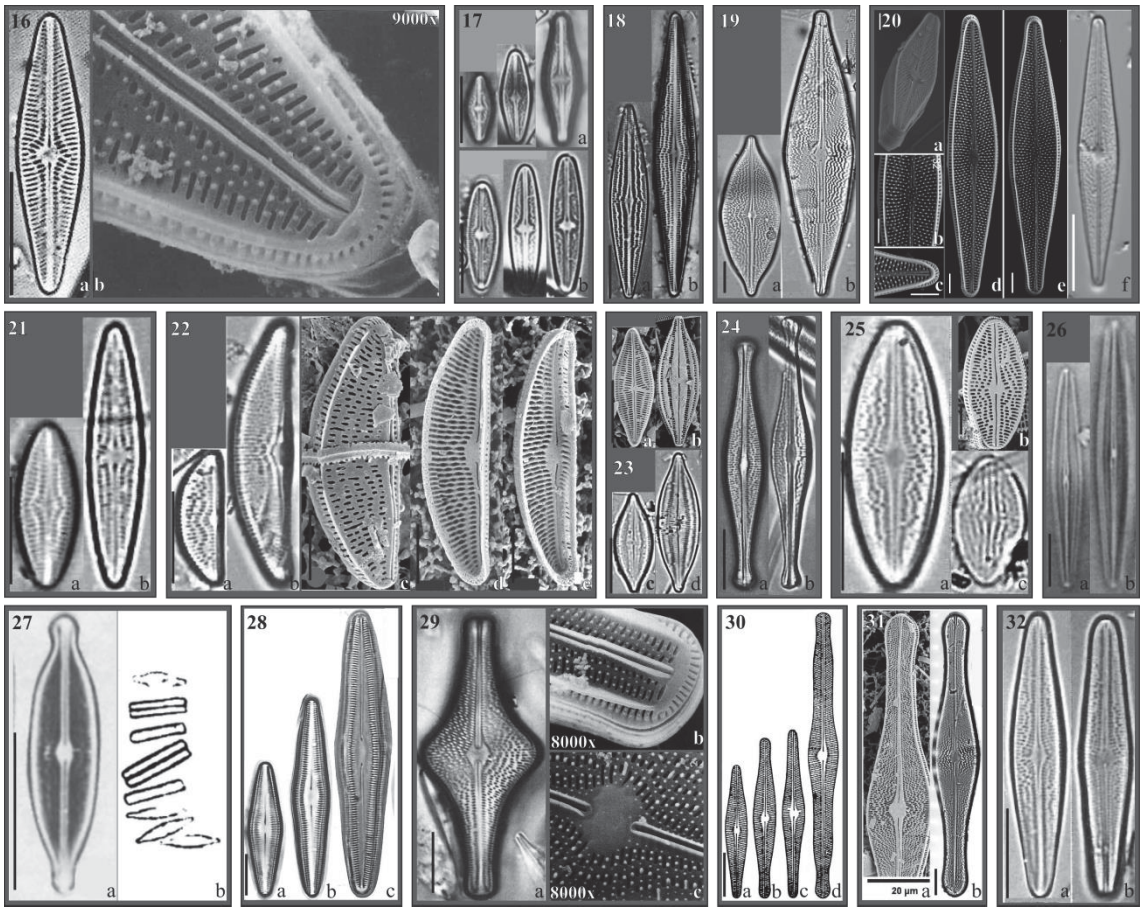
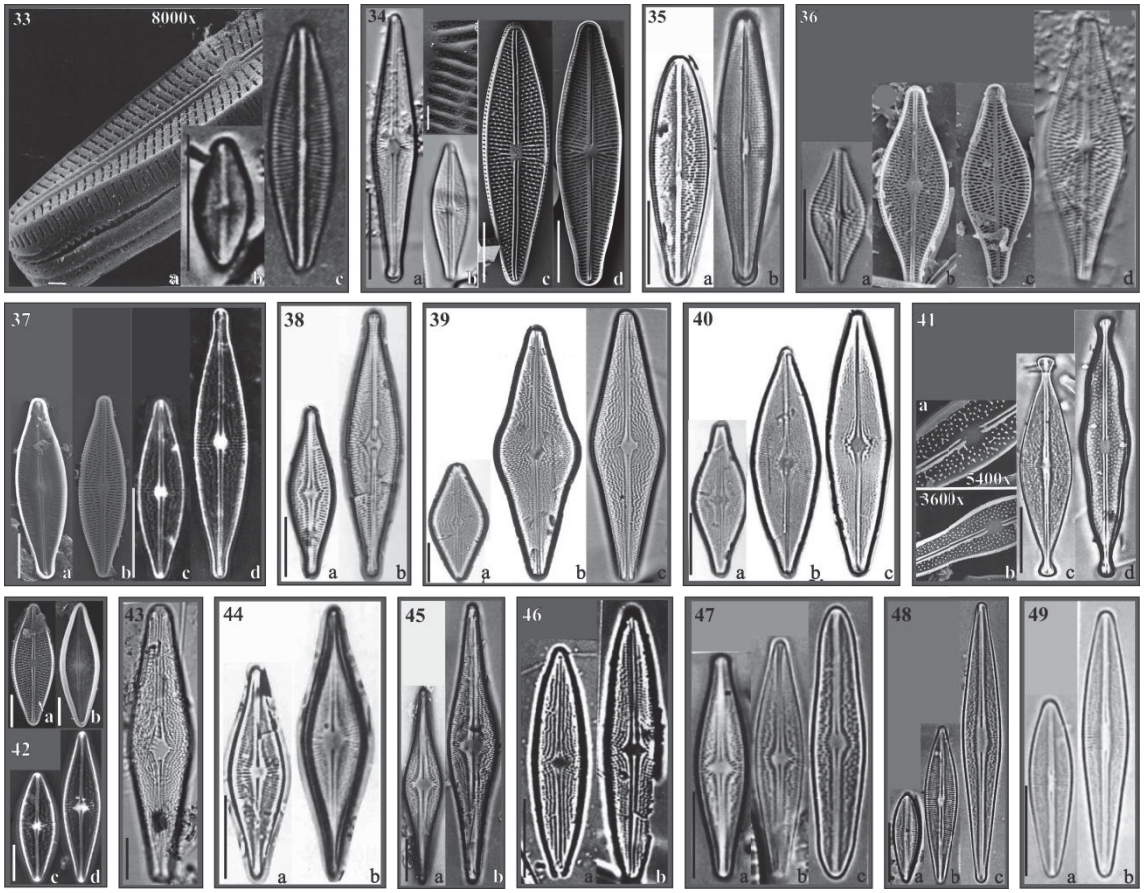


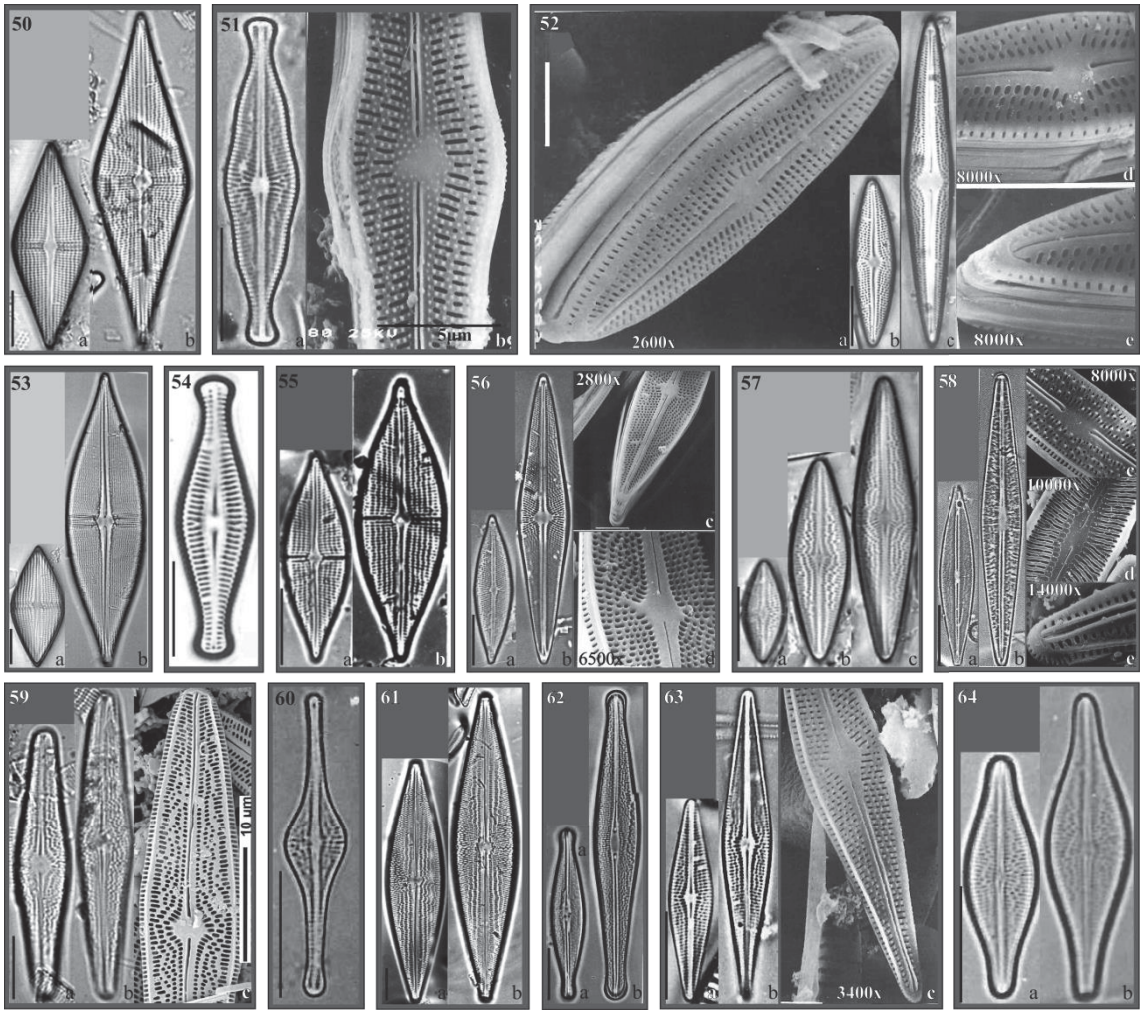
Figs 1–15. Species of the genus *Brachysira* and their morphological diversity (LM and SEM). 1. *Brachysira acutirhomboides* (Lange-Bertalot & Moser, 1994); 2. *B. acutíssima* (Moser et al., 1998); 3. *B. Altepetlensis* (D.Mora, R. Jahn & N. Abarca, 2017); 4. *B. amoena* (Lange-Bertalot & Moser 1994); 5. *B. angusta* (Lange-Bertalot & Moser, 1994); 6. *B. apiculata* (Lange-Bertalot & Moser, 1994); 7. *B. aponina* (Kützing, 1836); 8. *B. archibaldii* M. Coste & M. Ricard; 9. *B. arctoborealis* (Wolf and King, 2001); 10. *B. atacamae* (Hustedt) D.G. Mann, 1990; 11. *B. australofollis* (Lange-Bertalot & Moser, 1994); 12. *B. babuschka* (Moser et al., 1998); 13. *B. bacillifer* (Lange-Bertalot & Moser, 1994); 14. *B. blancheana* Lange-Bertalot & Moser, 1994; 15. *B. bouletiana* (Moser et al., 1998). Scale bars = 10 μm (Figs 1–15).



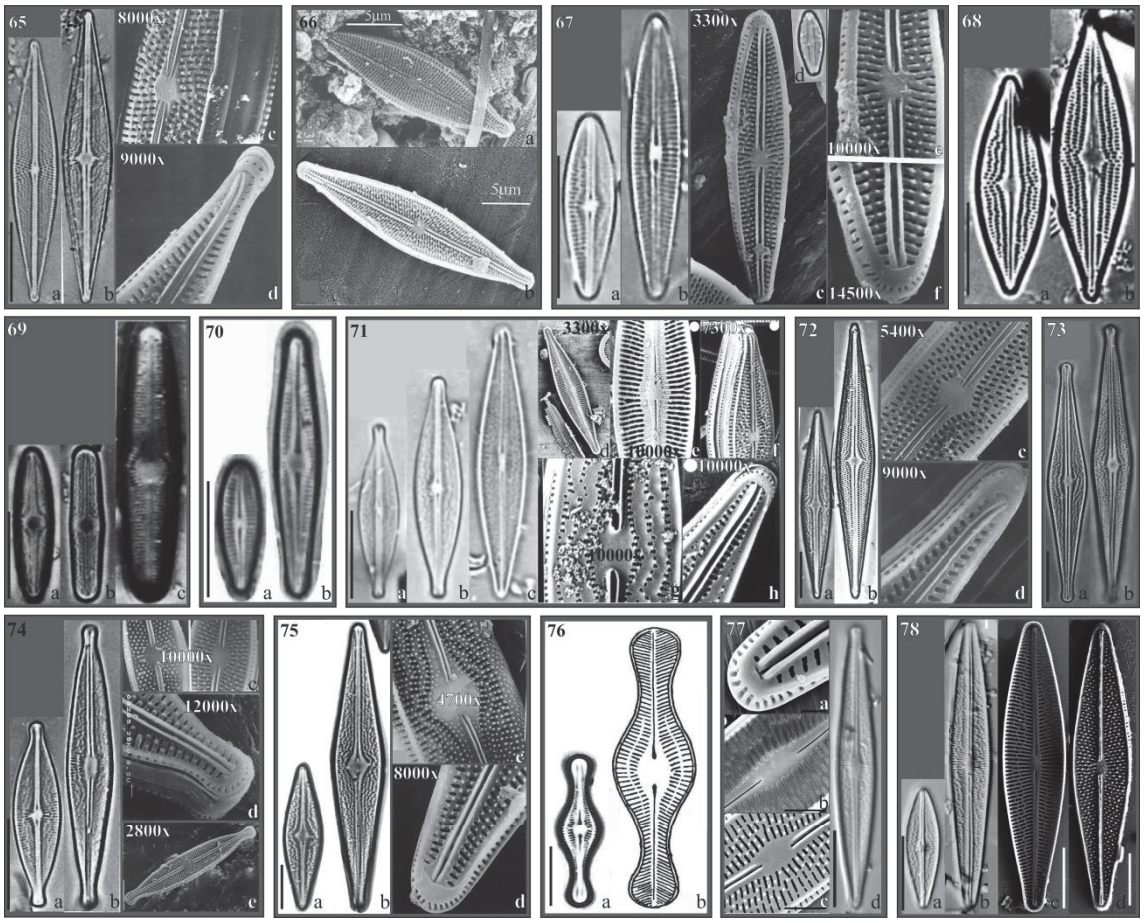
Figs 16–32. Species of the genus *Brachysira* and their morphological diversity (LM and SEM). 16. *Brachysira brebissonii* (R.Ross 1986); 17a, b. *B. calcícola* (Lange-Bertalot & Moser, 1994); 18. *B. calligraphica* (Lange-Bertalot & Moser, 1994); 19. *B. charlesreimeri* (Metzeltin & Lange-Bertalot, 2007); 20. *B. conamarae* (Kennedy & Allot, 2017); 21. *B. coralina*; 22. *B. cymbelliformis* (Metzeltin & Lange-Bertalot; xxx); 23. *B. delicatissima* (Metzeltin & Lange-Bertalot, 2007); 24. *B. dumbeana* (Lange-Bertalot & Moser, 1994); 25. *B. elliptica* (Metzeltin and Lange-Bertalot, 2007)*;* 26. *B. estonarium* (Witkowski *et al*., 2000); 27a, b. *B. exilis* (Round & Mann, 1981); 28. *B. feickertiae* (Moser *et al*., 1995); 29. *B. follis* (Ehrenberg) R.Ross, 1986); 30. *B. formidulosa* (Cholnoky) Lange-Bertalot & Gerd Moser 1994); 31. *B. fossilis* ((Reimer) Lange-Bertalot & Gerd Moser 1994*)*; 32. *B. frenguelli* (Metzeltin & Lange-Bertalot, 2007). Scale bars = 10 µm (Figs 16–30 e 32).



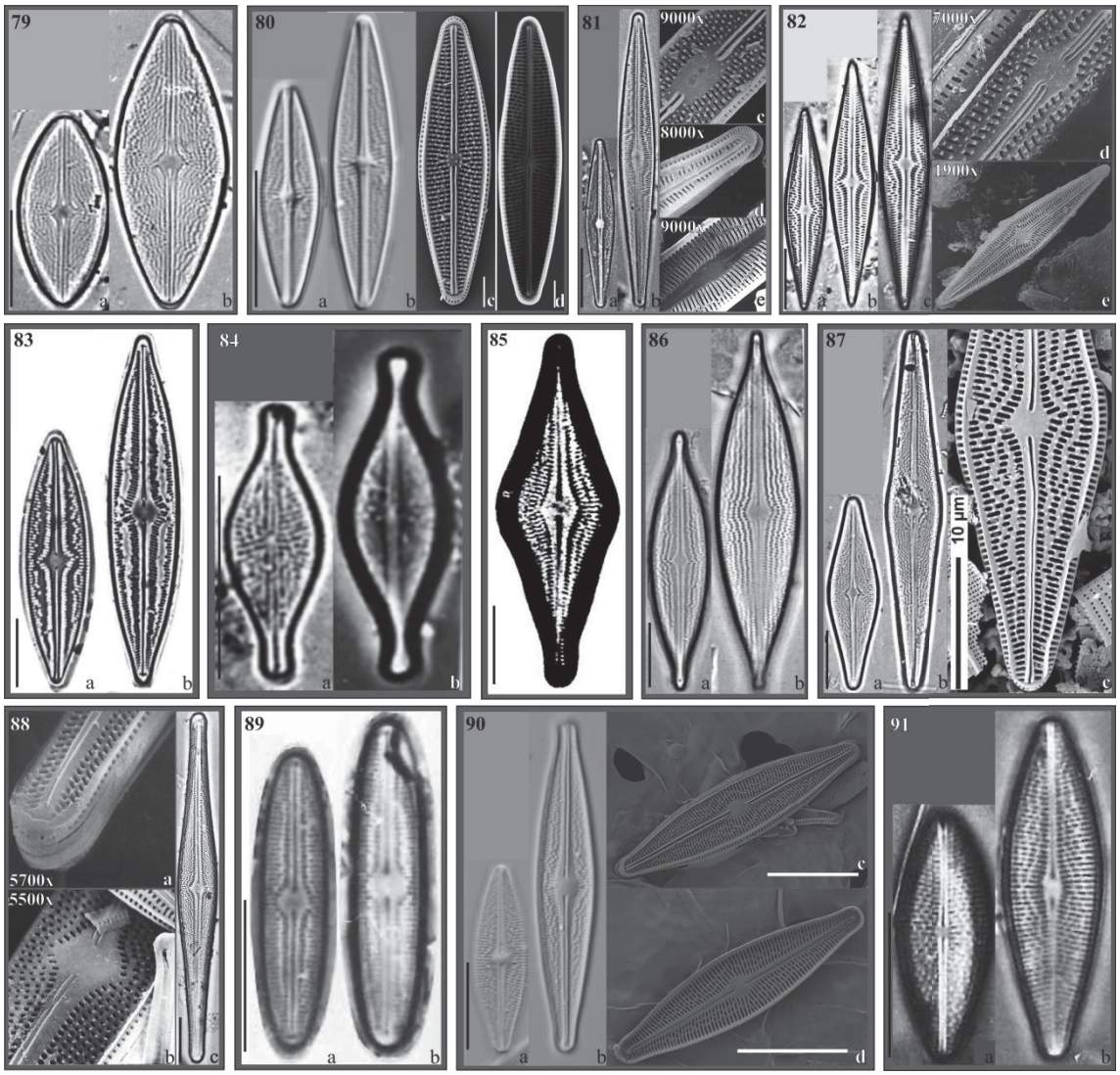
Figs 33–49. Species of the genus *Brachysira* and their morphological diversity (LM and SEM). 33. *Brachysira garrensis* (Lange-Bertalot & Moser, 1994); 34. *B. gatesii* (Kociolek & Lowe, 2013); 35. *B. gomphonemoides* (Lange-Bertalot & Moser, 1994); 36. *B. gravida* (Shayler & Siver 2004); 37. *B. guarrerai* (Vouilloud *et al*., 2014); 38. *B. guttiformis* (Moser et al., 1998); 39. *B. hannae* ((Reimer) Lange-Bertalot & Moser 1994); 40. *B. hedyklingiae* (Metzeltin & Lange-Bertalot, 2007); 41. *B. hofmanniae* (Lange-Bertalot & Moser, 1994); 42. *B. huitotarum* (Vouilloud *et al*., 2014); 43. *B. Inamoena* (Metzeltin & Lange-Bertalot, 1998); 44. *B. incognita* (Moser, Lange-Bertalot & Metzeltin, 1998); 45. *B. inflata* (Lange-Bertalot & Moser, 1994); 46. *B. insulsa* (Metzeltin & Lange-Bertalot 1998); 47. *B. intermedia* (Lange-Bertalot & Moser, 1994); 48. *B. irawanae* (Lange-Bertalot & Moser, 1994); 49 *B. irawanoides* (Lange-Bertalot & Moser, 1994). Scale bars = 10 μm (Figs 33–49).



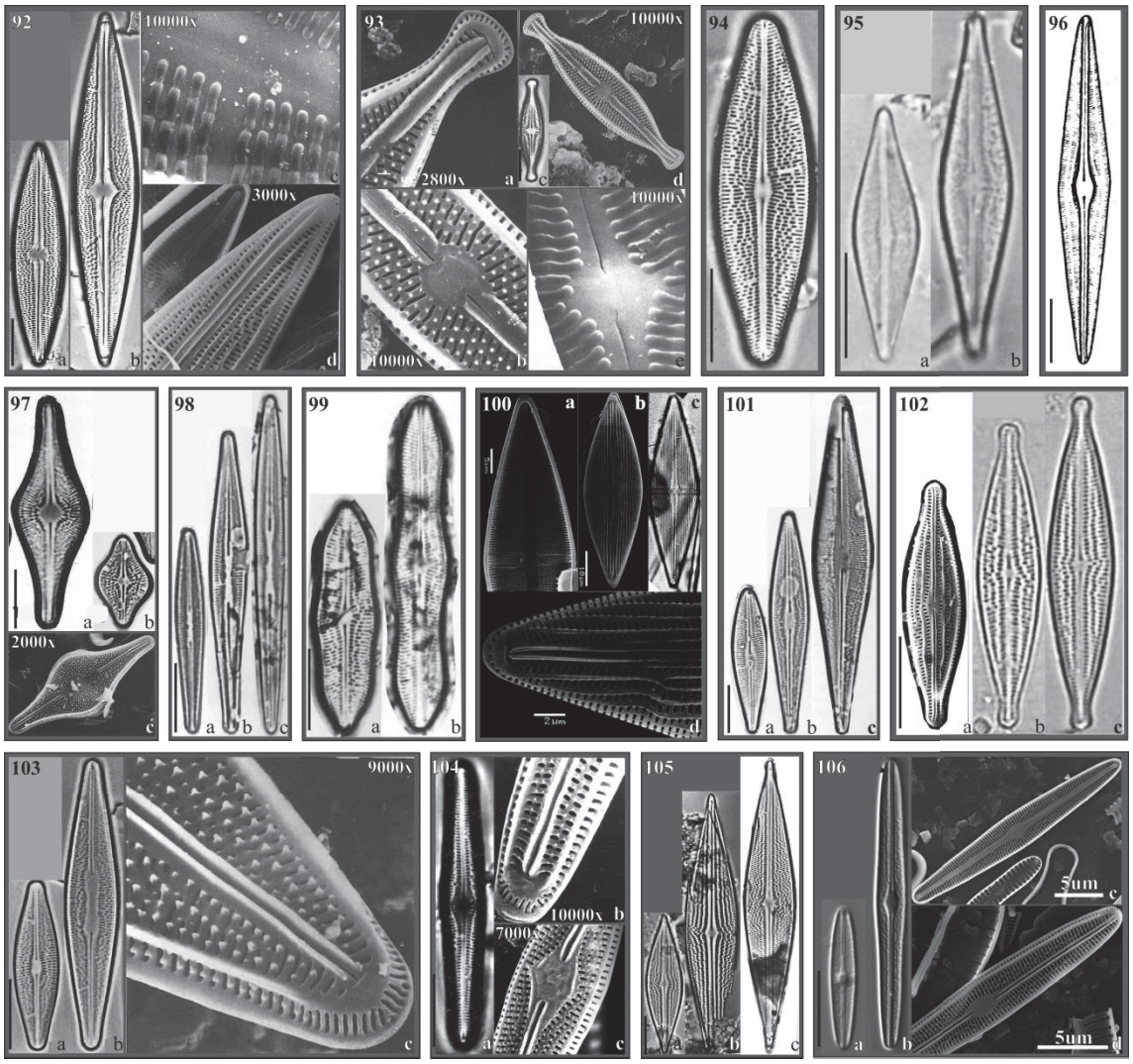
Figs 50–64. Species of the genus *Brachysira* and their morphological diversity (LM and SEM). 50. *Brachysira jankae* (Metzeltin & Lange-Bertalot 2007); 51. *B. Julio* (Lange-Bertalot & Moser, 1994; 52. *B. krammeri* (Lange-Bertalot & Moser, 1994); 53. *B. kuntzei* ((Reichelt) Metzeltin & Lange-Bertalot 1998); 54. *B. labrata* (Moser *et al.,* 1995); 55. *B. lange-bertalotii* (Metzeltin & Lange-Bertalot, 1998); 56. *B. lecohui* (Lange-Bertalot & Moser, 1994); 57. *B. lehmanniae* (Lange-Bertalot & Moser, 1994); 58. *B. liliana* (Lange-Bertalot & Moser, 1994); 59. *B. linearilanceolata* (Metzeltin & Lange-Bertalot, 2007); 60. *B. longirostris* (Hustedt) Mann, 1990; 61. *B. macroserians* (Metzeltin & Lange-Bertalot, 1998); 62. *B. maillardii* (Lange-Bertalot & Moser, 1994); 63. *B. manfredii* (Lange-Bertalot & Moser, 1994); 64. *B. manoylovae* (Metzeltin & Lange-Bertalot, 2007). Scale bars = 10 µm (Figs 50–64).



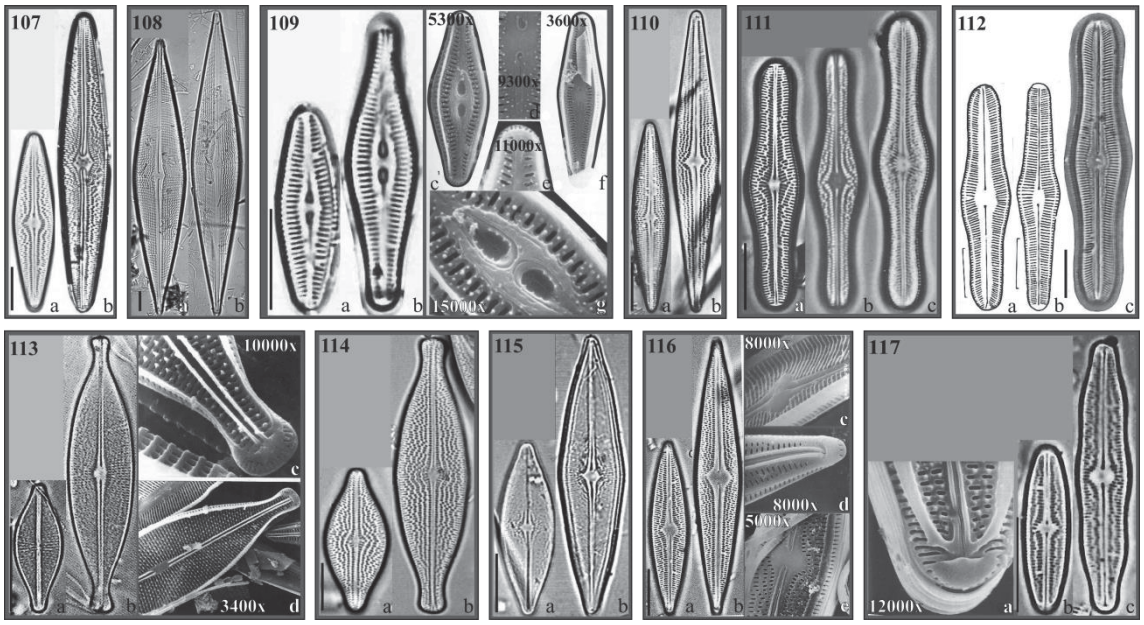
Figs 65–78. Species of the genus *Brachysira* and their morphological diversity (LM and SEM). 65. *Brachysira metzeltinii* (Lange-Bertalot & Moser, 1994); 66. *B. microcephala* (Grunow) Compère 1986; 67. *B. microclava* (Lange-Bertalot & Moser 1994); 68. *B. microserians* (Metzeltin & Lange-Bertalot, 1998); 69. *B. minor* (Lange-Bertalot & Moser, 1994); 70. *B. nanoclava* (Moser *et al*., 1998); 71. *B. neglectissima* (Werum & Lange-Bertalot 2004); 72. *B. neoacuta* (Lange-Bertalot & Moser, 1994); 73. *B. neocaledonica* (Lange-Bertalot & Moser, 1994); 74 *B. neoexilis* (Lange-Bertalot & Moser, 1994); 75. *B. nubigena* (Lange-Bertalot & Moser, 1994); 76. *B. obesa* (Moser et al., 1995); 77. *B. ocalanensis* (Shayler & Siver 2004); 78. *B. ontonageniana* (Kociolek & Lowe, 2013). Scale bars = 10 μm (Figs 65–78).



Figs 79–91. Species of the genus *Brachysira* and their morphological diversity (LM and SEM). 79 *Brachysira potapovae* (Metzeltin & Lange-Bertalot, 2007); 80. *B. praegeri* (Kennedy & Allott, 2017); 81. *B. procera* (Lange-Bertalot & Moser, 1994); 82. *B. pseudoacuta* (Lange-Bertalot & Moser, 1994); 83. *B. pulchra* (Lange-Bertalot & Moser, 1994); 84. *B. pumila* (Metzeltin & Lange-Bertalot 1998); 85. *B. rhomboides* ((Hustedt) Mann 1990); 86. *B. rostrata* (Metzeltin & Lange-Bertalot, 1998); 87. *B. ruckiae* (Metzeltin & Lange-Bertalot, 2007); 88. *B. rumrichiae* (Lange-Bertalot & Moser, 1994); 89. *B. ruppeliana* (Moser *et al*., 1998); 90. *B. sandrae* (Van de Vijver, 2014); 91. *B. seippii* (Lange-Bertalot & Moser, 1994). Scale bars = 10 µm (Figs 79–91).



Figs 92–106. Species of the genus *Brachysira* and their morphological diversity (LM and SEM). 92. *Brachysira serians* ((Brébisson) Round & Mann 1981); 93. *B. silvícola* (Lange-Bertalot & Moser, 1994); 94. *B. simplex* (Lange-Bertalot & Moser, 1994); 95. *B. sinaiensis* (Werum & Lange-Bertalot, 2004); 96. *B. spectabilis* ((Manguin) Lange-Bertalot & Moser, 1994*)*; 97. *B. speluncola* (Lange-Bertalot & Moser, 1994); 98. *B. spicula* (Moser *et al*., 1998); 99. *B. starmuehlneri* (Moser *et al*., 1998); 100. *B. staurophora* (Souza & Comperè, 1999); 101. *B. steindorfiana* (Moser *et al*., 1998); 102. *B. steinitziae* (Metzeltin & Lange-Bertalot, 1998); 103. *B. styriaca* ((Grunow) Ross, 1986); 104. *B. sublinearis* (Metzeltin & Lange-Bertalot, 1998); 105. *B. subrostrata* (Lange-Bertalot & Moser, 1994); 106. *B. subtile* (Potapova, Hamilton & Kopyrina, 2014). Scale bars = 10 μm (Figs 92–106).



Figs 107–117. Species of the genus *Brachysira* and their morphological diversity (LM and SEM). 107. *Brachysira superba* (Moser *et al*., 1998); 108. *B. superserians* (Metzeltin & Lange-Bertalot, 2007); 109 *B. supriniana* (Moser *et al*., 1998); 110. *B. tenuiserians* (Lange-Bertalot & Moser, 1994); 111. *B. trinodis* (Lange-Bertalot & Moser, 1994); 112. *B. venter* (Moser *et al*., 1995); 113. *B. vitrea ((Grunow) R.Ross 1986)*; 114. *B. vixapiculata* (Metzeltin & Lange-Bertalot, 2007); 115. *B. wolfei* (Metzeltin & Lange-Bertalot, 2007); 116. *B. wygaschii* (Lange-Bertalot & Moser, 1994); 117. *B. zellensis* ((Grunow) Round & D.G.Mann 1981). Scale bars = 10 μm (Figs 107–117).

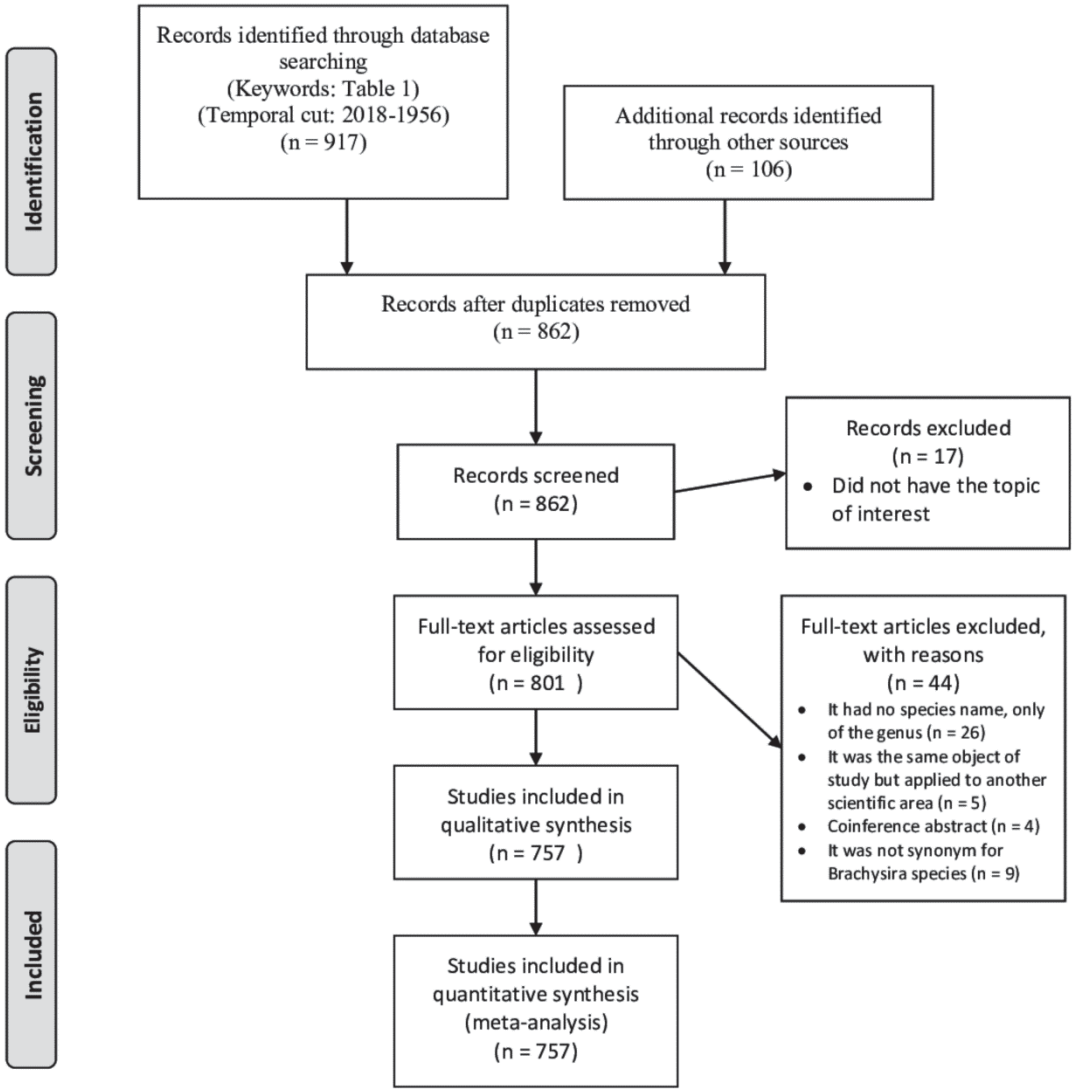


Fig. 118. PRISMA flow chart showing selection of articles for review. Produced using a downloadable template available at http://www.prisma-statement.org/ (Moher et al, 2009).

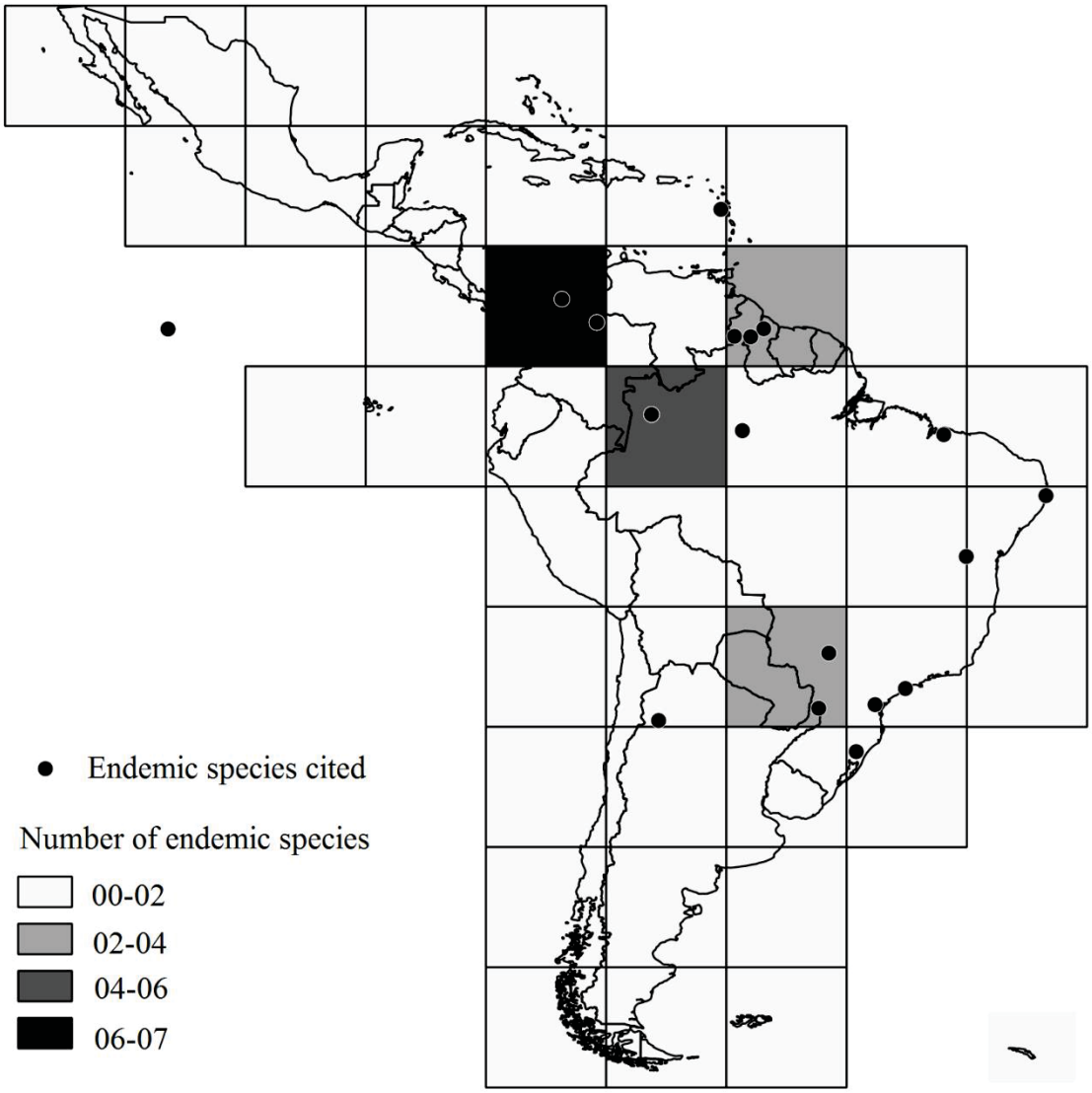


Fig. 119. Distribution of endemic species of *Brachysira* in the Neotropical. Squares shows the number of the different species mentioned in each region and the black circles shows the places where these species are mentioned within each region.

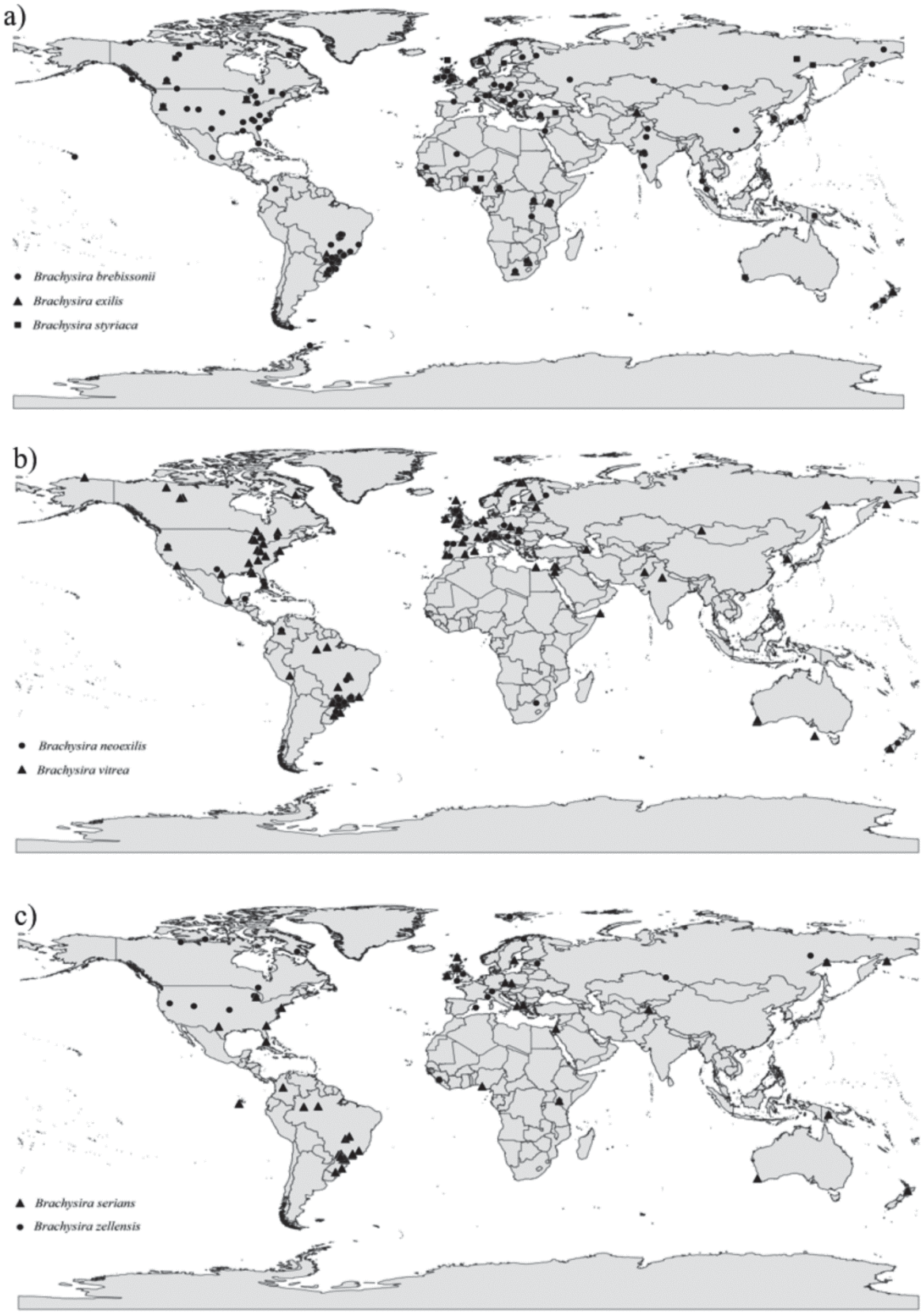


Fig. 120. Distribution of cosmopolitan *Brachysira* species in world: a) *Brachysira brebissoni, B. styriaca, B. exilis*; b) *B. vitrea, B. neoexilis*; c) *B. zellensis, B. serians*.

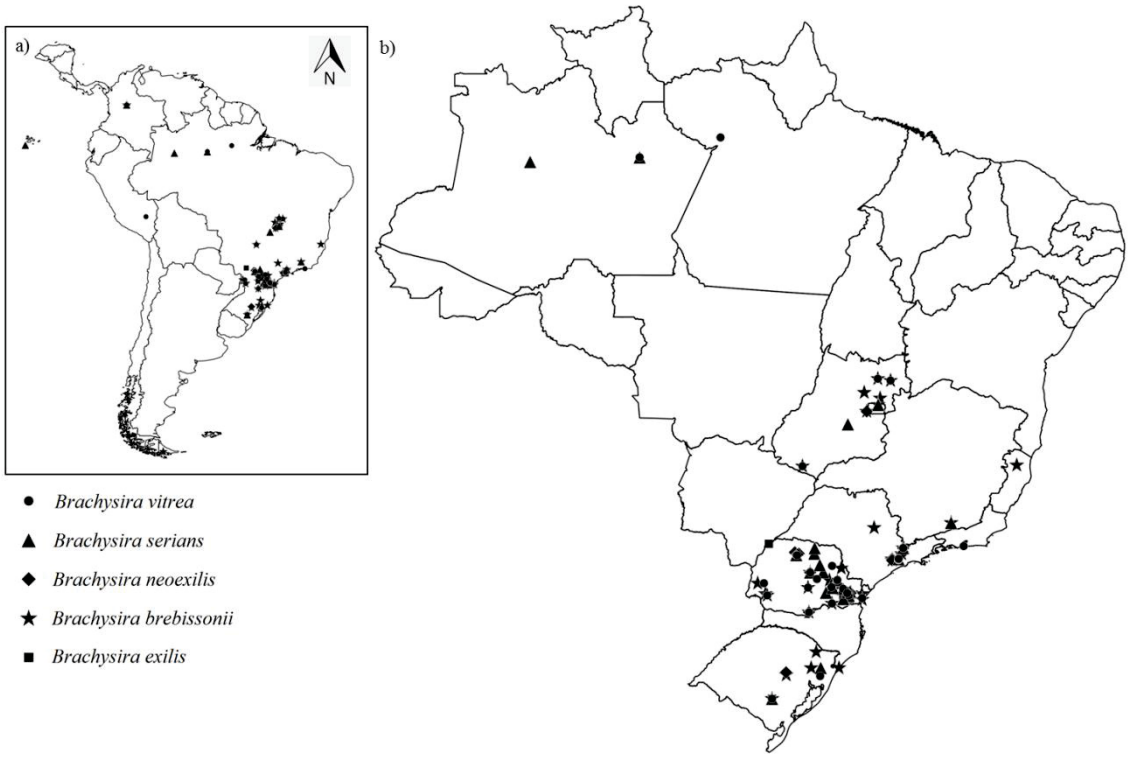


Fig. 121. Distribution of cosmopolitan *Brachysira* species in the Neotropical region (a) and in Brazil (b).

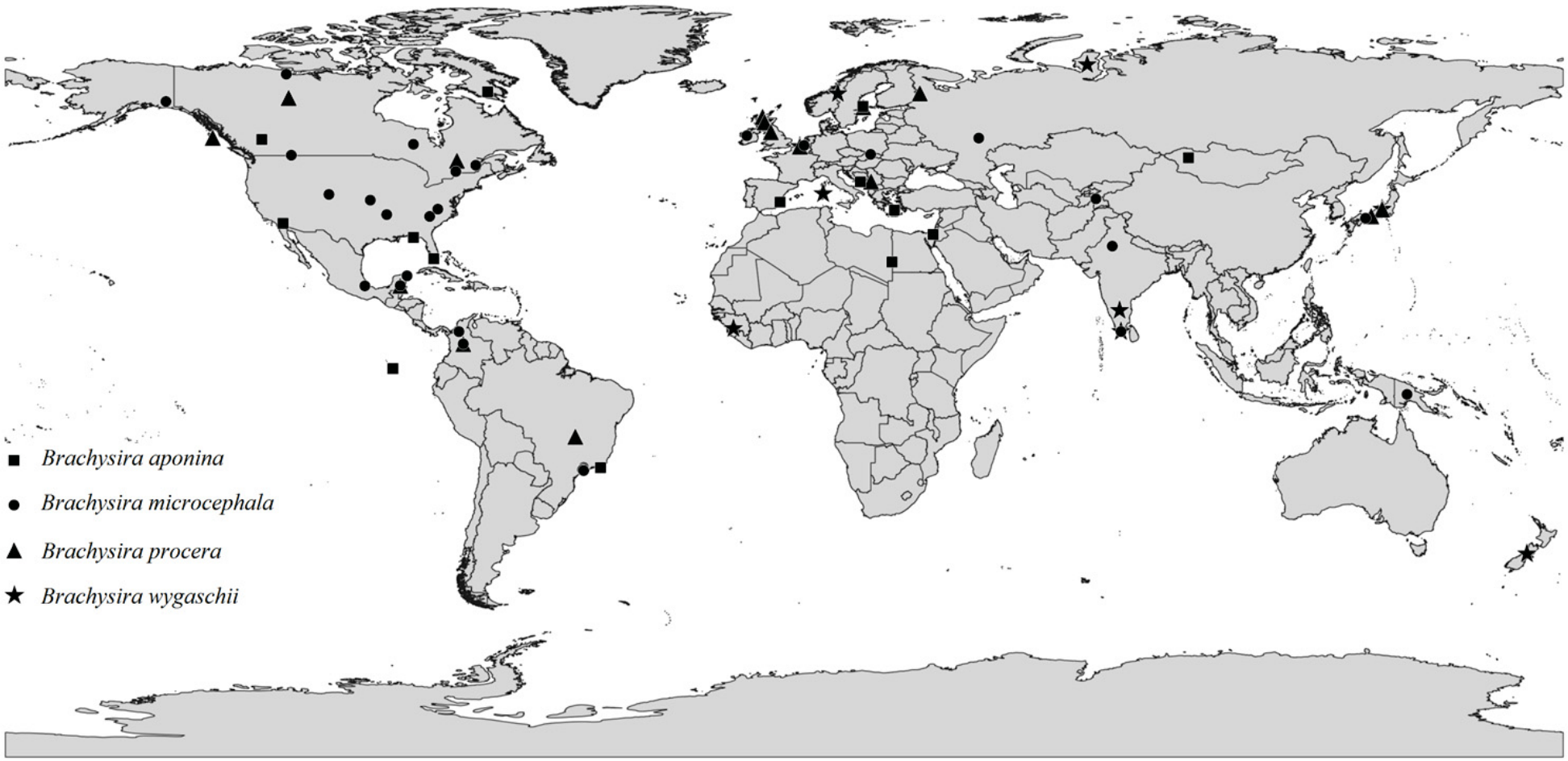


Fig. 122. Wide distribution *Brachysira* species in world. Black squares = *B. aponina*; black circles = *B. microcephala*; black triangle = *B. procera* and black star = *B. wygaschii*.

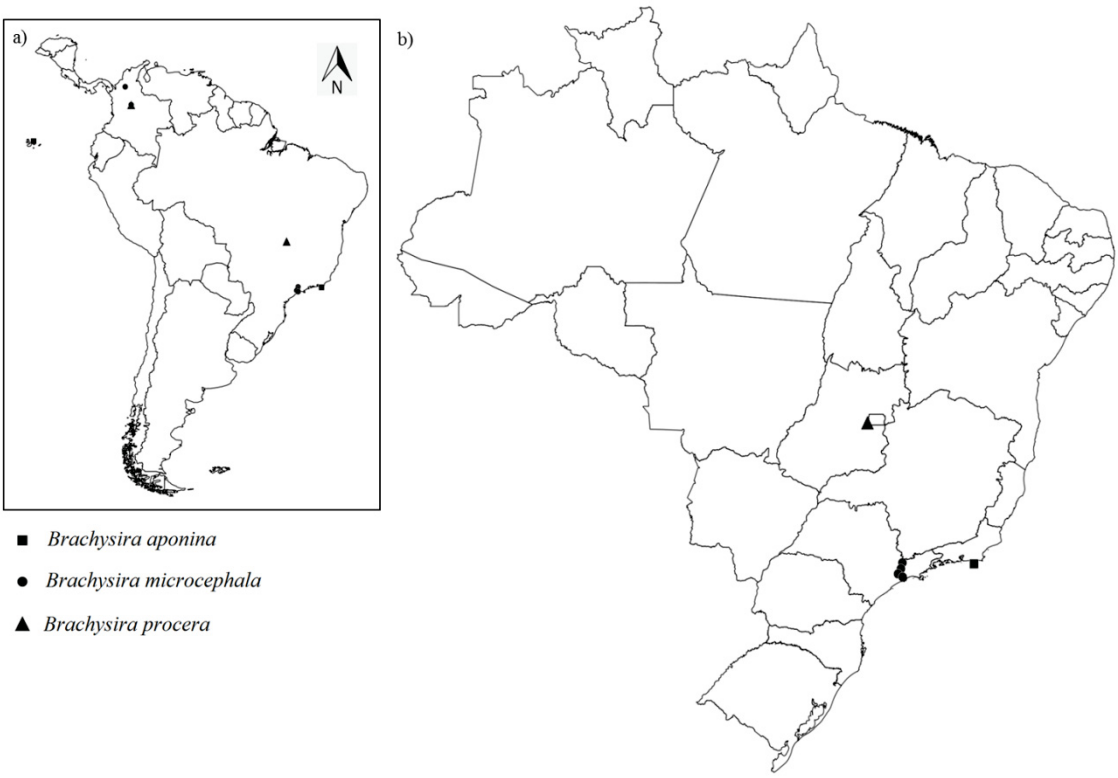


Fig. 123. Wide distribution *Brachysira* species in Brazil. Black squares = *B. aponina*; black circles = *B. microcephala* and black triangle = *B. procera.* Note: *B. wygaschii* is not found in Brazil.

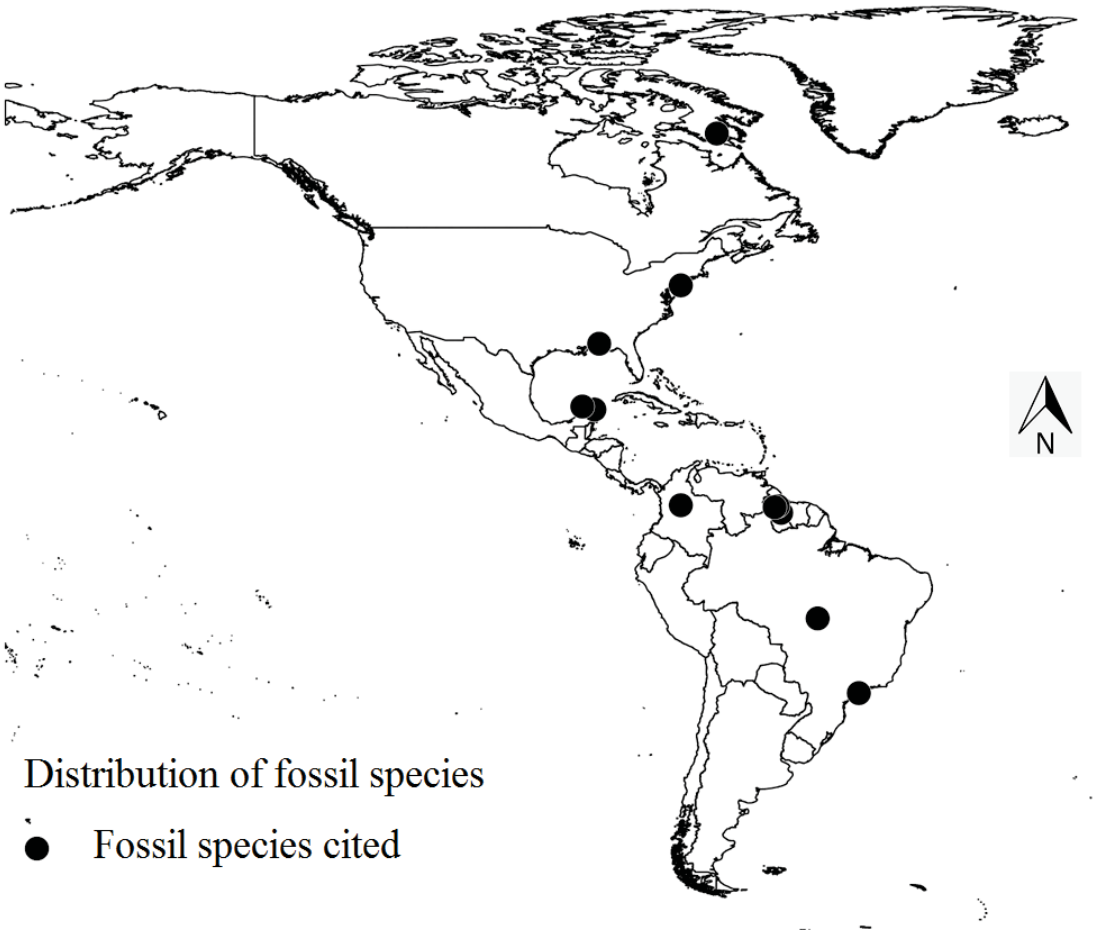


Fig. 124. Distribution of fossil species (black circles) of the genus *Brachysira* in the American continent.

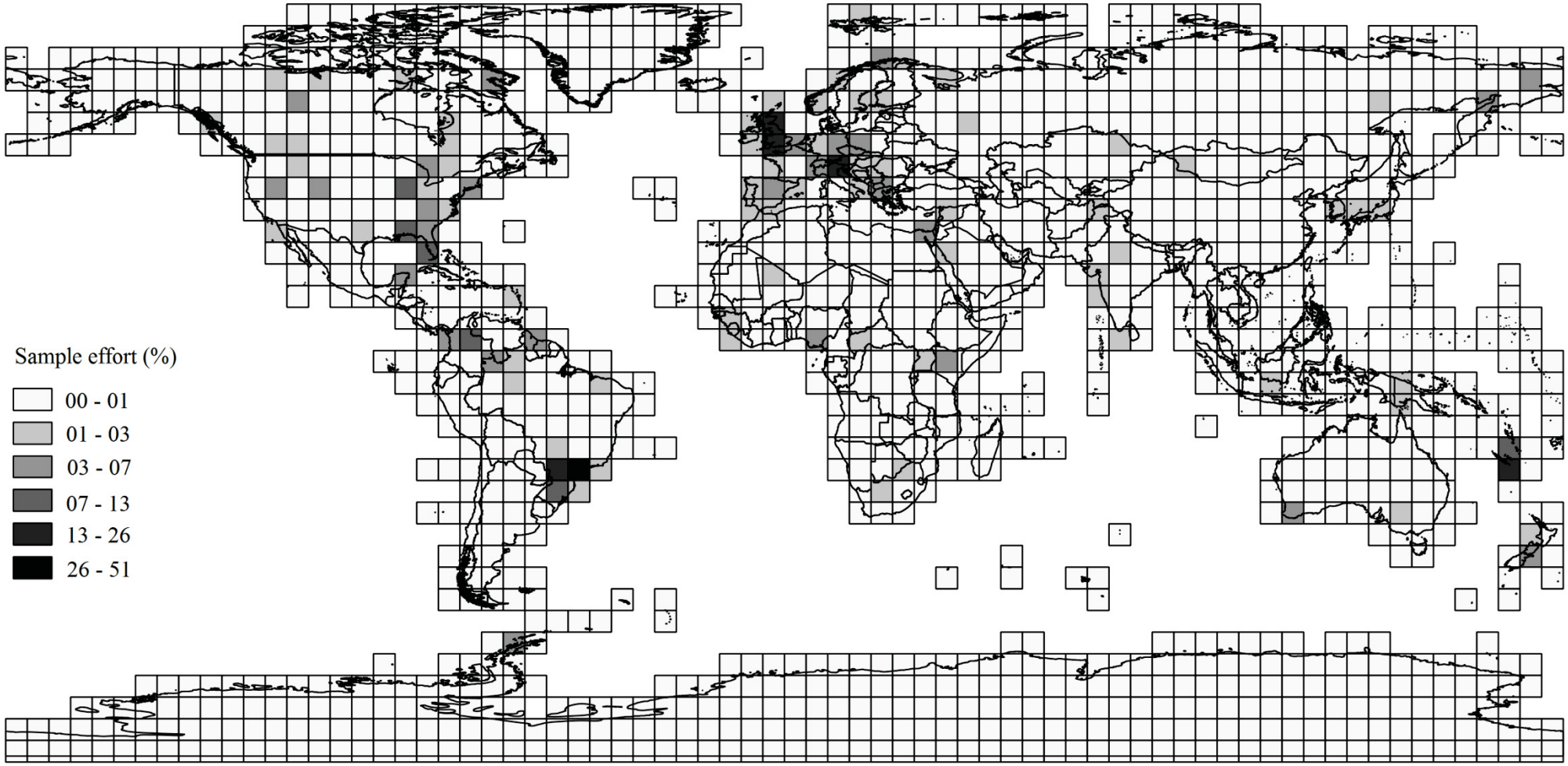


Fig. 125. Distribution of sample effort in the world, Darker squares indicate higher citation by genus and lighter squares indicate lower citation by genus.

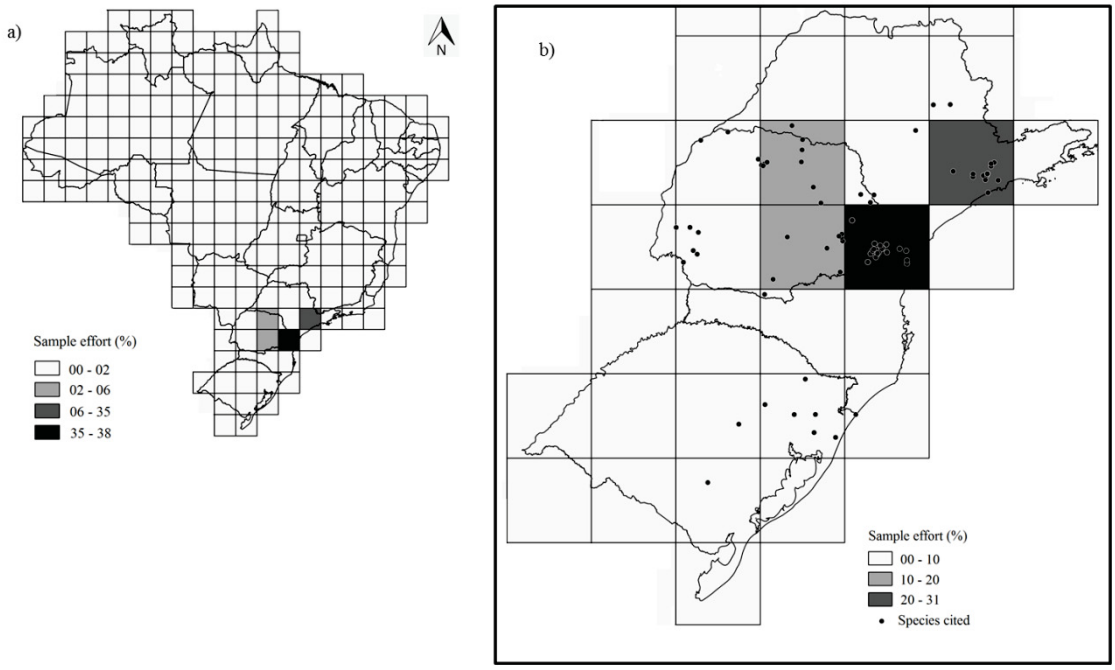


Fig. 126. Distribution of the sampling effort in Brazil, the darker squares indicate a higher citation by genus and the lighter squares indicate a lower citation by genus: a) sampling effort in Brazil and b) highlight on the states of Paraná and São Paulo, which has a greater representation of effort sample for Brazil; black circles indicate the location of species citations within each region.

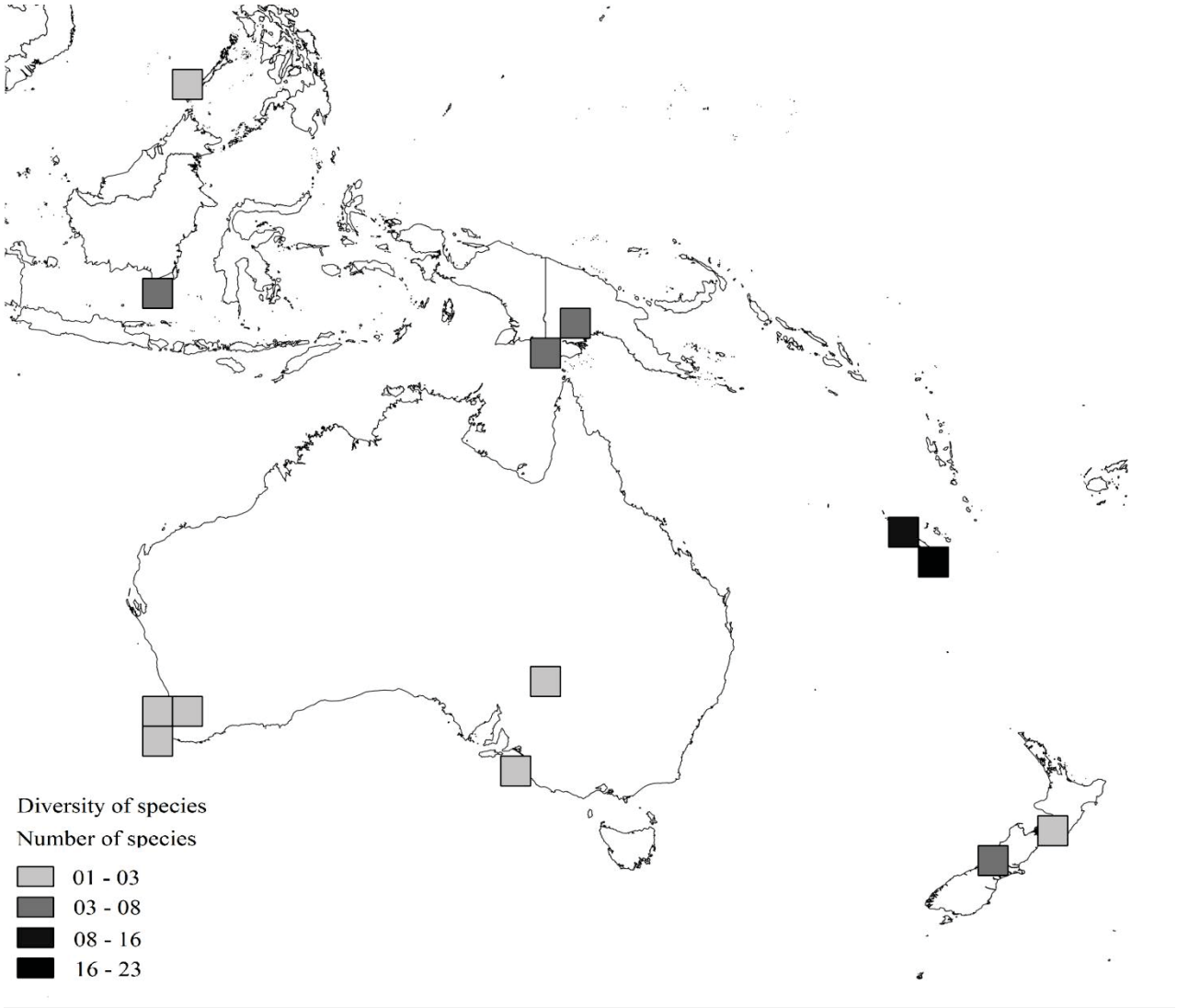


Fig. 127. Distribution of diversity in Australian region, darker squares indicate higher species diversity for *Brachysira* and lighter squares indicate lower diversity for genus.

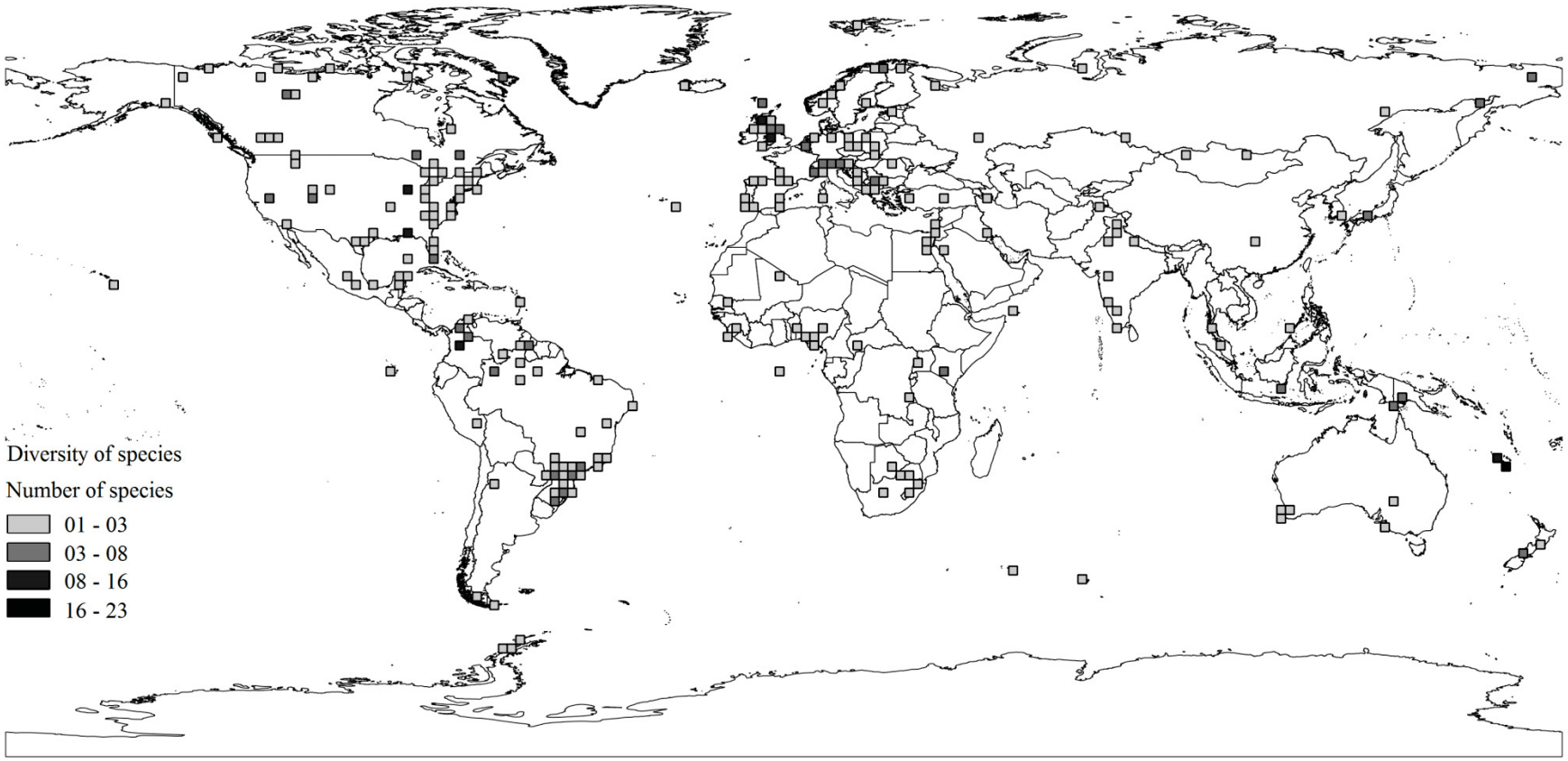


Fig. 128. Distribution of diversity in the world, darker squares indicate higher species diversity for *Brachysira* and lighter squares indicate lower diversity for genus.

Table 1. Keywords used together or alone in different online databases to search for articles/ records that had species of the genus *Brachysira* or species of other genus that are currently considered *Brachysira*.

	Anomoeoneis	*Brachysira*	*Caloneis*	*Gomphonema*	*Navicula*
Brazil	X	X	-	-	-
Taxonomy	X	X	-	-	-
Flora	X	X	-	-	-
Ecology	X	X	-	-	-
Bioindicator	X	X	-	-	-
Distribution	X	X	-	-	-
Diversity	X	X	-	-	-
Richness	X	X	-	-	-
Phylogeny	X	X	-	-	-
Molecular	X	X	-	-	-
Gene	X	X	-	-	-
Genetic	X	X	-	-	-
Caloneis savitschii	-	-	X	-	-
Caloneis silicula var. *blancheana*	-	-	X	-	-
Caloneis silicula var. *subundulata*	-	-	X	-	-
Caloneis obesa	-	-	X	-	-
Gomphonema blancheana	-	-	-	X	-
Gomphonema dumbeana	-	-	-	X	-
Gomphonema dumbeana var. *distans*	-	-	-	X	-
Gomphonema dumbeana var. *octensis*	-	-	-	X	-
Navicula aponina	-	-	-	-	X
Navicula. dvorachekii	-	-	-	-	X

Table 2. Current species status of the genus *Brachysira*: Valid names, localization of vouchers of the type species and the synonyms and/ or basionym described in classic and modern bibliographic references. Information not found or does not apply (-)

Current name	Vouchers	Basionym/ Synonym
Brachysira acutirhomboides Lange-Bertalot & Moser 1994	Holotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Synonym: *Anomoeoneis follis* var. *hannae* Reimer sensu Maillard 1978
B. acutissima Moser, Lange-Bertalot & Metzeltin 1998	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B altepetlensis D. Mora, R. Jahn & N. Abarca 2017	Holotype: Universidad Autonoma de Querétaro, Centro Universitario (QMEX) Isotype: Universidad Autonoma de Querétaro, Centro Universitario (QMEX)	-
B. amoena Lange-Bertalot, 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. angusta (Mail.) Lange-Bertalot & Moser 1994	Lectotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Basionym: *Anomoeoneis neocaledonica* var. *angusta* Maillard. nom. Inval. *Brachysira neocaledonica* var. *angusta* (Maillard) Le Cohu 1985
B. apiculata (Boyer) Lange-Bertalot & Moser 1994	-	Basionym: *Anomoeoneis serians* var. *apiculate* Boyer 1927
B. aponina Kützing 1836	Holotype: Collection Kützing, The Natural History Museum (BM)	Synonym: *Navicula aponina* Kützing 1844 *Navicula perlepida* Grunow 1884 *Navicula interruptestriata* Schwab & Simonsen 1961 *Caloneis savitschii* Karayeva 1974 *Navicula dvorachekii* 1978
B. archibaldii Coste & Ricard 1982	Holotype: Muséum National d'Histoire Naturelle	-

	(PC)	
B. arctoborelis Wolfe et H.J. Kling 2001	Holotype: Canadian Museum of Nature (CANA)	Synonym: *Brachysira brebissonii* R. Ross "Morphotyp *latior*" in Lange-Bertalot & Moser 1994; *Brachysira brebissonii* R.Ross "Morphotyp *major*" in Lange-Bertalot 1994; *Anomoeoneis* "species 3 PIRLA" in Camburn et al 1986 *Navicula serians* var. *minor* Grunow in Van Heurck 1880
B. atacamae (Hustedt) Mann 1990	-	Basionym: *Navicula atacamae* Hustedt 1927
B. australofollis Lange-Bertalot & Moser 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	Synonym: *Anomoeoneis follis* Ehrenberg sensu Maillard. 1978
B. babuschka Moser, Lnge-Bertalot & Metzeltin 1998	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. bacillifer Lange-Bertalot & Moser 1994	Lectotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Basionym: *Anomoeoneis serians* (Breb.) Cleve var. *bacillifera* Maillard 1978
B. blancheana (Maillard.) Lange-Bertalot & Moser 1994	Lectotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Basionym: *Gomphonema blancheana* Maillard 1978 Synonym: *Gomphonema dumbeana* var. *octensis* Maillard 1978 (nom. inval.)
B. brebissoni R. Ross in Hartley 1986	-	Basionym: *Navicula aponina* var. *brachysira* Kützing 1849 Synonym: *Anomoeoneis brachysira* (Brébisson ex Rabenhorst) Grunow in Cleve 1895. *Navicula brachysira* Brébisson ex Rabenhorst 1853. *Anomoeoneis serians* var. *brachysira* (Brébisson ex Rabenhorst) 1853.

		Anomoeoneis serians var. *brachysira* (Brébisson ex Rabenhorst) 1930.
B. bouletiana Gerd Moser, Lange-Bertalot & Metzeltin, 1998	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. calcicola Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. calligraphica Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. charlesreimeri Metzeltin & Lange-Bertalot	Type: Coll. Lange-Bertalot (FR) Holotype: Lange-Bertalot personal collection, Senckenberg Museum Frankfurt	-
B. conamarae Kennedy & Allott 2017	Holotype: Hustedt Collection, The Alfred Wegener Institute (AWI).	-
B. coralina Metzeltin & Lange-Bertalot, 1998	Type: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. cymbelliformis Metzeltin and Lange-Bertalot, 2007	Type: Coll. Lange-Bertalot (FR), Holotype: Lange-Bertalot personal collection Senckenberg Museum Frankfurt	-
B. delicatissima Metzeltin and Lange-Bertalot, 2007	Type: Coll. Lange-Bertalot (FR) Holotype: Lange-Bertalot personal collection Senckenberg Museum Frankfurt	-
B. dumbeana (Maillard) Lange-Bertalot & Moser 1994	Lectotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Basionym: *Anomoeoneis dumbeana* Maillard 1978
B. elliptica Metzeltin & Lange-Bertalot 2007	Type: Coll. Lange-Bertalot (FR) Holotype: Lange-Bertalot personal collection Senckenberg Museum Frankfurt	-
B. estonarium Witkowski, Lange-Bertalot &	Typus: Collection Lange-Bertalot, Botanical	-

Metzeltin, 2000	Institut, University, Frankfurt/ Main	
B. exilis (Kützing) Round & D.G. Mann 1981	-	Basionym: *Navicula exilis* Kützing
B. feickertiae Lange-Bertalot & Moser 1995	Lectotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Synonym: *Caloneis silicula* var. *blancheana* fo. Robusta Maillard *Caloneis silicula* var. *subundulata* Maillard (Grunow in Cleve & Grunow 1880) Mayer 1971
B. follis (Ehrenberg) Ross in Hartley 1986	-	Basionym: *Navicula follis* Ehrenberg 1838 Synonym: *Anomoeoneis follis* (Ehrenberg) Cleve 1895
B. formidulosa (Cholnoky) Lange-Bertalot & Moser 1994	-	Basionym: *Anomoeoneis formidulosa* Cholnoky 1959
B. fossilis (Reimer) Lange-Bertalot & Moser 1994	-	Basionym: *Anomoeoneis follis* var. *fossilis* Reimer 1961
B. frenguelli (Manguin) D. Metzeltin & Lange-Bertalot 2007	-	Basionym: *Anomoeoneis frenguellii* Manguin in Bourrelly & Manguin 1952 Synonym: *Brachysira intermedia* (Østrup) Lange-Bertalot in Lange-Bertalot & Moser 1994.
B. *garrensis* (Lange-Bertalot & Krammer) Lange-Bertalot 1994	-	Basionym: *Anomoeoneis* garrensis Lange-Bertalot & Krammer 1985
B. gatesii Kociolek et Lowe	Holotype: JPK Collection. University of Colorado (COLO)	-
B. gomphonemoides (Maillard) Lange-Bertalot & Moser 1994	Lectotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Basionym: *Anomoeoneis gomphonemoides* Maillard 1978 Synonym: *Gomphonema dumbeana* Maillard 1978
B. gravida Shayler & Siver 2004	Type/ Holotype: California Academy of Sciences. Isotype: Canadian Museum of Nature (CAS)	-

B. guarrerai Vouilloud, Sala & Núñez-Avellaneda 2014	Holotype: División Científica Ficología, Facultad de Ciencias Naturales y Museo (UNLP), La Plata, Argentina.	-
B. guttiformis Gert Moser, Lange-Bertalot & Metzeltin	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. hannae (Reimer) Lange-Bertalot & Moser 1994	-	Basionym: *Anomoeoneis follis* var. *hannae* Reimer 1961
B. hedyklingiae Metzeltin & Lange-Bertalot, 2007	Type: Coll. Lange-Bertalot (FR)	-
B. hofmanniae Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. huitotarum Vouilloud, Sala & Núñez-Avellaneda 2014	Holotype: División Científica Ficología, Facultad de Ciencias Naturales y Museo (UNLP), La Plata, Argentina.	-
B. inamoena Metzeltin & Lange-Bertalot, 1998	Type: Collection Krasske, Naturhist. Museum Ottoneum, Kassel	-
B. incognita Gerd Moser, Bertalot & Metzeltin, 1998	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/	-
B. inflata Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. insulsa Metzeltin & Lange-Bertalot, 1998	Type: Collection Krasske, Naturhist. Museum Ottoneum, Kassel	-
B. intermedia (*Oestrup*) Lange-Bertalot 1994	Holotype: Collection Oestrup	Basionym: *Anomoeoneis intermedia* Oestrup 1910
B. irawanae (Podzorski & HÅkansson) Lange-Bertalot & Podzorski 1994	-	Basionym: *Anomoeoneis irawanae* Podzorski & HÅkansson 1987
B. irawanoides Lange-Bertalot & Moser 1994	Holotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Synonym: *Gomphonema dumbeana* Maillard partim (nom. inval.)
B. jankae Matzeltin & Lange-Bertalot, 2007	Type: Coll. Lange-Bertalot (FR)	-
B. julio Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches	-

	Institut, Universität Frankfurt/Main	
B. krammeri Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. kuntzei Metzeltin & Lange-Bertalot, 1998	-	Basionym: *Navicula kuntzei* Reichelt
B. labrata Lange-Bertalot & Moser 1995	Lectotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Synonym: *Caloneis silicula* var. *blancheana* Maillard 1978
B. lange-bertalotii Metzeltin & Lange-Bertalot, 1998	Type: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. lecohui Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. lehmanniae Lange-Bertalot & Moser 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. liliana Lange-Betalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	Synonym: (?) *Anomoeoneis exilis* var. *lanceolate* Mayer 1919
B. linearilanceolata Metzeltin & Lange-Bertalot, 2007	Type: Coll. Lange-Bertalot (FR)	-
B. longirostris (Hustedt) Mann 1990	-	Basyonim: *Anomoeoneis longirostris* Hustedt 1942
B. macroserians Metzeltin & Lange-Bertalot, 1998	Type: Collection Krasske, Naturhist. Museum Ottoneum, Kassel	-
B. maillardii Lange-Bertalot & Moser 1994	Holotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Synonym: *Gomphonema dumbeana* Maillard 1978 (nom.inval.) *Gomphonema dumbeana* var. *distans* Mailard 1978 (nom.inval.) *Gomphonema dumbeana* var. *octensis* Mailard 1978 (nom.inval.)
B. manfredii Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. manoylovae Metzeltin & Lange-Bertalot 2007	Typus: Collection Manguin, Muséum National d'Histoire Naturelle, Paris (PC)	-

B. metzeltinii Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. microcephala (Grunow) Compère	Holotype: Coll. W. Smith V. Hochstetter (Im Taupo-See auf Neuseeland) (Herbarium Henri Van Heurck Museum -AWH) Lectotype (*N. microcephala*): Coll. W. Smith V. Dr. Dickie (Near Aberdeen) (Herbarium Henri Van Heurck Museum -AWH) – Holotype (*Colletonema exile*) : Grunow 157 (Naturhistorisches Museum Wien - W)	Synonyms: *Navicula microcephala* Grunow 1867 *Anomoeoneis microcephala* Ake Berg 1945 *Anomoeoneis exilis* (Grunow) Cleve 1895 *Anomoeoneis variabilis* (R. Ross) Reimer 1961 *Anomoeoneis vitrea* (Grunow) R.Ross in Patrick & Reimer 1966 *Brachysira neoexilis* Lange-Bertalot et Gerd Moser 1994 *Brachysira exilis* (Kützing) Round et D.G. Mann 1981 *Brachysira. vitrea* (Grunow) R. Ross in Hartley 1986
B. microclava Lange-Bertalot & Moser 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. microserians Metzeltin & Lange-Bertalot, 1998	Type: Collection Lange-Bertalot, Botanisches Institut Universität Frankfurt/Main	-
B. minor (Krasske) Lange-Bertalot 1994	Lectotype: DTII 113 (Chile): Krasske Herb.	Basionym: *Anomoeoneis minor* Krasske 1939
B. nanoclava Gerd Moser, Lange-Bertalot & Metzeltin, 1998	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. neglectissima Lange-Bertalot, 2004	Type: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. neoacuta Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	Synonym: (?) *Anomoeoneis serians* var. *acuta* Hustedt 1937

B. neocaledonica (Maillard) Lange-Bertalot & Moser 1994	Lectotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Basionym: *Anomoeoneis neocaledonica* Maillard 1978
B. neoexilis Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	Synonym: *Navicula exilis* Kützing 1844 *Navicula exilis* Kützing sensu Gunow, in Van Heurck 1880 *Anomoeoneis exilis* (Kützing) Cleve 1895 *Anomoeoneis exilis* (Kützing) Grunow sensu Berg 1945 *Navicula variabilis* var *capitate* Ross 1947 (?)*Anomoeoneis exilis* f. *undulata* Kisselev 1955
B. nubigena Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. obesa (Maillard) Lange-Bertalot & Moser 1995	Lectotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Basionym: *Caloneis obesa* Maillard 1978
B. ocalanensis H.A. Shayler & P.A. Siver 2004	Holotype: California Academy of Science (624785) Isotype: Canadian Museum of Nature (CANA 76142)	-
B. *ontonageniana* Kociolek et Lowe	Holotype: JPK Collection, University of Colorado (COLO)	-
B. potapovae Metzeltin & Lange-Bertalot 2007	Type: Coll. Lange-Bertalot (FR)	-
B. praegeri Kennedy & Allott	Holotype: Hustedt Collection, Alfred Wegener Institute.	-
B. procera Lange-Bertalot & Moser 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	Synonym: *Anomoeoneis exilis* var. *lanceolate* Mayer 1919 *Anomoeoneis exilis* var *azorica* Manguin 1942

B. pseudoacuta Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. pulchra Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. pumila Metzeltin & Lange-Bertalot, 1998	Type: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. rhomboides (Hustedt) Mann 1990	Holotype: Herbarium of Queen's university (QK), 397/47b.	Basionym: *Anomoeoneis rhomboides* Hustedt 1942
B. rostrata (Krasske) Metzeltin & Lange-Bertalot 1998	Lectotype: Coll. Kraske D IV 130 from Avenida Paulista, Brazil.	Basionym: *Anomoeoneis serians* var. *rostrata* Krasske 1948 Synonym: *Brachysiria serians* var. *rostrata* Lange-Bertalot & Moser 1994
B. ruckiae Metzeltin & Lange-Bertalot, 2007	Type: Coll. Lange-Bertalot (FR)	-
B. rumrichiae Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. ruppeliana Gerd Moser, Lange-Bertalot & Metzeltin, 1998	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. sandrae B. Van de Vijver	-	-
B. seippii Lange-Bertalot & Moser 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. serians (Brébisson) Round & Mann 1981	-	Basionym: *Navicula serians* Brébisson in Kützing 1844
B. silvicola Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. simplex Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. sinaiensis Lange-Bertalot 2004	Type: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. spectabilis (Maguin) Lange-Bertalot &	Type: Muséum National d'Histoire Naturelle (PC)	Basionym: *Anomoeoneis spectabilis*

Moser 1994		Manguin 1942
B. speluncola Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	Synonym: *Anomoeoneis follis* Ehrenberg sensu Schoeman 1970
B. spicula Gerd Moser, Lange-Bertalot & Metzeltin, 1998	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. starmuehlneri (Maillard.) Gerd Moser Lange-Bertalot & Metzeltin 1998	Neotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	*Anomoeoneis starmuehlneri* Maillard 1978
B. staurophora Souza & Compère 1999	Holotype: Collection Herbarium UPCB Isotype: Herbarium ANSP, BM, BR and BRM	-
B. steindorfiana Moser, Lange-Bertalot & Metzeltin, 1998	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. steinitziae Metzeltin & Lange-Bertalot, 1998	Type: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. styriaca (Grunow) Ross in Hartley 1986	-	Basionym: *Navicula styriaca* Grunow in Van Heurck 1880 Synonym: *Anomoeoneis styriaca* (Grunow Hustedt 1930
B. sublinearis Metzeltin & Lange-Bertalot, 1998	Type: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. subrostrata Lange-Bertalot	Collection Krasske, Nat. Hist. Mus., Kassel	Synonym: *Anomoeoneis serians* var. *rostrate* Krasske 1948
B. subtile Potapova, Hamilton & Kopyrina	Holotype and Isotype: Russia, Sakha (Yakutia) Republic, ANSP.	-
B. superba (Lange-Bertalot & Gerd Moser) Gerd Moser, Lange-Bertalot & Metzeltin, 1998	Holotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Basionym: *Brachysira brebissonii* ssp. *superba* Lange- Bertalot e Moser, 1994. Synonym: *Anomoeoneis serians* var. *brachysira* Maillard 1978
B. superserians Metzeltin & Lange-Bertalot, 2007	Type: Coll. Lange-Bertalot (FR)	-
B. supriniana Gerd Moser, Lange-Bertalot	Holotype: Collection Lange-Bertalot, Botanisches	-

& Metzeltin, 1998	Institut, Universität Frankfurt/Main	
B. tenuiserians Lange-Beratalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	-
B. trinodis Lange-Bertalot & Moser 1994	Holotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	-
B. venter Lange-Bertalot & Moser 1995	Lectotype: Collection Maillard, Muséum National d'Histoire Naturelle, Paris (PC)	Synonym: *Caloneis silicula* var. *blanchenan* Maillard 1978
B. vitrea (Grunow Ross in Hartley 1986	-	Basionym: *Gomphonema* ? *vitrea* Grunow in Schneider 1878 Synonym: *Navicula gomphonemacea* Grunow in Van Heurck 1880 *Navicula variabilis* Ross 1947 *Anomoeoneis variabilis* (Ross) Reimer 1961 *Anomoeoneis vitrea* (Grunow) Ross in Patrick & Reimer 1966
B. vixapiculata Metzeltin & lange-Bertalot 2007	Type: Coll. Lange-Bertalot (FR)	-
B. wolfei Metzeltin & Lange-Bertalot 2007	Type: Coll. Lange-Bertalot (FR)	-
B. wygaschii Lange-Bertalot 1994	Holotype: Collection Lange-Bertalot, Botanisches Institut, Universität Frankfurt/Main	Synonym: *Anomoeoneis serians* sensu Hein 1990 et auct. nonnull. *Anomoeoneis serians* var. *acuta* Hustedt sensu auct. nonnull.
B. zellensis (Grunow) Round & Mann 1981	-	Basionym: *Navicula zellensis* Grunow 1860 Synonym: *Anomoeoneis zellensis* (Grunow) Cleve 1895 *Anomoeoneis brachysira* var. *zellensis* (Grunow) Krammer 1985

Table 3. Comparison of the morphometric data of *Brachysira* species, consulted references and figure indication for each species. Measurements in micrometre (µm).

Taxon	Valve length (µm)	Valve width (µm)	Striae in 10 µm	Valve	Central area	Valve ends	Reference	Local type specie	Image
Brachysira acutirhomboides Lange-Bertalot & Gerd Moser	34-56	12-15	23-24	Rhombic to lanceolate	Distinctly rhombic	Rounded to acute	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 1
B. acutissima Gerd Moser, Lange-Bertalot & Metzeltin 1998	35-60	9-13	22-24	Lanceolate to rhombic-lanceolate. Axial ribs and silicified marginal ribs on the valve surface Presence of papillae.	Present in larger individuals and absent or very small in smaller individuals	acute	Moser, Lange-Bertalot & Metzeltin, 1998	New Caledonia	Fig. 2
B. altepetlensis D. Mora, R. Jahn & N. Abarca 2017	12.6-23.1	3.2-4.5	34-37	Lanceolate to linear-lanceolate. Ribs on the edge of the raphe. Presence of papillae.	Round to elliptical	Rostrate	Mora et al., 2017	Mexico	Fig. 3
B. amoena Lange-Bertalot 1994	40-71	9-12	24	Linear-elliptical. Ribs on the edge of the raphe. Presence of papillae.	Rhombic	Wide rounded	Lange-Bertalot & Moser 1994	Venezuela	Fig. 4

B. angusta (Maillard) Lange-Bertalot & Gerd Moser	23-34	5-6	34-38	Rhombic-lanceolate. Presence of papillae.	Rhombic transversal	Narrow and capitate	Lange-Bertalot & Moser 1994 & Moser et al. 1995	New Caledonia	Fig. 5
B. apiculata (Boyer) Lange-Bertalot & Gerd Moser	35-60 (80)	12,5-20	20-24	Elliptical-lanceolate. Valvae with very slightly wavy edges	Small to moderately elliptic or dilated rhombic	Almost flat rounded, subcapitate to capitate	Lange-Bertalot & Moser 1994	Venezuela	Fig. 6
B. aponina Kützing 1836	14-62	3.5-6	32-42	Narrow lanceolate to rhombic-lanceolate	little extended	Rounded	Lange-Bertalot & Moser, 1994.		Fig. 7
B. archibaldii Coste & Ricard 1982	28-50	4.7-6.5	29-30	Heteropolar-lanceolate. Central pores far apart between them.	Elongated transapical.	Rounded	Lange-Bertalot & Moser, 1994.		Fig. 8
B. arctoborealis Wolfe et H.J. Kling 2014	17-31	7-10	19-25	Rhombic. Ribs on the edge of the raphe. Presence of papillae.	Central area small, symmetrical to slightly asymmetric, and occasionally bearing a large occluded areola.	Broadly rounded to slightly rostrate.	Vouillod et al., 2014 Wolfe & Kling, 2001	Canada	Fig. 9
B. atacamae (Hustedt) Mann	30.66	4.5	40	Rhombic-lanceolate		Capitate	Lange-Bertalot & Moser 1994		Fig. 10

1990									
B. australofollis Lange-Bertalot & Gerd Moser 1994	20-40	6.5-10	19-21	Rounded to elliptical. Presence of papillae.	Small, rounded to elliptical	Rounded, capitate	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 11
B. babuschka Gerd Moser, Lange-Bertalot & Metzeltin 1998	8.5-18	4.0-5.5	27-30	Lanceolate, elliptic-lanceolate to rhombic-lanceolate. No longitudinal striae. Median part inflated in large individuals	central area of rhombic shape with rounded	Rounded wide	Moser, Lange-Bertalot & Metzeltin, 1998	New Caledonia	Fig. 12
B. bacillifer (Maillard) Lange-Bertalot & Gerd Moser 1994	45-88	12-18	23-26	Rhombic-lanceolate. Valvae with distinctly convex sides	Rhombic-lanceolate	Rounded, not separated from the rest of the valvae.	Lange-Bertalot & Moser 1994 & Moser et al. 1995	New Caledonia	Fig. 13
B. blancheana (Maillard) Lange-Bertalot & Gerd Moser	22-36	4-5	28-30	Narrow little lanceolate, gomphonemoid.	Elliptical and very little expanded	Broadly rounded.	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 14
B. bouletiana Gerd Moser, Lange-Bertalot & Metzeltin 1998	30-45	12-16	18-20	Narrow-lanceolate with a strongly rounded center. Centrally inflated valve with striae interrupted in the	Narrow-rhombic and rhombic-lanceolate	Broadly protracted rounded	Moser, Lange-Bertalor & Metzeltin, 1998	New Caledonia	Fig. 15

				central area					
B. brebissonii R. Ross 1986	18.2-27.7	4.9-5.8	24-27	Acutely rounded. Presence of papillae.	Very small rounded to rhombic		Kennedy & Allott 2017; Van de Vijver 2014		Fig. 16
B. calcicola Lange-Bertalot 1994	10-27	4.5-6	32-34	Lanceolate to rhombic-lanceolate	Rhombic for discontinuous transverse elliptical.	Two morphotypes. Rounded and protracted.	Lange-Bertalot & Moser 1994	Baviera (German)	Fig. 17a,b
B. calligraphica Lange-Bertalot 1994	31-53	6-7.5	23-24	Fairly narrow lanceolate. Longitudinal striae with siliceous thickening	Slightly expanded	Rounded to capitate	Lange-Bertalot & Moser 1994	Venezuela	Fig. 18
B. charlesreimeri Metzeltin & Lange-Bertalot, 2007	50-90	20-22	20-22	Lanceolate-rhombic. Discreet undulations at the edge of the valve.	Rounded	Rostrate to subrostrate	Metzeltin & Lange-Bertalot, 2007	Florida (USA)	Fig. 19
B. conamarae Kennedy & Allott 2017	22.9-40.0	4.8-5.8	34-37	Lanceolate, tapering uniformly to weakly or hardly.	Indistinctly lanceolate and only slightly wider than axial area	Slightly protracted rounded	Kennedy & Allott, 2017	Galway (Irelan)	Fig. 20

B. coraliana Metzeltin & Lange-Bertalot, 1998	15-19	3.8-5.5	27	Lanceolate	Small and rounded	Rounded to cuneiform	Metzeltin & Lange-Bertalot, 1998	Santos (Brazil)	Fig. 21
B. cymbelliformis Metzeltin and Lange-Bertalot, 2007	12.7-30	4-7	21-22	Strongly dorsiventral	Small, expanded towards the dorsal side	Obtusely rounded	Metzeltin & Lange-Bertalot 2007	Florida (USA)	Fig. 22
B. delicatissima Metzeltin and Lange-Bertalot, 2007	18-26	6.6-8.0	26-28	Elliptical-lanceolate. Pores densely spaced by longitudinal striae	Indistinct	Protracted rostrate to subrostrate	Metzeltin & Lange-Bertalot 2007	Florida (USA)	Fig. 23
B. dumbeana (Maillard) Lange-Bertalot & Gerd Moser	39-45	6-8	28-30	Rhombic-lanceolate, in the middle weakly bulbous, narrowing toward the poles relatively quickly	Elliptical, often very indistinct.	Broadly protracted-rounded	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 24
B. elliptica Metzeltin & Lange-Bertalot 2007	6-20	5.3-6.6	28-30	Elliptical. Presence of papillae.	Small or almost lacking	Broadly rounded	Metzeltin & Lange-Bertalot 2007	Florida (USA)	Fig. 25
B. estonarium Witkowski, Lange-Bertalot &Metzeltin, 2000	11.5-29	3-3.5	40-45	Narrowly lanceolate	Absence	Obtusely rounded	Witkowsky, Lange-Bertalot & Metzeltin	Crete (Greece)	Fig. 26

B. exilis (Kützing) Round & D.G. Mann, 1981	17,98	4,55		Lanceolate	Small, rounded	Capitate to subcapitate	Archibald & Schoeman, 1987; Round & Mann, 1981	Santorini (Greece)	Fig. 27a, b
B. feickertiae Lange-Bertalot & Gerd Moser 1995	33-75	7-11	16-20	Lanceolate	Widely expanded longitudinal (apex to apex)	Rounded	Moser et al. 1995	New Caledonia	Fig. 28
B. follis (Ehrenberg) Ross 1986	(12)20-54	12-20	23-26	Rhombic, central area strongest extendido. Presence of papillae.	Elliptical-rhombic	Rounded	Lange-Bertalot & Moser, 1994	South Africa	Fig. 29
B. formidulosa (Cholnoky) Lange-Bertalot & Moser 1994	28-65	5-6	28	Linear-lanceolate, in larger individuals it is triondulated lanceolate	Irregular rounded	Rounded to subcapite-rounded	Lange-Bertalot & Moser, 1994; Potapova et al. 2019 (symbiont.ansp.org)	Kaap-Provinz (South Africa)	Fig. 30
B. *fossilis* (Reimer) Lange-Bertalot & Gerd Moser, 1994	50-107	13-22		Lanceolate	Small, rounded to rhombic	Rounded-subcapitate	Lange-Bertalot & Moser, 1994; Metzeltin & Lange-Bertalot 2007	Florida (USA)	Fig. 31
B. frenguelli (Manguin) D. Metzeltin & Lange-Bertalot 2007	32	6	25	Elliptical-lanceolate	Small, rounded or rhombic	Rounded	Lange-Bertalot & Moser 1994; Metzeltin & Lange-Bertalot 2007		Fig. 32
B. garrensis (Lange-Bertalot	8.3-32	3-6.5	36-40	Lanceolate to elliptical-	Less expanded to	capitate to non-capitate	Lange-Bertalot & Moser 1994		Fig. 33

& Krammer) Lange-Bertalot 1994				lanceolate	almost completely absent				
B. gatesii Kociolek et Lowe	16-32	4-5	29-32	Valves lanceolate, barely clavate	Small but distinctly rounded	Headpole rounded and footpole protracted	&erson et al. 2013	Michigan county (USA)	Fig. 34
B. gomphonemoides (Maillard) Lange-Bertalot & Gerd Moser 1994	(23?)-26-40 (43?)	6-8	28-30	Gomphonemoid	imperceptible	Broadly rounded	Lange-Bertalot & Moser 1994/ Moser et al.1995	New Caledonia	Fig. 35
B. gravida Shayler & Siver, 2004	15-30	6-9	27-31	Elliptical-rhombic. Ribs on the edge of the raphe/ mantle. Presence of papillae.	Rounded to narrow and elongated	Protracted, rostrate	Shayler & Siver 2004	Florida (USA)	Fig. 36
B. guarrerai Vouilloud, Sala & Núñez-Avellaneda 2014	19-38	5.5-7	26-32	Lanceolate, slightly asymmetrical in relation transapical axis	Elliptical, asymmetrical.	Rostrate to subcapitate	Vouillod et al., 2014	Colombia	Fig. 37
B. guttiformis Gert Moser, Lange-Bertalot & Metzeltin	27-44	7.0-8.5	22-24	Rhombic-lanceolate. Longitudinal striae irregularly arranged.	Rhombic-lanceolate	Protracted, rounded	Gert Moser, Lange-Bertalot & Metzeltin 1998	New Caledonia	Fig. 38
B. hannae (Reimer) Lange-	68-26	18-12	22-23	Rhombic	Rhombic	Rounded-rostrate	Lange-Bertalot & Moser 1994/ Moser et	Florida (USA)	Fig. 39

Bertalot & Moser 1994							al.1995 Metzeltin e Lange-Bertalot, 2007		
B. hedyklingiae Metzeltin & Lange-Bertalot, 2007	25-42	8.6-11.3	27-29	Valvas rombical-lanceolate. Gently triondulate margins	Small, rhombic	acutely rounded	Metzeltin & Lange-Bertalot 2007	Florida (USA)	Fig. 40
B. hofmanniae Lange-Bertalot 1994	30-46	5.5-7	28-32	Rhombic-lanceolate, triondulate. Ribs on the edge of the raphe. Presence of papillae.	Ellipitical	Protracted	Lange-Bertalot & Moser 1994	Lustsee, Upper Bavaria (German)	Fig. 41
B. huitotarum Vouilloud, Sala & Núñez-Avellaneda 2014	17-28.5	5-7.5	25-30	Broadly lanceolate to rhomboid. Ribs on the edge of the raphe/ mantle. Presence of papillae.	Rhomboid and slightly irregular to asymmetrical	Rounded to subrostrate	Vouilloud *et al.* 2014	Colombia	Fig. 42
B. inamoena Metzeltin & Lange-Bertalot, 1998	40-74	10-14.5	24-28	Lanceolate	Small	Protracted rounded	Metzeltin & Lange-Bertalot 1998	Brazil	Fig. 43
B. incognita Gerd Moser & Lange-Bertalot 1998	27-32	6-8	28-30	Rhombic to rombic-lanceolate.	Rhombic-lanceolate	Protracted, acutely rounded	Lange-Bertalot & Moser 1994; Moser, Lange-Bertalot & Metzeltin 1998	New Caledonia	Fig. 44

B. inflata Lange-Bertalot 1994	45-63	8.5-11.5	20-22	Lanceolate. Middle part of valve is distinctly inflated	Wide, rhombic to transverse elliptical	Rounded	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 45
B. insulsa Metzeltin & Lange-Bertalot, 1998	29-36	6-7	27-29	Lanceolate. Margin slightly triondulated	Small, approximately rhombic or transverse ellipticals	Rounded slightly acute. Moderately protracted	Metzeltin & Lange-Bertalot, 1998	Brazil	Fig. 46
B. intermedia (Oestrup) Lange-Bertalot 1994	31-26	6		Lanceolate	Small elliptical	Rounded to subcapitate	Lange-Bertalot & Moser 1994 (sem as medidas)		Fig. 47
B. irawanae (Podzorski & HÅkansson) Lange-Bertalot & Podzorski 1994	15-50	4.5-6.5	30-32	Valve lanceolate, gomphonemoid. Central pores of rafe is always clearly distant	Smaller individuals (not visible), larger individuals (elliptical-lanceolate)	Rounded	Lange-Bertalot & Moser, 1994	Philippinen	Fig. 48
B. irawanoides Lange-Bertalot & Gerd Moser 1994	19-30	4.2-4.8	30-32	Lanceolate to rhombical-lanceolate, gomphonemoid. Central pores of rafe is always clearly distant from each other.	Imperceptible or contracted, in larger individuals little expanded	Obtuse to rounded	Lange-Bertalot & Moser, 1994	New Caledonia	Fig. 49
B. jankae Matzeltin & Lange-Bertalot,	36-60	14-17	21-22	Broad-rhombical	Smaller	Acutely cuneate	Metzeltin & Lange-Bertalot 2007	Bahia (Brazil)	Fig. 50

2007									
B. julio Lange-Bertalot 1994	24-32	5.5-6.5	24-26	Rhombic-lanceolate, triondulate. Presence of papillae.	Small	Capitate-rostrate	Lange-Bertalot & Moser, 1994	Laguna de la Plaza (Colombia)	Fig. 51
B. krammeri Lange-Bertalot 1994	17-55	5-8	22-24	Lanceolate	Rhombic	Obtuse rounded	Lange-Bertalot & Moser, 1994	New Caledonia	Fig. 52
B. kuntzei Metzeltin & Lange-Bertalot, 1998	55-95	20-28	20-21	Rhombic-lanceolate. Transverse siliceous thickening	Elliptical transversally	Rounded-capitate	Metzeltin & Lange-Bertalot 1998		Fig. 53
B. labrata Lange-Bertalot & Gerd Moser 1995	26-32	7-8	17-19	Rhombic-lanceolate		Acute-rounded	Moser et al. 1995	New Caledonia	Fig. 54
B. lange-bertalotii Metzeltin & Lange-Bertalot, 1998	17-44	8.5-14	24-27	Rhombic-lanceolate. Transverse siliceous thickening	Elliptical transversally	Acute-rounded	Metzeltin & Lange-Bertalot 1998	Potaro river (Guyana)	Fig. 55
B. lecohui Lange-Bertalot 1994	27-62	7-11	23-26	Rhombic to rhombical-lanceolate, triondulated. Presence of papillae.	Rhombic	Acute-rounded	Lange-Bertalot & Moser, 1994	New Caledonia	Fig. 56
B. lehmanniae Lange-Bertalot &	24-30	8-9.5	23-25	Rhombic-lanceolate	Absence or small and	Obtuse-rounded	Lange-Bertalot & Moser 1994	Sierra Nevada	Fig. 57

Gerd Moser 1994					rounded			(Colombia)	
B. liliana Lange-Betalot 1994	23-50	5-7	36-40	Rhombic-elliptic. Presence of papillae.	Narrowly elliptical or absence in small species	Obtuse-rounded	Lange-Bertalot & Moser 1994	(German) Lustsee, Upper Bavaria	Fig. 58
B. linearilanceolata Metzeltin & Lange-Bertalot, 2007	39-47	7.3-8.7	23-25	Linear-lanceolate. Presence of papillae.	Moderately large, circular or rhombical	Obtusely rounded	Metzeltin & Lange-Bertalot 2007	Florida (USA)	Fig. 59
B. longirostris (Hustedt) Mann 1990	24	4		Rhombic	Absence	Rounded	Lange-Bertalot & Moser 1994 Simonsen (1987, p.272 fig 406:3-8)	Sulawesi Island (Indonesia)	Fig. 60
B. macroserians Metzeltin & Lange-Bertalot, 1998	75-96	17-20	18-21	Lanceolate, large areolas	Small, transverse elliptical to rhombic	Obtuse-rounded	Metzeltin & Lange-Bertalot, 1998	Brazil	Fig. 61
B. maillardii Lange-Bertalot & Gerd Moser 1994	30-60	6-8.5	27-29 (30)	Lanceolate to linear-lanceolate. Central pores of raphe almost always distant	Slightly elliptical	Rounded-Capitate	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 62
B. manfredii Lange-Bertalot 1994	23-40	(4.5)5-6	24.5-26.5 (30-35)	Lanceolate rhombic–lanceolate. Presence of papillae. Ribs on the edge of the	Elliptical transverse, and slightly asymmetrical	Acute-rounded	Lange-Bertalot & Moser 1994, Vouillod et al 2014	Beedelup Falls (Australia)	Fig. 63

raphe/ mantle.

B. manoylovae Metzeltin & Lange-Bertalot 2007	20-26	6.0-7.3	27-29	Broadly lanceolate or elliptic-lanceolate	Very small or absence	Long-protracted, Sub-rostrate	Metzeltin & Lange-Bertalot, 2007	Guadeloupe Island	Fig. 64
B. metzeltinii Lange-Bertalot 1994	26-37	5.3-6.5	27-30	Lanceolate to rhombical-lanceolate. Presence of papillae. Ribs on the edge of the raphe/ mantle.	Small rhombic	Short rounded. Raphe curved at the apex	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 65
B. microcephala (Grunow) P. Compère 1986	13-28	4-6.5	32-39	Lanceolate. Presence of papillae. Ribs on the edge of the raphe/ mantle.	Rounded or Distinctly rounded or lanceolate appearing	Rostrate to subcapitate	Vouillod et al 2014, (Olhar Compere 1986 e 1988) Kennedy & Allott 2007		Fig. 66
B. microclava Lange-Bertalot & Gerd Moser 1994	10-30	3.6-4	27-29	Narrow Gomphonemoid. Presence of papillae. Ribs on the edge of the raphe/ mantle.	Rounded to elliptical	Rounded	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 67
B. microserians Metzeltin & Lange-Bertalot,	24-31	7-8	24-27	Lanceolate	Small and rounded	Acute-rounded, slightly	Metzeltin & Lange-Bertalot, 1998	Monte Roraima (Venezuela)	Fig. 68

1998						capitate			
B. minor (Krasske) Lange-Bertalot 1994	12-28	3-5	(30) 36-39	Linear-elliptic	Large, rounded, transverse-elliptical or rhombic.	Broadly rounded	Krasske1939, Lange-Bertalot & Moser 1994	Rizo patron lake (Chile)	Fig. 69
B. nanoclava Gerd Moser, Lange-Bertalot & Metzeltin, 1998	9-22	2.5-3.5	26-28	Gomphonemoid. Striae are usually interrupted at the edge of the mantle	Elliptic-lanceolate	Obtuse to rounded	Moser, Lange-Bertalot e Metzeltin, 1998	New Caledonia	Fig. 70
B. neglectissima Lange-Bertalot, 2004	18-30	4.3-5.4	36-40	Lanceolate to rhombic-lanceolate	Variable, indistinct to very small	Protracted-rostrate to subcapitate	Werum & Lange-Bertalot 2004, Kennedy & Allot 2017	Weitsee, Upper Bavaria (German)	Fig. 71
B. neoacuta Lange-Bertalot 1994	40-65	6-9	24-26	Lanceolate. Presence of papillae	Small, rhombic	Acute-rounded	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 72
B. neocaledonica (Maillard) Lange-Bertalot & Moser 1994	45-62	8-10	28-30	Rhombic-lanceolate	Rounded	Subcapitate to rounded	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 73
B. neoexilis Lange-Bertalot 1994	12-34(36)	3-5(7)	30-36	Lanceolate, elliptical or Rhombic-lanceolate. Presence of papillae.	Small circular to moderately rhombic	Mostly distinctly rostrate or subcapitate	Lange-Bertalot & Moser 1994, Kennedy & Allott 2007	Tirol (Austria)	Fig. 74
B. nubigena Lange-Bertalot	40-70	9-12	22-23	Lanceolate. Presence of	Rhombic	Obtuse-rounded to	Lange-Bertalot & Moser 1994	Colombia	Fig. 75

1994				papillae.		protracted			
B. obesa (Maillard) Lange-Bertalot & Moser 1995	16-26	6-7	23-30	Divided into three portions: central area and expanded apex	Rounded	Capitate, broadly rounded	Moser et al., 1995	New Caledonia	Fig. 76
B. ocalanensis Shayler & Siver 2004	21.5-35.5	4-6	33-43	Valves heteropolar and laceolate to linear lanceolate Papillae often present.	Rounded to elongate	Rounded, slightly subcapitate	Vouillod et al., 2014; Shayler & Siver 2004	Florida (USA)	Fig. 77
B. ontonageniana Kociolek et Lowe	16-40	4.5-5.5	28-31	Valvas lanceolate-clavate	Indistinct narrow lanceolate	Rounded, never protracted. Heteropolar outline	Anderson et al. 2013	Michigan county (USA)	Fig. 78
B. potapovae Metzeltin & Lange-Bertalot 2007	19-30	9.5-12	30-33	Rhombic-elliptic or broadly rhombic-lanceolate. Striae conspicuously densely spaced	Small and rhombic	Cuneate, rounded.	Metzeltin & Lange-Bertalot, 2007	Florida (USA)	Fig. 79
B. praegeri Kennedy & Allott 2017	16-22.6	4.2-5.4	32-36	Lanceolate, convex margins tapering evenly to non-protracted. Presence of papillae also in the central area	Indistinct rounded or absent often with papillae.	Rounded and never protracted	Kennedy & Allott, 2017	Galway (Irelan)	Fig. 80

B. procera Lange-Bertalot & Moser 1994	25-60	4.5-6	27-30	Valvae narrow and lanceolate. Ribs on the edge of the raphe. Presence of papillae.	Barely transversely dilated but apically elongate in larger valves	Obtuse-rounded	Lange-Bertalot & Moser 1994	Scotland	Fig. 81
B. pseudoacuta Lange-Bertalot 1994	32-57	6.5-8.5	21-23	Rhombic-lanceolate. Ribs on the edge of the raphe/ mantle. Presence of papillae.	Rhombic	Acute-rounded	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 82
B. pulchra Lange-Bertalot 1994	36-55	9-10.5	22-24	Lanceolate. Two hyaline longitudinal lines on each side of the irregularly expanded raphe. Elongated areolas	Rhombic	Slightly protracted	Lange-Bertalot & Moser 1994	Colombia	Fig. 83
B. pumila Metzeltin & Lange-Bertalot 2007	12-17	4-5	34-36	Lanceolate	Absence	Long/ broadly protracted	Metzeltin & Lange-Bertalot, 1998	Yucatan (Mexico)	Fig. 84
B. rhomboides (Hustedt) Mann 1990	45	15	18	Rhombic-lanceolate	Moderately expanded	Slightly acute	Hustedt, F. 1942 (http://symbiont.ansp.org)	Sulawesi Island (Indonésia)	Fig. 85
B. rostrata (Krasske)	40-60	9-14	20-25	Lanceolate	Transversely elliptical	Narrowly rostrate	Metzeltin & Lange-Bertalot, 1998;	São Paulo (Brazil)	Fig. 86

Metzeltin & Lange-Bertalot 1998							Metzeltin & Lange-Bertalot, 2007		
B. ruckiae Metzeltin & Lange-Bertalot, 2007	33-62	8.7-10	24-26	Rhombic-lanceolate. Ribs on the edge of mantle. Presence of papillae.	Elliptical	Obtuse-rounded	Metzeltin & Lange-Bertalot, 2007	Florida (USA)	Fig. 87
B. rumrichiae Lange-Bertalot 1994	75-80	7-10	24	Linear-lanceolate. Ribs on the edge of the raphe. Presence of papillae.	Rhombic	Rounded-subcapitate	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 88
B. ruppeliana Gerd Moser, Lange-Bertalot & Metzeltin, 1998	20-21	4	29-33	Linear. Ribs on the edge of the raphe but do not unite at the apex. Presence of papillae.	Rounded	Obtuse-Rounded	Moser, Lange-Bertalot & Metzeltin, 1998	New Caledonia	Fig. 89
B. sandrae B. Van de Vijver 2014	21–40	5.4–7.1	29–31	Narrowly lanceolate to rhombic lanceolate. Presence of papillae.	Narrow, linear	Protracted rostrate and rounded	Van de Vijver 2014	Iles Kerguelen (sub-Antarctica)	Fig. 90
B. seippii Lange-Bertalot & Moser 1994	17.5-25	6.5-7.5	25-27	Lanceolate, gomphonemoid	Smallest or absence	Cuneate	Lange-Bertalot & Moser, 1994	Nova Caledonia	Fig. 91
B. serians							Lange-Bertalot &	Santorini	Fig.

(Brébisson) Round & Mann 1981							Moser, 1994	(Israel)	92
B. silvicola Lange-Bertalot 1994	20-26	5-6	25-26	Elliptic-lanceolate. Ribs on the edge of the raphe. Presence of papillae.	Small rounded or rhombic	Subcapitate to capitate-constrict	Lange-Bertalot & Moser, 1994	New Caledonia	Fig. 93
B. simplex Lange-Bertalot 1994	32-38	15-16	22-24 in10	Elliptic-lanceolate	Absence	Rounded, slightly protracted.	Lange-Bertalot & Moser, 1994	Venezuela	Fig. 94
B. sinaiensis Lange-Bertalot, 2004	20-34	6-6.6	40-44	Rhombic	Small, elliptical	Acutely rounded	Werum & Lange-Bertalot, 2004	Sinai (Egypt)	Fig. 95
B. spectabilis (Manguin) Lange-Bertalot & Moser 1994	54,5	8	28 in 10	Rhombic-lanceolate	Elliptical-lanceolate	Rounded	Lange-Bertalot & Moser, 1994		Fig. 96
B. speluncola Lange-Bertalot 1994	12-40	8-15	21-23 in 10	Rhombic to rhombic-lanceolate. Ribs on the edge of the raphe/ mantle. Presence of papillae.	Rounded to rhombic	Protracted-rounded	Lange-Bertalot & Moser, 1994	South Africa	Fig. 97
B. spicula Gerd Moser, Lange-Bertalot & Metzeltin, 1998	29-44	4.0-5.3	24-26	Narrow, gomphonemoid. Shortened raphe	Absent or small transapically narrow and	Acute-rounded	Moser, Lange-Bertalot & Metzeltin, 1998	New Caledonia	Fig. 98

B. starmuehlneri (Maillard.) Gerd Moser Lange-Bertalot & Metzeltin 1998	29-40	6-8	22-24	Elliptical triondulate	elongated Small redonded	Rounded	Moser et al., 1995; Moser, Lange-Bertalot & Metzeltin, 1998	New Caledonia	Fig. 99
B. staurophora Souza & Compère	53-90	16-20	18-24	Rhombic to rhombic lanceolate,	Slightly transversally widened (stauros-like appearance),	Acutely rounded, sometimes showing a more or less developed pseudoseptum at one end	Souza & Compère, 1999	Federal District (Brazil)	Fig. 100
B. steindorfiana Moser , Lange-Bertalot & Metzeltin, 1998	40-70	7.0-12	22-24	Lanceolate to linear-lanceolate, gomphonemoid (heteropolar). Central area with rafes distant between them	Transapically expanded	Acute-rounded	Moser, Lange-Bertalot & Metzeltin, 1998	New Caledonia	Fig. 101
B. steinitziae Metzeltin & Lange-Bertalot, 1998	29-36	6-6.5	24-26	Lanceolate. Prominent interstrias (silicon thickening)	Small rounded	Protracted-rostrate to subcapitate	Metzeltin & Lange-Bertalot, 1998	Venezuela	Fig. 102
B. styriaca (Grunow) Ross in Hartley 1986	14-50	5-8	27-28 (30)	Rhombic, rhombic-lanceolate to linear-lanceolate.	Rounded to linear (elliptical)	Rounded	Lange-Bertalot & Moser 1994	Hemisfério Norte	Fig. 103

				Presence of papillae.					
B. sublinearis Metzeltin & Lange-Bertalot, 1998	38-42	5.5-6	25	Linear-lanceolate. Ribs on the edge of the raphe. Presence of papillae.	Irregulary rhombic	Rounded	Metzeltin & Lange-Bertalot, 1998	Potaro river (Guyana)	Fig. 104
B. subrostrata Lange-Bertalot 1994	35-67	9-14	19-24 in 10	Rhombic-lanceolate	Small, rhomboidal, irregular	Acute-rostrate, slightly protracted	Lange-Bertalot & Moser 1994; Vouillod et al 2014	Santanna (Brazil)	Fig. 105
B. subtile Potapova, Hamilton & Kopyrina	17-42	3.2-4.4	32-40	Valves linear-lanceolate	small, elliptic, apically elongated.	Apex not protracted.	Potapova et al. 2014	Sakha, Yakutia Republic (Russia)	Fig. 106
B. superba (Lange-Bertalot & Gerd Moser) Gerd Moser, Lange-Bertalot & Metzeltin, 1998	40-55	10-12	19-22	Rhombic-lanceolate	Rhombic-elliptic	Broadly rounded	Lange-Bertalot & Moser 1994;	New Caledonia	Fig. 107
B. superserians Metzeltin & Lange-Bertalot, 2007	145-165	26-28	13-15	Rhombic-lanceolate	Expanded	Usually rounded	Metzeltin & Lange-Bertalot, 2007	Florida (USA)	Fig. 108
B. supriniana Gerd Moser, Lange-Bertalot & Metzeltin, 1998	18-27	6.0-7.5	19-21	Lanceolate to Rhombic-lanceolate. Presence of	Lanceolate	Broadly rounded or protracted	Gerd Moser, Lange-Bertalot & Metzeltin, 1998	New Caledonia	Fig. 109

				papillae. Some depressions in the central area near raphe					
B. tenuiserians Lange-Bertalot 1994	40-75	7-10	22-24 in 10	Narrow, lanceolate	Small, rhombic	Obtuse-rounded	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 110
B. trinodis Lange-Bertalot & Moser 1994	38-45	6.5-9	22-23	Linear-elliptic to linear, expanded in the central area	Small, rhombic	Broadly rounded	Lange-Bertalot & Moser 1994	New Caledonia	Fig. 111
B. venter Lange-Bertalot & Moser 1995	56-65	12-13	18-20	Elliptical, expanded in the central area	Rounded	Rounded	Moser et al., 1995	New Caledonia	Fig. 112
B. vitrea (Grunow Ross 1986	16-40	5.5-9	30-35	Lanceolate to elliptical-lanceolate. Presence of papillae.	Absence or only slightly Expanded, rounded	Capitate to occasionally rostrate	Lange-Bertalot & Moser 1994		Fig. 113
B. vixapiculata Metzeltin & lange-Bertalot 2007	30-60	15-18	19.5-20.5	Elliptical-lanceolate	Small, rounded	Protracted-capitate or subcapitate to short-subrostrate	Metzeltin & Lange-Bertalot, 2007	Venezuela	Fig. 114
B. wolfei Metzeltin & Lange-Bertalot 2007	(16) 26-48	(6.6) 8.7-10	25	Rhombical-lanceolate	Small, rhombical shaped	Acutely rounded	Metzeltin & Lange-Bertalot, 2007	Florida (USA)	Fig. 115
B. wygaschii Lange-Bertalot	30-65	7-12	20-24 in 10	Lanceolate, rhombic-	Transversely wide rhombic	Acute-Rounded	Lange-Bertalot & Moser 1994		Fig. 116

1994				lanceolate. Ribs on the edge of the raphe.	or elliptical			
B. zellensis (Grunow) Round & Mann 1981	15-45	4-7	27-32	Linear with parallel sides, slightly triondulated edges. Presence of papillae.	Rhombic to elliptic	Rounded, subrostrate	Round & Mann 1981 & Lange-Bertalot & Moser 1994	Fig. 117

Table 4. Classification of types of references screened without habit citations and types of references with the habit mentioned for species of the genus *Brachysira*.

Type of reference	Absence of citation of habit [1]	Presented citation of habit [2]
Atlas	0,00%	1,32%
Bioindication	0,26%	5,55%
Ecology	0,40%	17,57%
Flora	31,04%	2,77%
Review	21,14%	6,21%
Taxonomy	12,42%	1,32%

[1] Percentage values generated based on a total of 757 screened records.
The total number of records that did not present information on the habit was 280.
[2] Total number of records that contained information it was 477.

Table 5. Percentage of publications classified according to habit type citations. Percentage of all records screened (including those that did not mention the habit) and the total number of records that presented the type of habit. Periphyton = fixed to a substrate; Mixed = not defined the type of habit in the article; Phytoplankton = not adhered, found in the water column.

Type of habit	Citation[1]	Citation[2]
Periphyton	76.10%	47.95%
Mixed	14.47%	9.11%
Phytoplankton	9.22%	5.81%
Sub-air	0.21%	0.13%

[1] Percentage in 280 of records screened that presented de type of habit.
[2] Percentage in 757, total of records screened.

Table 6. Classification of types of references screened without environment citations and types of references with the environment mentioned for species of the genus *Brachysira*.

Type of reference	Absence of citation of habitat (%) [1]	Presence of citation of habitat (%) [2]
Atlas	0	1.32%
Bioindication	0	5.81%
Ecology	0	17.97%
Flora	0.92%	4.02%
Review	18.63%	81.03%
Taxonomy	3.43%	14.94%

[1] Percentage values generated based on a total of 757 sorted records. The total number of records that did not present information about the environment was 174.
[2] Percentage values generated based records that contain information it was 583.

Table 7. Citations of type of environment mentioned in the records screened. Comparison between the percentage of total records that mentioned the environment and the total of screened records.

Environment	Citation [1]	Citation [2]
Lentic	56.09%	43.20%
Lotic	29.50%	22.72%
Mixed	14.07%	10.83%
Other	0.34%	0.26%

[1] Percentage in 583 of records screened that presented de type of habit.
[2] Percentage in 757, total of records screened.

Table 8. Fossil species of the genus *Brachysira*, where they were found and in which key references were mentioned.

Fossil specie	Locality	Reference
B. delicatissima [1]	USA. Florida Santa Rosa county	Metzeltin and Lange-Bertalot (2007);
	Colombia	Montoya-Moreno et al. (2013).
B. elliptica	USA. Florida Santa Rosa county	Metzeltin and Lange-Bertalot (2007).
B. fossilis	USA. Florida Santa Rosa county	Lange-Bertalot and Moser (1994)
B. hannae	USA. New Jersey	Lange-Bertalot and Moser (1994);
	Canada. Baffin Island	Wolfe and Kling (2014).
B. hedyklingiae	USA. Florida Santa Rosa county	Meltzeltin and Lange-Bertalot (2007).
B. insulsa	Brazil. Santos	Metzeltin and Lange-Bertalot (1998).
	Guyana. Essequibo river	Metzeltin and Lange-Bertalot (1998).
	Guyana. Potaro river	Metzeltin and Lange-Bertalot (1998).
	Guyana. Kaieteur Falls	Metzeltin and Lange-Bertalot (1998).
	México. Yucatan. Cobá-see	Metzeltin and Lange-Bertalot (1998).
	México. Yucatan. Misol-Há	Metzeltin and Lange-Bertalot (1998).
B. macroserians	Brazil	Metzeltin and Lange-Bertalot (1998).
B. potapovae [2]	USA. Florida Santa Rosa county	Metzeltin and Lange-Bertalot (2007).
B. wolfei [2]	USA. Florida Santa Rosa county	Metzeltin and Lange-Bertalot (2007).

[1] Species cited as found only one locality, however it was found in other place.
[2] Species cited as found only one locality.

Table 9. Percentage of sample effort by citations of species of the genus *Brachysira* distributed by countries. Countries with greater relevance in the sampling effort are underline.

Countries	Citation %	Countries	Citation %
Albania	0.1%	Ireland	2.5%
Germany	0.7%	Iceland	0.1%
Antarctica	0.7%	Israel	0.7%
Argentina	0.3%	Italy	3.2%
Australia	1.1%	Japan	0.9%
Austria	0.8%	Kenya	0.1%
Azerbaijan	0.1%	Macedonia	0.4%
Belgium	0.7%	Malaysia	0.1%
Benin Republic	0.1%	Mexico	1.5%
Bosnia/ Herzegovina	0.4%	Mongolia	0.5%
Brazil	21.9%	Nigeria	0.9%
Canada	7.3%	Norway	0.9%
Chile	0.1%	New Zealand	1.3%
China	0.1%	Scandinavia	0.1%
Colombia	3.7%	Wales	0.5%
South Korea	0.3%	Papua New Guinea	1.6%
Cuba	0.1%	Peru	0.1%
Rep. Democ. Congo	0.4%	Poland	0.5%
Egypt	0.3%	Portugal	0.4%
Ecuador	0.4%	Kenya	0.7%
Scotland	2.4%	King United	1.2%
Spain	2.1%	Republic of Karelia	0.4%
Estonia	0.3%	Czech Republic	1.1%
Philippines	0.1%	Romania	0.1%
Finland	1.1%	Russia	2.5%
France [1]	5.7%	Senegal	0.1%
French Guyane	0.1%	Sierra Leone	0.9%
French Sudan	0.3%	Serbia	0.9%
Guyana	0.8%	South Africa	0.9%
Hawaii	0.1%	Sweden	1.1%
Netherland	0.4%	Switzerland	0.1%
Hungary and Sweden	0.1%	Thailand	0.1%
Iberian Peninsula	0.1%	Tajikistan	0.4%
Crozet Island	0.1%	Tanzania	0.1%
Kerguelen Island	0.1%	Turkey	0.5%
India	2.0%	USA	12.8%
England	1.6%	Venezuela	0.9%
Iraq	0.4%	Yemen	0.1%

[1]France has 2 records in Bordeaux plus records found in New Caledonia and Guadeloupe Island (territory that belongs to France).

Table 10. Percentage of sample effort by citations of species of the genus *Brachysira* distributed in Brazil. States with greater relevance in the sampling effort are underline.

States	Citation %	States	Citation %
Amazonas	2.4%	Minas Gerais	1.2%
Bahia	0.6%	Pará	0.6%
Distrito Federal	4.2%	Paraná	51.5%
Espírito Santo	0.6%	Rio de Janeiro	1.2%
Goiás	5.5%	Rio Grande do Sul	7.9%
Maranhão	1.8%	São Paulo	21.8%

5. Title: A brief review of the genus *Gomphonema* Ehrenberg (Bacillariophyceae): Distribution and Character Evolution

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Abstract

The genus *Gomphonema* has remarkable characteristics in its morphology, such as heteropolar valves, presence of stigma in the central area and pore field used to adhere to the substrate. This taxon has a large number of species and a wide distribution of the genus. Its species have wide morphological diversity and together with its wide distribution it is considered a good tool for bioindication of the aquatic environment. However, correct taxon identification is necessary since specimens with the same genotypes can be identified as different species, for which genetic data is proving to be a good tool for solving this problem. The aim of our study was: a) to verify the evolution of morphological characters in different species and relate them to ancestry; b) to analyze the distribution of species through the analysis of sampling effort and diversity. Two morphological characters were more consistent to present an ancestral relationship. The sampling effort was greater in the northern hemisphere and the diversity of species was highlighted in three points in the world (Canada, India and part of Europe). The diversity was related to the sampling effort and in places considered hotspots of biodiversity. However, more data is needed to analyze the evolution of characters, mainly of endemic species, increasing the diversity of morphological data. In which it would assist us in more robust results in the relationship of ancestry and diversity distribution, mainly in places with India, which still has a relationship with other continents due to its connection in the past and which probably brought great diversity and endemism of species, as in the genus *Gomphonema*.

Keywords: diversity, endemic, filogeny, synapomorphy

1. Introduction

The genus *Gomphonema* Ehrenberg (1832) is characterized by having heteropolar valves, stigma in the central area of the valve, striae that can vary from uni- to biseriatedas composed of areolas with different patterns that can occur solitary or in colonies. In addition, this taxon has a pore field responsible for the production of a mucilage stem, to which the cell adheres to the substrates (periphytic habit) (Round et al. 1990, Abarca et al. 2014, 2020).

It is considered one of the genus with the largest number of species, with 535 species accepted taxonomically according Guiry & Guiry, 2020. In the last 20 years, there has been a significant increase in the number of taxa for this genus (Ponader et al 2017), in which several reviews separated complexes and/ or identified new species (Reichardt 2005, 2009, 2015), as in Levkov et al. (2016) who described 30 new taxa for the genus. In addition, new species are often identified in different locations around the world (Karthick & Kociolek 2012, Kociolek et al. 2016, Kociolek et al 2018, Liao & Li 2018).

Another striking feature of this taxon is the wide distribution of the genus worldwide (Kociolek 2018). Although for freshwater diatoms the distribution pattern is more complex, relating their evolutionary history and the Earth's history (Kociolek and Spaulding, 2000).

Due to the wide distribution and the great morphological diversity of the species, *Gomphonema* is already widely used in studies involving bioindication, since for the characterization of aquatic environments, we can use diversity measures to verify the ecological status mainly after changes by abiotic factors (Blanco et al. 2012).This taxon presents some species that have a wide tolerance spectrum and are found in almost all environments, for example, *G. parvulum* in the indication of the level of pollution (Larras et al. 2014), as well as *G. intricatum* related to the alkalinity of the environment (Galovic et al. 2018). However, also there are other species that are considered endemic (Karthick et al 2011, Li et al 2006) and that can be limited by different habitats (Kociolek et al 2004).

Even though the genus has striking characteristics (for example, heteropolar), species delimitation is not so simple and the accurate identification is necessary for several studies, as in bioindication (Rimet and Bouchez, 2012). It is already known that changes in the morphology of specimens with the same genotype can result in the

identification of different species (Rose & Cox 2014), so it is necessary to apply new tools together with the existing ones, for a more coherent distinction of these taxa, such as the addition of molecular investigations (Cox et al., 2012) that, has already been applied in some studies in the last decade (Abarca et al. 2020, 2014, Jahn et al 2019, Kulikovskiy et al 2019). However, the use of molecular data is still in the introductory phase of the routine of identifying diatoms.

Even if there are already some studies involving *Gomphonema* and genetic data, there are still no studies directly relating the ancestry of morphological characters. However, this type of study, such as character evolution, can also be of great value to understand the differentiation of species of the genus *Gomphonema*, knowing the origin of the diversity of this taxon through the relationship of ancestry of morphological characters.

The aim of our study was: a) to verify the evolution of morphological characters in different species and relate them to ancestry; b) to analyze the distribution of species through the analysis of sampling effort and diversity

2. Material and methods

2.1 *Character Evolution – Genotypic and Phenotypic data*

Sequences of *18S* was screened in the NCBI (www.ncbi.nlm.nih.gov/genbank/) for the genus Gomphonema and only the most informative sequence was chosen for each species (Table S1). The sequences were converted into FASTA format and were later aligned in the MEGA 7 program (Kumar et al, 2015) using both *ClustalW* and *Muscle* tools.

Bayesian inference analyzed consisted of multiple executions with the evolutionary model GTR +G+I, analyzed on the Cipres (Miller et al 2010) on line interface (MrBayes).

After choosing the best sequences (18S) of the species available in the database (Table S1). We defined six morphological characters for the taxa through the classic and modern reference query and building a character matrix in the Mesquite 3.4 program (Maddison & Maddison 2019). These six characters were classified according to the morphology presented in binary and multibinary: one character with four states (multibinary), three characters presented three states (multibinary) and two presented two states (binary) (Table 1).

After obtaining the phylogenetic tree, we used the Mesquite program to visualize the cladogram and subsequently reconstruct the state of the ancestral character for the six morphological characters.

2.2 *Sampling effort/ Diversity*

The records were screened in different databases (NCBI, Research gate, Google scholar, Science Direct) using the word "Gomphonema" alone or together with other different words (for example: bioindication , distribution, diversity, genetics, molecular, phylogeny, richness).

Records that presented duplicated data, no species identification that does not has the species *Gomphonema* in the results, species used for more the one study and study that does not has the data about the localization (e.g. laboratorial studies, culture), were excluded.

The material screened from 1942 to 2020 had its information classified into different variables (Table S1).

For analysis of sampling effort, richness and diversity, a previous "taxonomic cleaning" was done, adding the frequencies of synonyms of each species to the name of the current species; coordinates were added for citations without this data; coordinates that were not in decimals were converted and finally a table with all species and their respective decimal coordinates was built (Table S2).

Sampling effort was analyzed in the Qgis program and the diversity of species in the R program, which generated a shape that was configured in the Qgis program.

3. Results

3.1 *Character evolution*

According 18S sequences available at the database, we selected 33: 28 species for the genus *Gomphonema*, 04 *Gomphonella*, 01 *Gomphoneis* and 01 *Cymbela affinis* Kützing (outgroup) (Figs 1-91) (Table S1). We cannot found the image that show the areolae structure in *Gomphonema intricatum* so, lack this information at the analyze, *Gomphoneis minuta*, we assume that this species has the rounded areolae since all *Gomphoneis* present this areolae pattern. (Fig. 73)

Phylogenetic analyses, the results support monophyly of the genus *Gomphonella* with Bayesian posterior probability (PP) of 1.0. There is a group *Gomphonema* (PP 1.0),

however with excession *G. micropus* and *G. rosenstockianum*, that are presented near to group *Gomphonella* (Fig. 92)

In the analysis of the combined data, the valve margin formed a "undulate" group. And in the morphological character of stigma there was a clear grouping of species of the genus *Gomphonella* that "do not have stigma" (Figs. 93-97)

A total of 1635 records were screened, 1084 were excluded, 551 studies were selected generating 2266 frequency by species that were added to the main table (Fig. 98) (Table S2).

Of the species that were mentioned there was a preliminary "*taxonomic cleanliness*" and some species were excluded and their frequency was added to the current and correct name of the taxon, such as synonymization. 335 species were not found in any of the selected works and were classified as "uncertain", "not found", "undocumented", "invalid name" or "alternative name" (according to Worms and Potapova et al 2020 - ANSP) (Table S2). Of the total number of species, 21 changed to another genus as shown in table 2.

3.2 *Sampling effort*

The sampling effort rate was higher in Canada and southern Europe (Fig. 99). In the neotropical region, the south region of Brazil was the one with the highest rate (Fig. 100).

3.3 *Diversity of species*

Analysis of species diversity in the world (Fig. 101) showed two countries that showed greater diversity, India and Canada (Figs. 102, 103) followed by part of Europe (Fig. 104). However, in Brazil, diversity was not widely distributed in the country, being concentrated in some regions (Fig. 105).

4. Discussion

4.1 *Character evolution*

In the phylogenetic analysis, it was observed that, despite the low support (PP 0.58), *G. carolinense* is close to *G. pseudoaugur* (Fig. 92), this is interesting, as the two species are similar, differing mainly in the amount of stigma present (from 2 stigma and 1 stigma, respectively) (Figs. 29, 58-59). This situation makes us wonder if *G. carolinense* is a variation of *G. pseudoaugur*, since it is already known that the habitat

can alter the morphological characters (Cox 2010) of some species. We also emphasize that, since its first description (Hagelstein, 1939), the records of *G. carolinense* are very scarce (Gjini et al 2015, Ivanov et al 2006, Stoyneva-Gärtner et al., 2015, UTEX FD285 and 330), resulting in a species considered endangered (Stoyneva-Gärtnet et al., 2015). However, if this species is a variation of another species, *G. carolinense* would not be in extinction but would be a morphological response to some environmental variable. Another unlikely situation was the presence of two species of the genus *Gomphonema* (*G. micropus* PP0.94; *G. rosenstockianum* PP 1) in the *Gomphonella* group. This can be explained by the evolution of the characters related to the shape of the areola since both have rounded areolas (Novais et al. 2009) (Figs 42-45, 62-64).

Regarding the analysis of character evolution, “Areolae shaped” (Fig. 95) did not define an older common ancentral, however, it is possible to verify a polyphyletic group with most species presenting “two or more shapes”areolae and within this group there was a grouping of species that have “c shape” areolae, so it is possible to define that "c-shaped" is character derived from the group of "two or more shapes" areolae and within “c-shaped” areolae there was a reversion with the species *G. minutum*, since this returned to present the ancestral character. Meanwhile, some species were outside the group to which they corresponded morphologically like *G. clavatum* (Reichardt 2015), that is presented as a synonym for *Gomphonella olivacea* (Jahn et al 2019), so it should be grouped with the species of that genus, however in the phylogenetic analysis this grouping was not possible. This can be explained since according to Cox (2010), the different shapes of the areolae should not be considered as different characters, but as variants in the type of occlusion. *Gomphonema rosenstockianum* and *G. micropus* were grouped in *Gomphonella*, showing an interesting situation, as according to Kermarrec et al. (2011) the generic position of *G. micropus* is not yet well defined, because this species may be close to another genus, such as *Reimeria*, *G. rosenstockianum*, however, it may be in another supra-generic taxa, since it presents divergence between Cymbellaceae and Gomphonemataceae families.

In the character of striae patterns it was clearly defined that the oldest ancentral had uniseriate striae (Fig. 97) and it was possible to observe evident grouping of the genus *Gomphonella*. However, we must consider that *Gomphonema clavatum* belongs to the genus *Gomphonella* (Reichardt, 2015, Jahn et al 2019). In addition, the character of “biseriate striae” is also present in the genus *Gomphoneis* (Jahn et al. 2019, Skibbe et al 2018), therefore, in this analysis, the only representative specimen of the genus

Gomphonema that has this character is *G. minutum*. In this way, the result in the formation of the *Gomphonella* group (biseriate striae) cannot be considered a "natural group". The occurrence of the character uni-biseriate striae can be indicative of parallelism, occurring more than once independently from the same common ancestor for other characters.

In some species of Gomphonemataceae, the presence/ absence of stigma is considered a good indicator of the relationships between species (Kulikovskiy et al., 2019), in our analysis the formation of the *Gomphonella* group can be classified as a monophyletic group that presented synapormophy “without stigma” since the oldest ancestral had a stigma. *Gomphonema carolinense* can be classified as autapomorphic, presenting more than one stigma and was in the “one stigma group” and the same situation can be applied to *G. clavatum* a species without stigma that is also present in the group “one stigma” (Fig. 94) this if we consider that this specimen has the morphology presented by Reichardt 2015.

Analyzing the “valve margin”, the ancestral of this group had a plan margin and the character “undulate margin” is an apomorphic character. However, the margin undulate group can be considered as a polymorphic group, having the presence *G. clavatum* that presented reversion keeping the valve margin plan (Fig. 93).

From the phylogenetic analysis to the character evolution analysis, in all results the species *G. clavatum* (= *Gomphonella olivaceae*) was grouped with species that did not correspond to its morphology, we emphasize that the morphological data used in this analysis was based on Reichardt (2015), since in the article referenced in the database (NCBI) to which the genetic sequence was selected, there was no image for morphological comparison.

It is important to verify the correct morphological identification of the species used in genetic studies, so that there are no trends in the results of other subsequent studies. In the case of *G. clavatum*, there are records with different morphological descriptions, such as the presence of a stigma (Figs 2: 10-13 Wojtal 2003) and the absence of stigma in the description given by Reichardt (2015), who analyzed Ehrenberg's material (Figs 83-91).

4.2 *Sampling effort*

The sampling effort can be used for different purposes, such as water quality indices, for which the sampling effort should not necessarily be high and the difference between phytoplankton and perifiton is insignificant for this type of study (Lane et al. 2007, Zhao et al 2016). However, the same does not apply when the objective is to estimate species diversity. Since benthic diatoms, the sampling effort has a greater influence than when compared to macroorganisms, since the spatial extent, distribution and number of samples in this area define the rate of species accumulation (Azovsky 2011). To estimate diversity, we depend on the representativeness of the sample and, consequently, this is reflected not only in the number of samples, but also in the different types of substrates (Hassan, 2018).

For *Gomphonema*, the local sampling effort in each country was concentrated mainly in Europe and Canada, other points were also evident, such as in Brazil and India and in countries close to India. In our data collected, only slightly more than half of the records have information that the total sample is of periphytic habit (54.37%), the rest being between records without information (22.27%), phytoplankton (6, 47 %) and others (16.99%) (Table S3).

However, it is important to note that, periphyton and phytoplankton presented the biggest difference between species in the assemblies collected (Lane et al. 2007). For *Gomphonema* ssp, the relevant type of sampling is the periphytic, due to its sessile habit, but it can be detached from the periphyton and found in planktonic samples, as observed in our data.

Other issues should also be highlighted in a sample that aims to match the diversity of species in a habitat. In the case of perifiton, it is known that there is no difference between epiliton and epidendron that depending on the substrate it will not provide phosphorus for the development of the adherent periphyton. In the case of the epifiton, different species of macroalgae may have different assemblages and different factors can influence the formation of the assemblages, such as herbivorism and water flow (Townsend Gell 2005) which can affect the composition of species of the adhered diatom assemblage.

In general what is observed is that the occurrence of common taxon can occur in different substrates, however the occurrence of a species in a single periphytic substrate should be viewed with caution, and may be the result of an insufficient sampling effort (Townsend ang Gell 2005).

As important as a good sampling effort in one study, are also local records of different studies over time. The sampling effort for Canada is the result of many studies done in a long time (1972-2019), mainly Laurentian Great Lakes which has a great contribution from Stoemer and Kreis (1978) and Stoermer et al (1999), totaling 157 records for the genus *Gomphonema*.

Studies in India as opposed to Canada started a little later (1991-2020), but it already reaches around 72% (114) of records compared to Canada. This increase in work in this region may be related due to the great diversity in some regions located in that country, in addition to the increase in the number of new species endemic to India and the region close to India (Karthick & Kociolek 2012, Karthick et al. 2011, Karthick et al. 2016, Jütnner et al 2018).

The sampling effort in part of Europe, on the other hand, may be the result of the joint sampling effort of Portugal and Spain, which has 129 records.

In Brazil, the sampling effort for this taxon was mainly concentrated in the south of the country (Fig. 100). What was already expected since the largest contribution of the genus to the country is concentrated in the state of Paraná (Medeiros et al. 2018, 2020, Ruwer et 2019, Tremarin et al. 2009). Of the total of 230 records for Brazil, more than half are registered in Paraná (141).

4.3 *Diversity of species*

The influence of different variables on the structuring of the diatom community, on the distribution of species and on the delimitation of their habitat has been studied for a long time (Patrick 1948).

In order to assess the diversity of species in a given location, a representative sampling effort is required, also collecting species with restricted distribution, as already mentioned above (see Sample effort) (Townsend ang Gell 2005).

For freshwater diatoms, such as Gomphonema, the environmental and spatial variables strongly influence their distribution at different spatial scales (Blanco et al 2012, Keck et al 2018, Marra et al. 2018, Remmer et al 2019, Soininen 2007).

Since these variables have a great influence on the diversity and distribution of species, according to Soininen 2007, they showed that the distribution of diatoms has clear biogeographic patterns. And that dispersion is limited on continental and intercontinental scales, impacting the identity of the diatom community among these different scales (Keck et al 2018).

Thus, we observe that the diversity for the genus *Gomphonema* agrees with the limitation on a continental scale to which we register greater diversity in India, Canada and part of Europe (Fig. 101).

Western Ghats in India are considered hotspots of species endemic to other organisms, such as animals and plants (Myers, 2000). According to Karthick et al. (2012) the genus *Gomphonema* for India may suggest that diatoms in the western Ghats share biogeographic patterns as in the organisms cited by Myers (2000), since this country is related to other continents such as Africa, Madagascar and South America to which had connected geologically in the past. This may explain the high rate of diversity found in western India in our studies (Fig. 102). In addition, *Gomphonema* ssp records for this country are still recent (1991-2020- in our survey) when compared to oldest *Gomphonema* record (1843 - according to our data) (Table S3).

The high diversity for Canada was contributed by several taxonomic studies, citations of new species and survey of species that occur in specific places, such as the Great Laurentian Lakes, located between Canada and the USA (Bahls et al., 2018, Kociolek & Kingston 1999, Ponader et al 2017, Stoermer et al 1999). However, it is possible to observe that the diversity of species is concentrated in the southeastern region of the country, highlighting the smaller number of studies in these other regions of Canada. In contrast, in the United States, species diversity is more distributed across the country (Fig. 103).

In Brazil, diversity is concentrated in the south of the country and in the south of the Southeast and Midwest regions (Fig. 105). This result can be directly related to the high rate of sampling effort for these regions in Brazil (Fig. 100), since the sampling effort can determine ecological variations of diatoms (Hassan 2018). Likewise, it is possible to observe this relationship (effort sample - diversity) in part of Europe, and we must also take into account that the oldest diatom taxonomists came from Europe (Metzeltin, Lange-Bertalot, Ehrenberg), bringing knowledge identification species.

5. Conclusion

By better understanding the evolution of characters it is possible to understand the origin of the biodiversity of the species of this taxon, especially in places like India that have endemic species related to the biogeographic evolution of the land (Karthick & Kociolek 2012).

The best characters to describe the evolutionary history of these species, were the presence / absence of stigma and the shape of the valve margin, which formed monophyletic groups, presenting more adequate results to verify the relationship between these species.

However, the lack of consistency in the other characters may be the result of the lack of more morphological and genetic data. So it would be interesting to add more data from different species, mainly from endemic species, which would help us to obtain more robust results in the relationship ancestry and also in the distribution of diversity, mainly in places like India, which still maintains a relationship with other continents due to its connection in the past and which probably brought great diversity and endemism of species, as in the genus *Gomphonema* (Karthick & Kociolek 2012).

In addition, a greater sampling effort between the number and type of substrate is necessary for the collection of these data, since the low representativeness of the species can also tend to the results of ecological standards, such as the diversity and distribution of species of the taxon, decreasing the chances of collecting species considered rare / endemic.

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Table 1. Character matrix crossing data between taxa and character status. Already included data of the outgroup (*Cymbella affinis*).

Táxon/ Character	**Areolae shaped**	**Dorsiventral**	**Headpole and Footpole**	**Margin valvae**	**Stigma**	**Striae**
Gomphonella olivacea	Rounded	Absence	Different	Plan	None	Biseriate
G. acsiae	Rounded	Absence	Similar	Plan	None	Biseriate
G. coxiae	Rounded	Absence	Similar	Plan	None	Biseriate
G. tegelensis	Rounded	Absence	Similar	Plan	None	Biseriate
Gomphoneis minuta	Rounded	Absence	Different	Plan	One	Biseriate
Cymbella affinis	Slit-shaped	Presence	Similar	Plan	One	Uniseriate
Gomphonema acuminatum	C-shaped	Absence	Strongly diferente	Undulate	One	Uniseriate
G. affine	Two/more shaped	Absence	Similar	Plan	One	Uniseriate
G. angustatum	Two/more shaped	Absence	Similar	Plan	One	Uniseriate
G. angusticephalum	C-shaped	Absence	Strongly different	Undulate	One	Uniseriate
G. angustum	Rounded	Absence	Similar	Plan	One	Uniseriate
G. augur	Two/more shaped		Different	Plan	One	Uni-biseriate
G. bourbonense	C-shaped	Absence	Similar	Plan	One	Uniseriate
G. brebissonii	C-shaped	Absence	Strongly different	Undulate	One	Uniseriate
G. capitatum	C-shaped	Absence	Strongly different	Undulate	One	Uniseriate
G. carolinense	Two/more shaped	Absence	Different	Plan	Two/ more	Uni-biseriate
G. clavatum	Rounded	Absence	Different	Plan	None	Biseriate
G. clevei	Two/more shaped	absence	Different	Plan	One	Biseriate
G. dichotomum	C-shaped	Absence	Similar	Plan	One	Uniseriate
G. exilissimum	Two/more shaped	Absence	Similar	Plan	One	Uniseriate
G. gracile	Two/more shaped	Absence	Similar	Plan	One	Uniseriate
G. intricatum	-	Absence	Similar	Plan	One	Uniseriate
G. lagenula	Two/more shaped	absence	Similar	Plan	One	Uniseriate
G. micropus	Rounded	Absence	Similar	Plan	One	Uniseriate

G. minutum	Two/more shaped	Absence	Similar	Plan	One	Biseriate
G. narodoense	Two/more shaped	Absence	Similar	Plan	One	Uniseriate
G. parvulum	Two/more shaped	Absence	Similar	Plan	One	Uniseriate
G. productum	Two/more shaped	absence	Similar	Plan	One	Uni-biseriate
G. pseudoaugur	Two/more shaped	absence	Different	Plan	One	Uniseriate
G. pumilum	C-shaped	Absence	Similar	Plan	One	Uniseriate
G. rosenstockianum	Rounded	Absence	Similar	Plan	One	Uniseriate
G. saprophilum	Two/more shaped	Absence	Similar	Plan	One	Uniseriate
G. subclavatum	Two/more shaped	Absence	Different	Plan	One	Uniseriate
G. truncatum	C-shaped	absence	Strongly different	Undulate	One	Uni-biseriate

Table 2. Species of the genus *Gomphonema* that were reviewed and later transferred to other genus (*Didymosphenia* ssp., *Gomphonella* ssp., *Gomphoneis* ssp., *Gomphosphenia* ssp.)

Current taxon genus	Old taxon genus
DYDIMOSPHENIA	
D. geminata (Lyngbye) M. Schmidt emend Antoine & Benson-Evans 1984	*Gomphonema geminatum* (Lyngbye) C. Agardh 1824
D. dentata (Dorogostaisky) Sktzow & K.I. Meyer 1928	*G. dentata* Dorogostaisky 1904
GOMPHONELLA	
G. densistriata (Levkov) R.Jahn & N.Abarca 2020	*Gomphonema densistriatum* Levkov 2007
G. fonticola (Hustedt) R.Jahn & N.Abarca 2020	*G. olivaceum* var. *fonticola* Hustedt 1945 *G. fonticola* (Hustedt) Levkov & Krstic 2007
G. linearoides (Levkov) R.Jahn & N.Abarca 2020	*G. linearoides* Levkov 2011
G. stauroneiformes (Grunow) R.Jahn & N.Abarca 2020	*G. stauroneiformes* Grunow 1878
G. olivacea (Hornemann) Rabenhorst 1853	*G. clavatum* Reichardt 2015
G. olivacea (Hornemann) Rabenhorst 1853	*G. olivaceum* (Hornemann) Brébissoni 1838. *Gomphoneis olivaceum* (Hornemann) P. Dawson ex Ross & Sims 1978
GOMPHONEIS	
G. curta (Hustedt) Lange-Bertalot 1978	*Gomphonema curtum* Hustedt 1945
G. eriense (Grunow Stoermer 1963	*G. eriense* Grunow 1878
G.	G. herculeano
G. quadripunctatum (Østrup) Cleve ex Dawson 1974	*G. quadripunctatum* (Østrup) Wislouch
G. olivaceoides (Hustedt) Carter 1980	*G. olivaceoides* Hustedt 1950
G. olivaceum (Hornemann) P. Dawson ex Ross & Sims 1978	*G. olivaceum* (Hornemann) Brébissoni 1838
G. septa (Moghadam) Kociolek, Stoermer & Bahls 1986	*G. septum* Moghadam 1969
GOMPHOSPHENIA	
G. grovei (M. Schmidt) Lange-Bertalot 1995	*Gomphonema grovei* M. Schmidt 1899
G. holmquistii (Foged) Lange-Bertalot 1995	*G. holmquistii* Foged 1968
G. lingulatiformis (Lange-Bertalot & Reichardt) Lange-Bertalot 1995	*G. lingulatiformis* Lange-Bertalot & Reichardt 1993
G. reicheltii (M. Schmidt) Lange-Bertalot 1995	*G. reichelti* M. Schmidt 1899
G. tenerrima (Hustedt) E. Reichardt 1999	*G. tenerrimum* Hustedt 1938

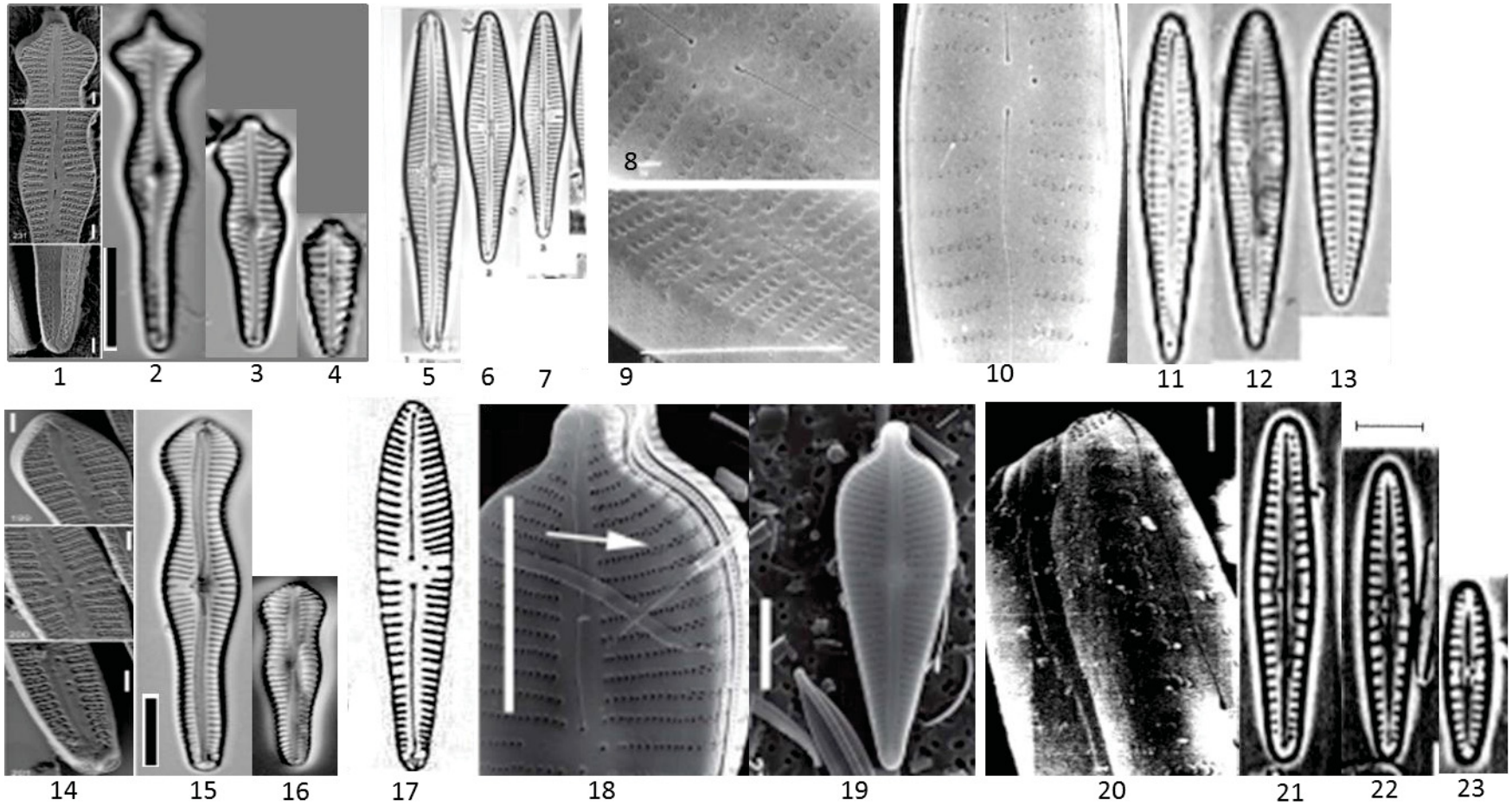

FIGURE 1-23. Figs 1-4 *Gomphonema acuminatum*. Figs 5-9. *G. affine*. Figs 10-13. *G. angustatum*. Figs 14-16 *G. angusticephalum*. Fig 17. *G. angustum*. Figs 18-19 *G. augur*. Figs 20-23. *G. bourbonense*.

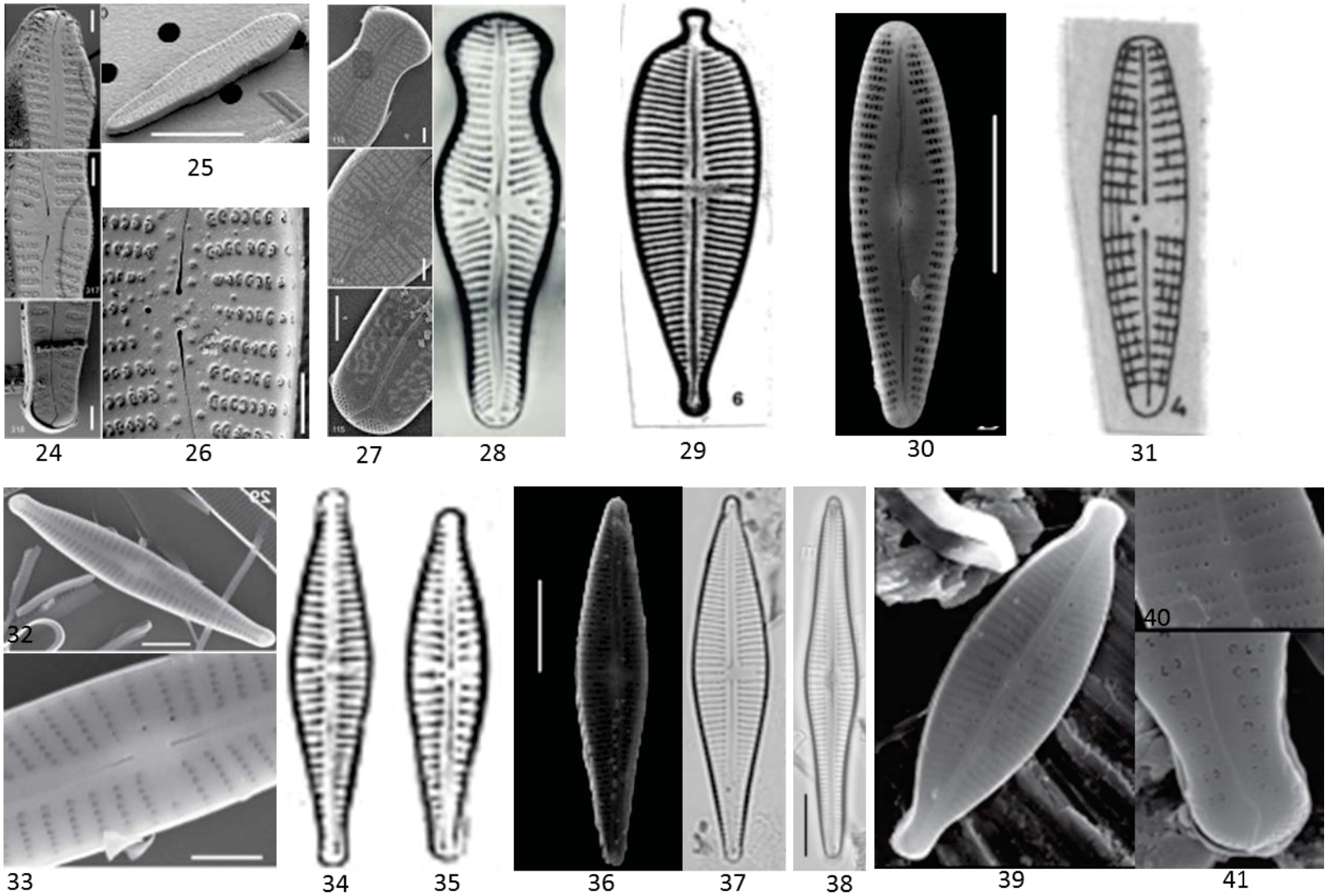

FIGURE 24-41. Figs 24-26 *G. brebissonii*. Figs 27-28 *G. capitatum*. Fig 29 *G. carolinense*. Fig 30 *G. clevei*. Fig 31 *G. dichotomum*. Figs 32-35 *G. exilissimum*. Figs 36-37 *G. gracile*. Fig 38 *G. intricatum*. Figs 39-41 *G. lagenula*.

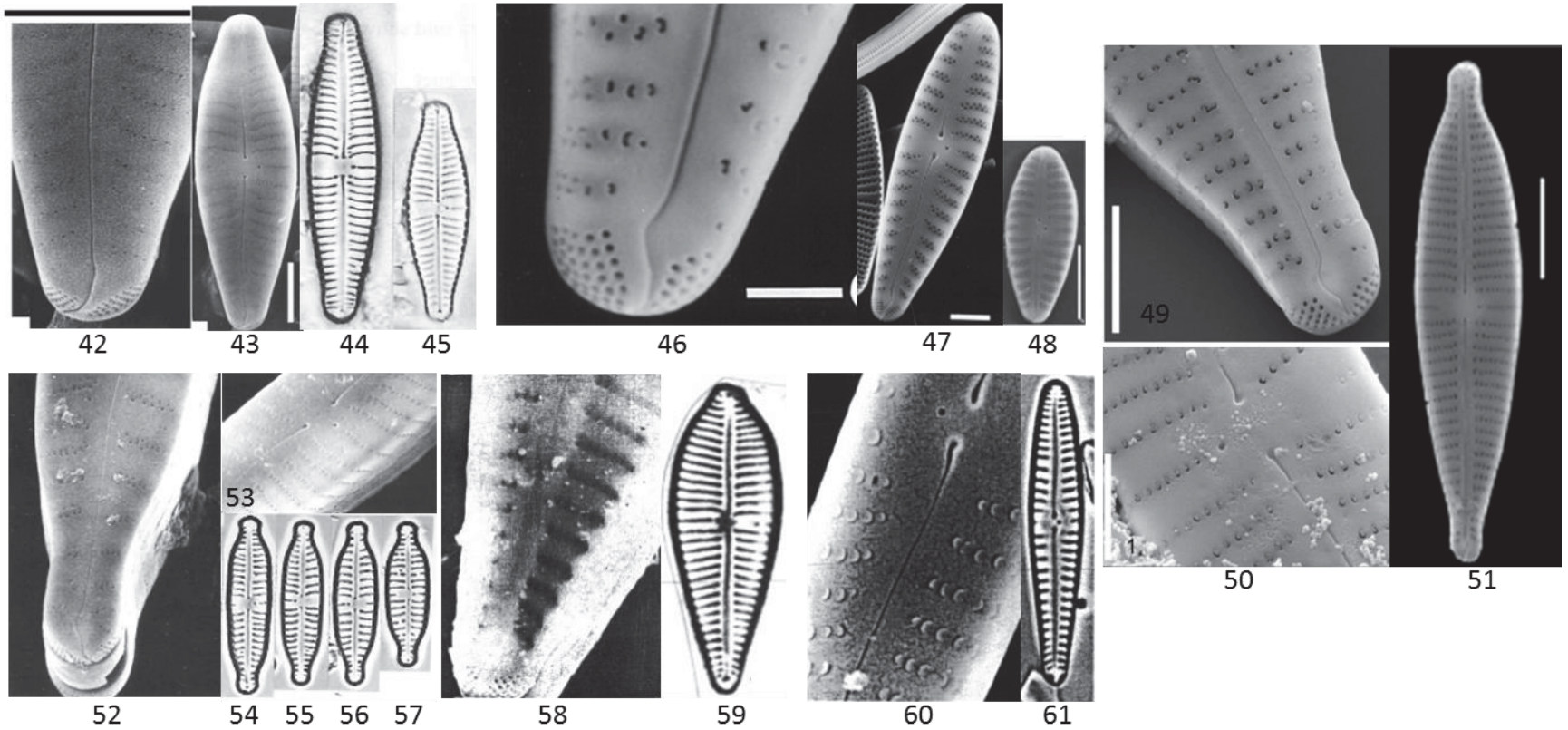

FIGURE 42-61. Figs. 42-45 *G. micropus*. Figs. 46-48 *G. minutum*. Figs. 49-51 *G. parvulum*. Figs. 52-57 *G. productum*. Figs. 58-59 *G. pseudoaugur*. Figs 60-61 *G. pumilum*

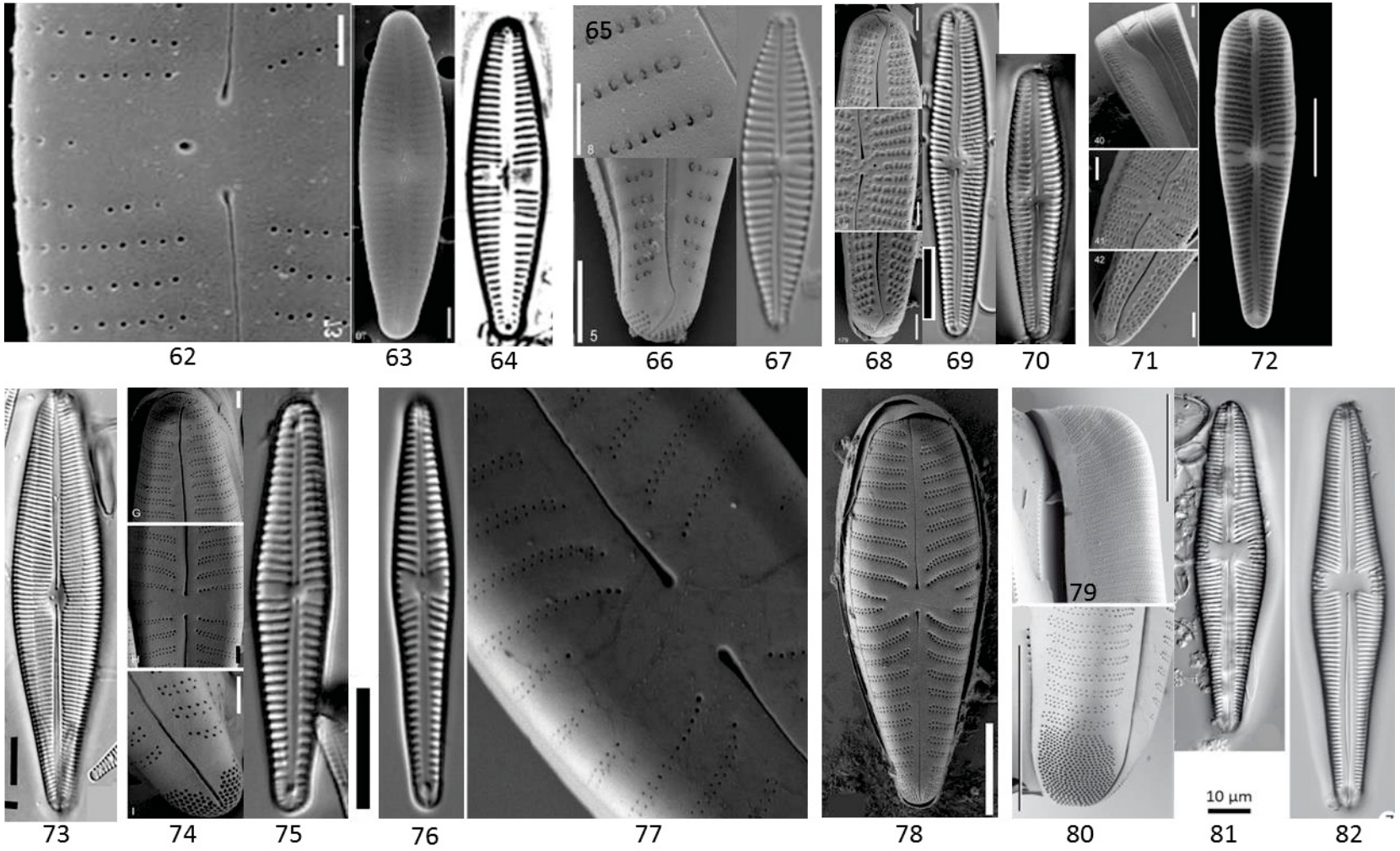

FIGURE 62-82. Figs 62-64 *Gomphonema rosenstockianum*. Figs 65-67 *G. saprophilum*. Figs 68-70 *G. subclavatum*. Figs 71-71 *G. truncatum*. Fig 73 *Gomphoneis minuta*. Figs 74-75 *Gomphonella acsiae*. Figs 76-77 *G. coxiae*. Fig 78 *G. olivaceae*. Figs 80-82 *G. tegelensis*.

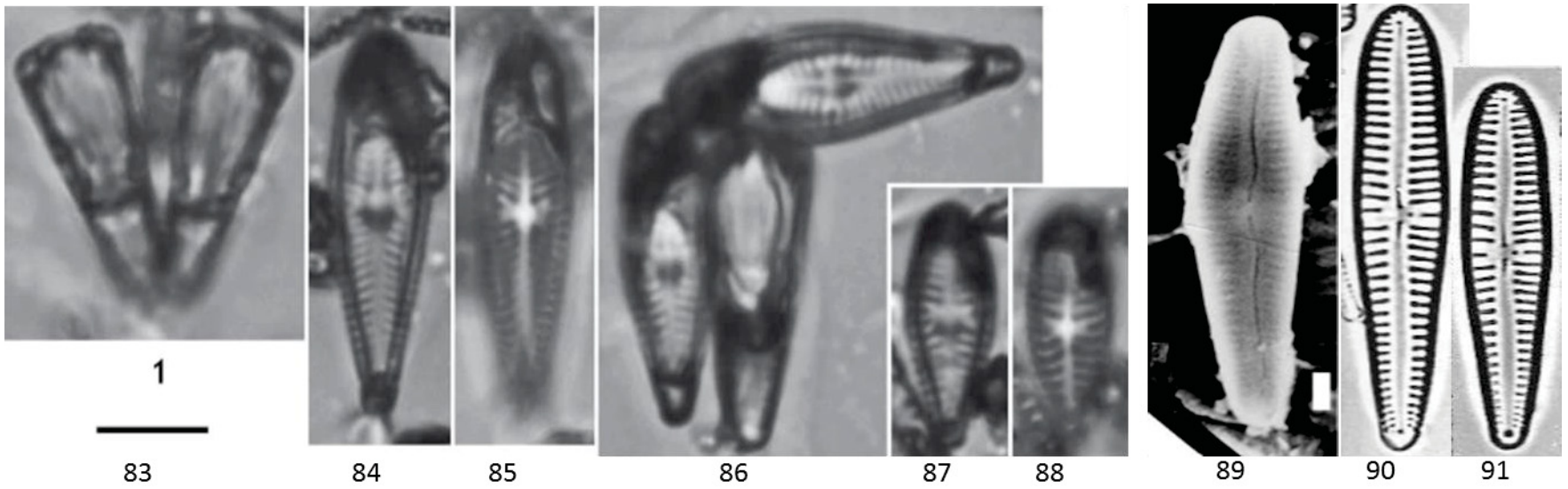

FIGURE 83-91. Figs 83-88. *Gomphonema clavatum* (Reichardt 2015 = *Gomphonella olivaceae*). Figs 89-91. *Gomphonema clavatum* (Wotjal et al. 2003).

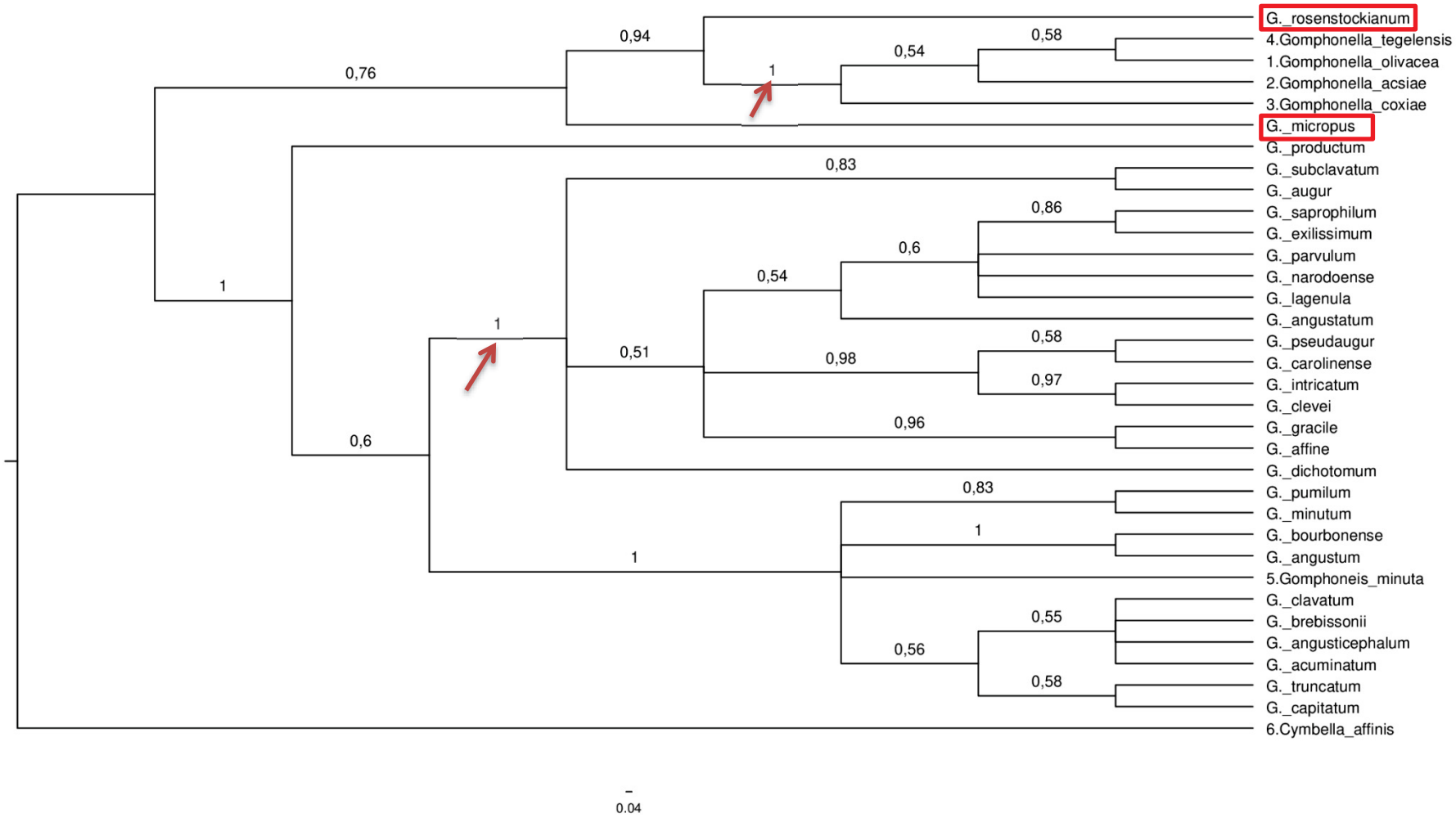

Figure 92. Bayesin inference phylogeny, inferred from an alignment 18S marker available at database. Posterior probabilities (PP)are shown at the nodes. The tree is rooted with the raphid pennate tax *Cymbella affinis*.

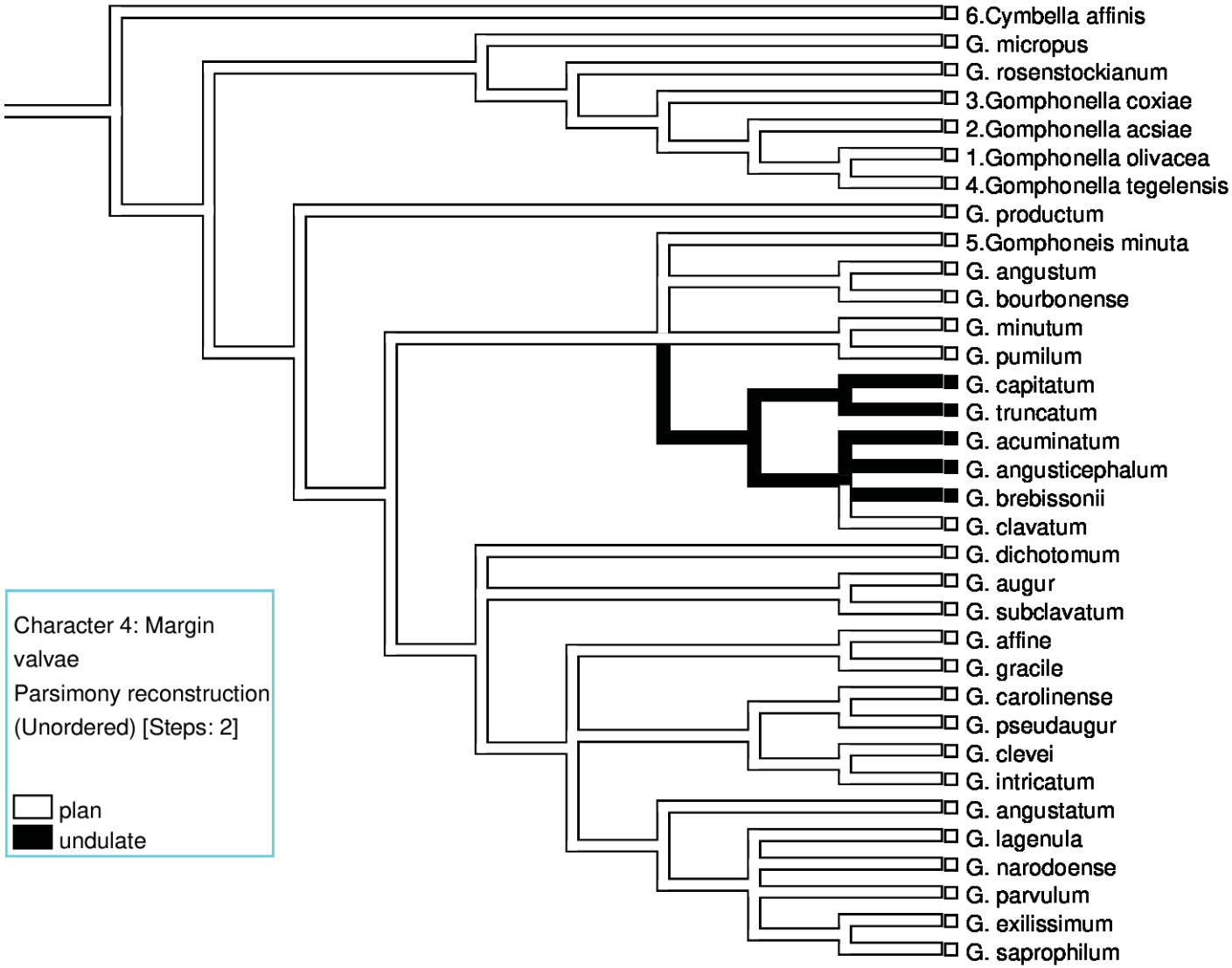

Figure 93. Combined cladogram between the morphological and molecular matrix. Black branches indicate species that present undulated margin valve and white branches plan margin valve.

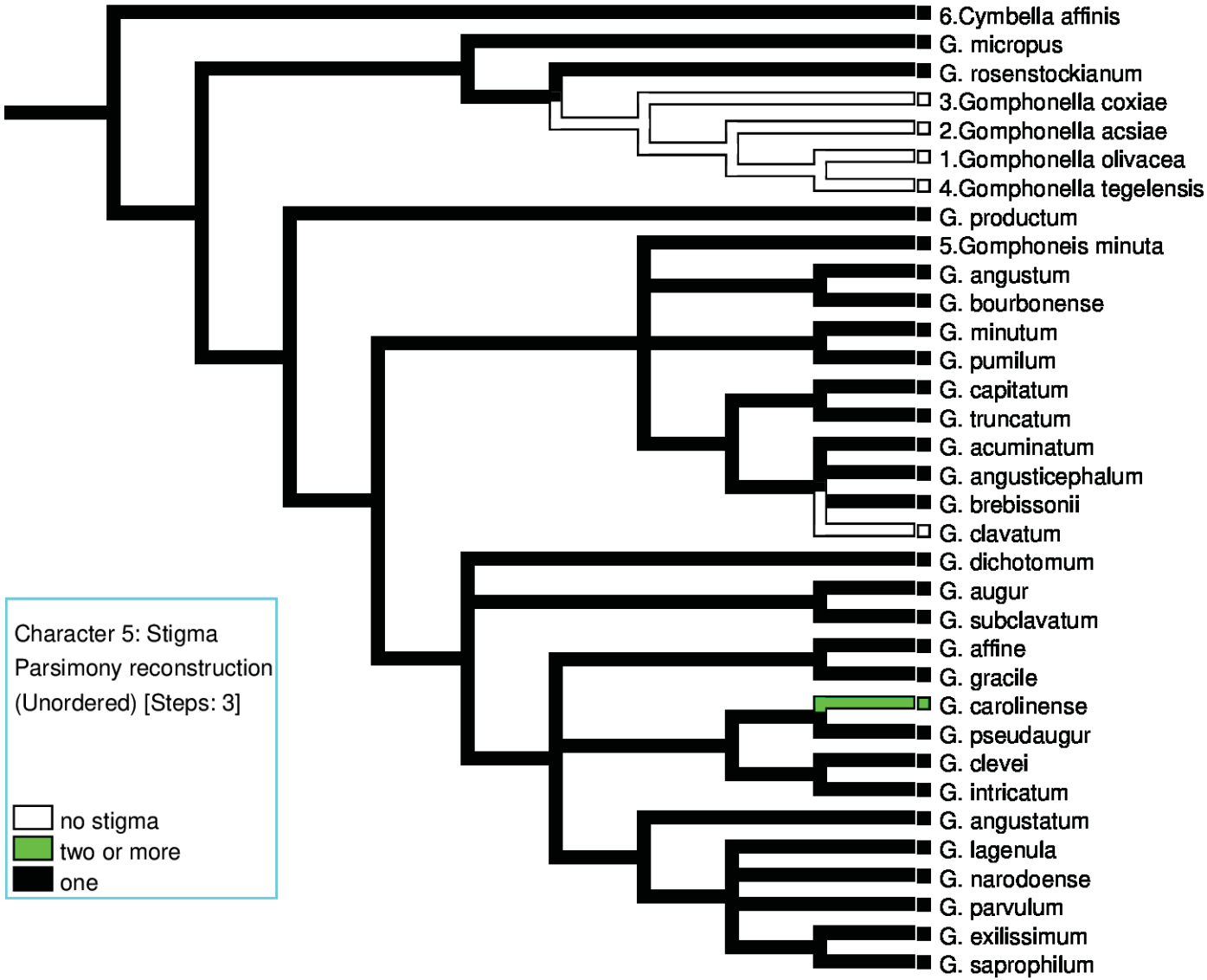

Figure 94. Combined cladogram between the morphological and molecular matrix. White branches indicate species that has no stigma, green branches two or more stigma and black branches one stigma.

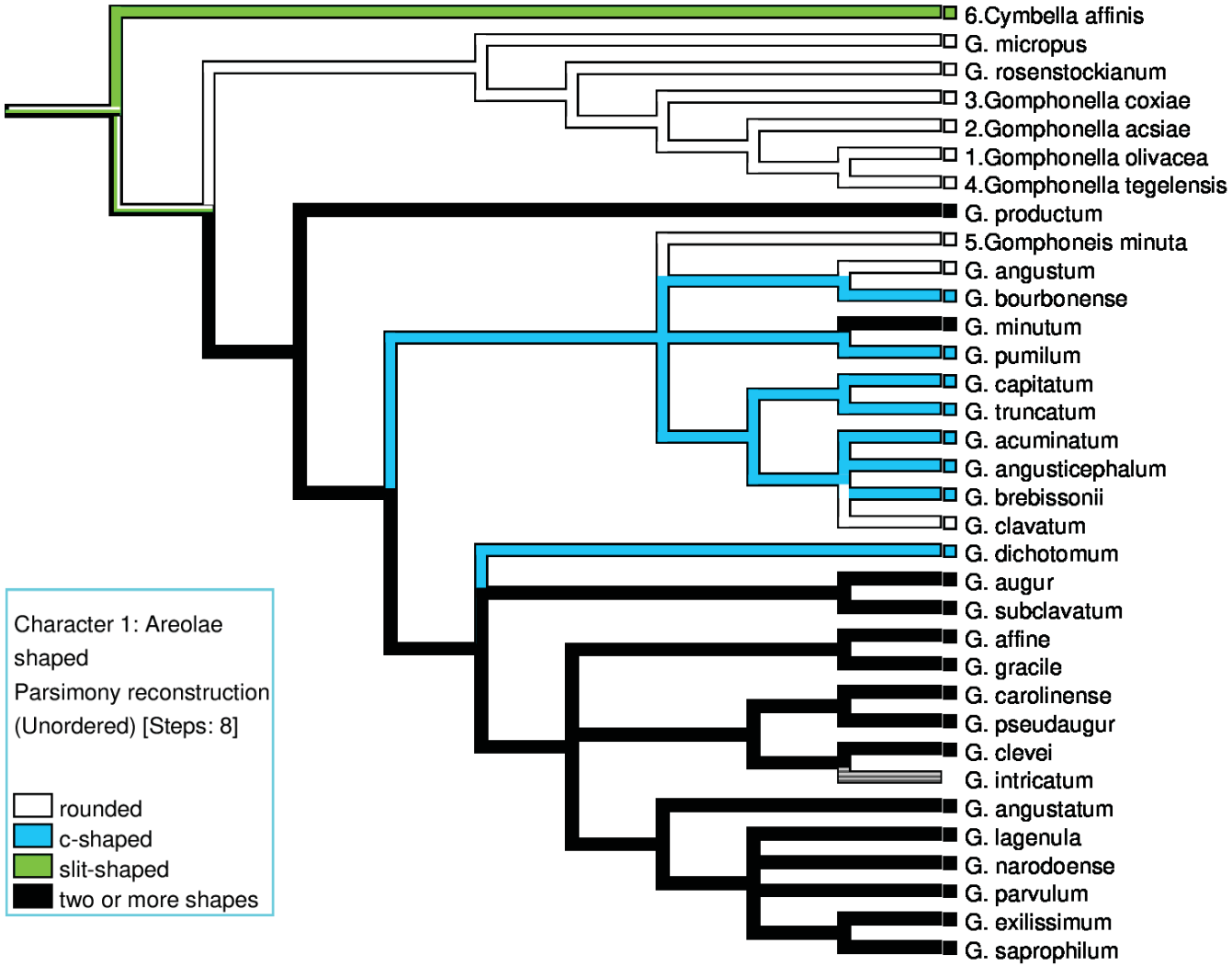

Figure 95. Combined cladogram between the morphological and molecular matrix. White branches indicate species that has rounded areolae, blue branches c-shaped areolae, green branches slit-areolae and black branches two or more shaped..

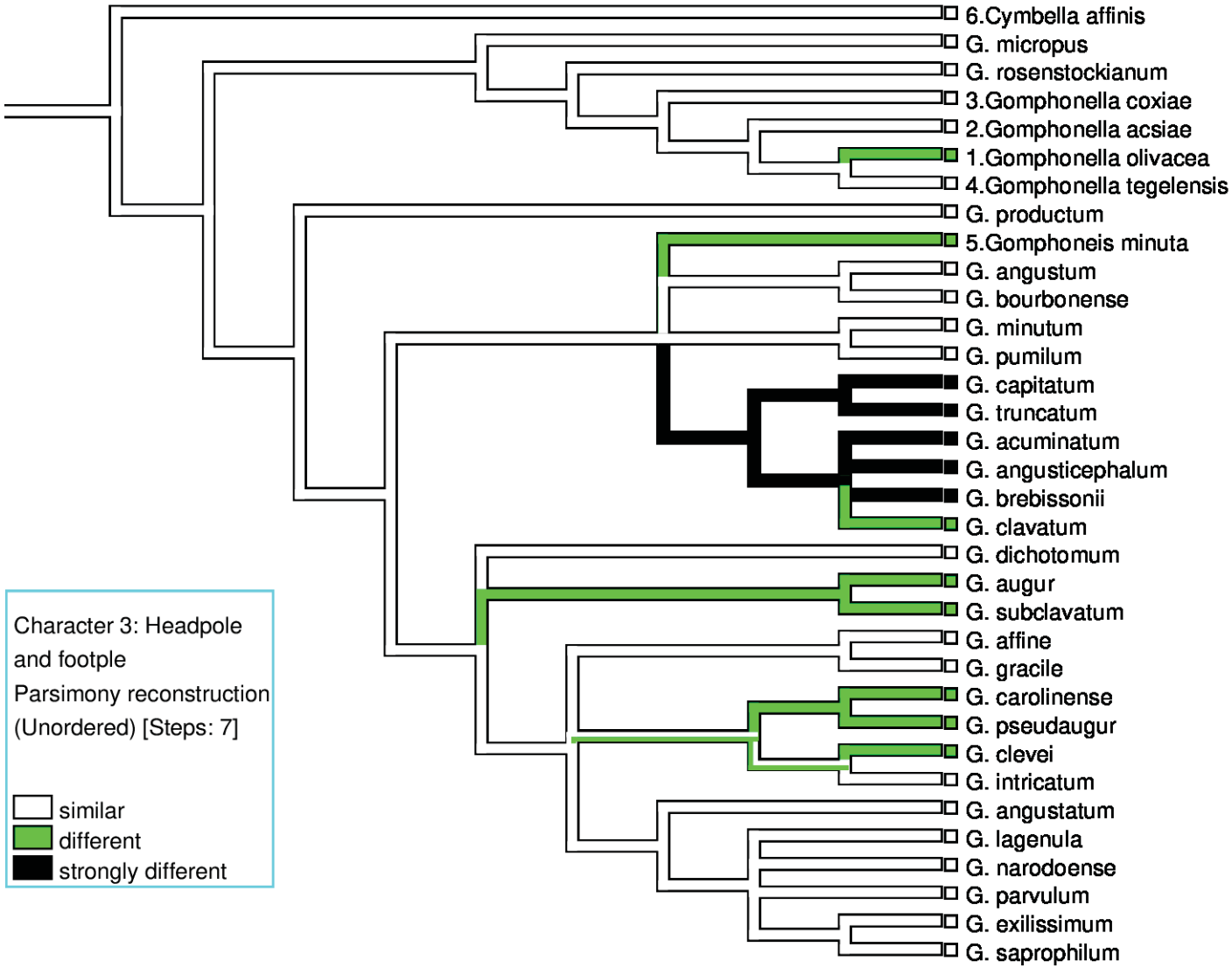

Figure 96. Combined cladogram between the morphological and molecular matrix. White branches indicate species that has similar headpole and footpole, green branches different headpole and fotpole and black branches strongly different headpole and footpole.

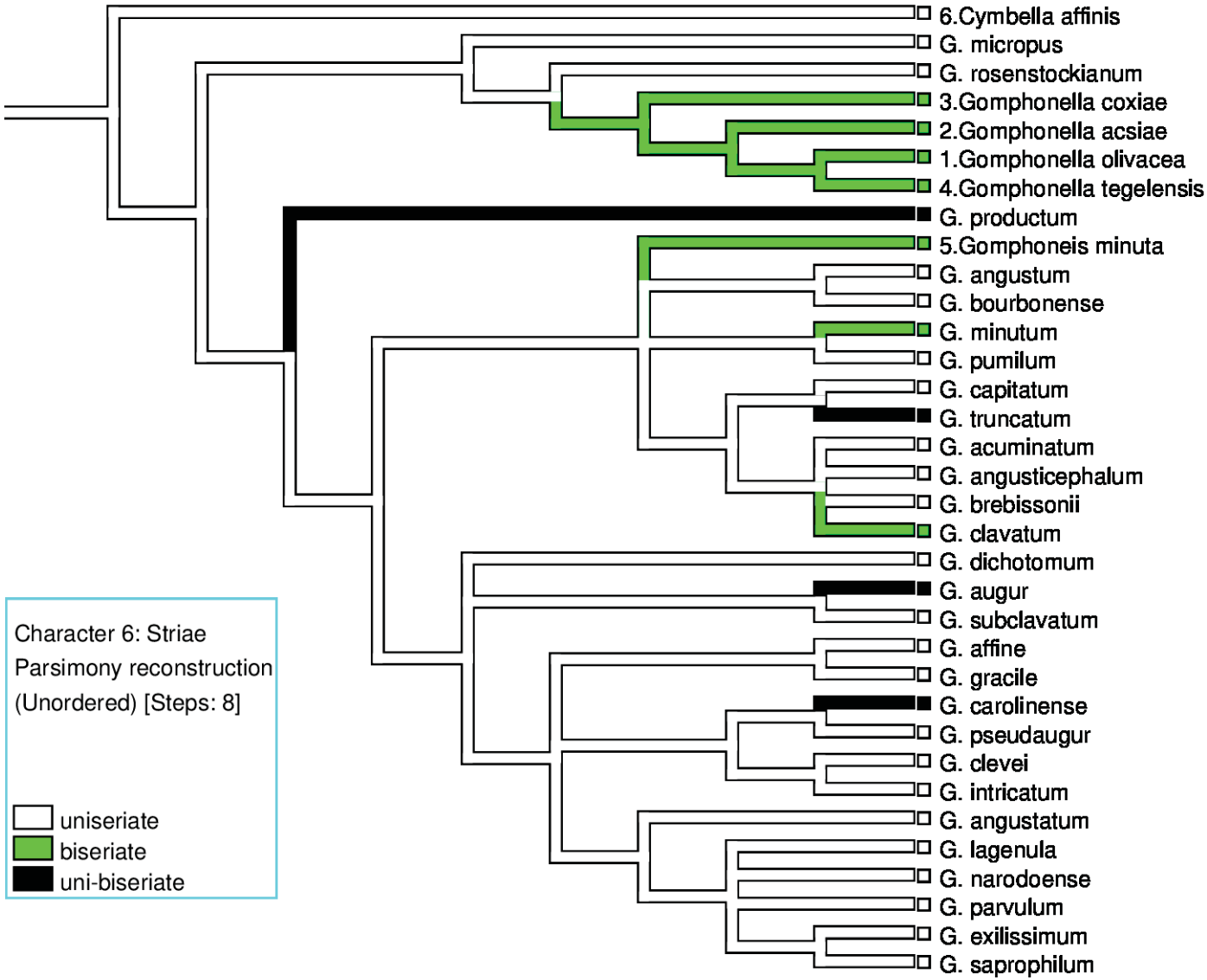

Figure 97. Combined cladogram between the morphological and molecular matrix. White branches indicate species that has uniseriate striae, green branches biseriate striae and black branches uni-biseriate striae.

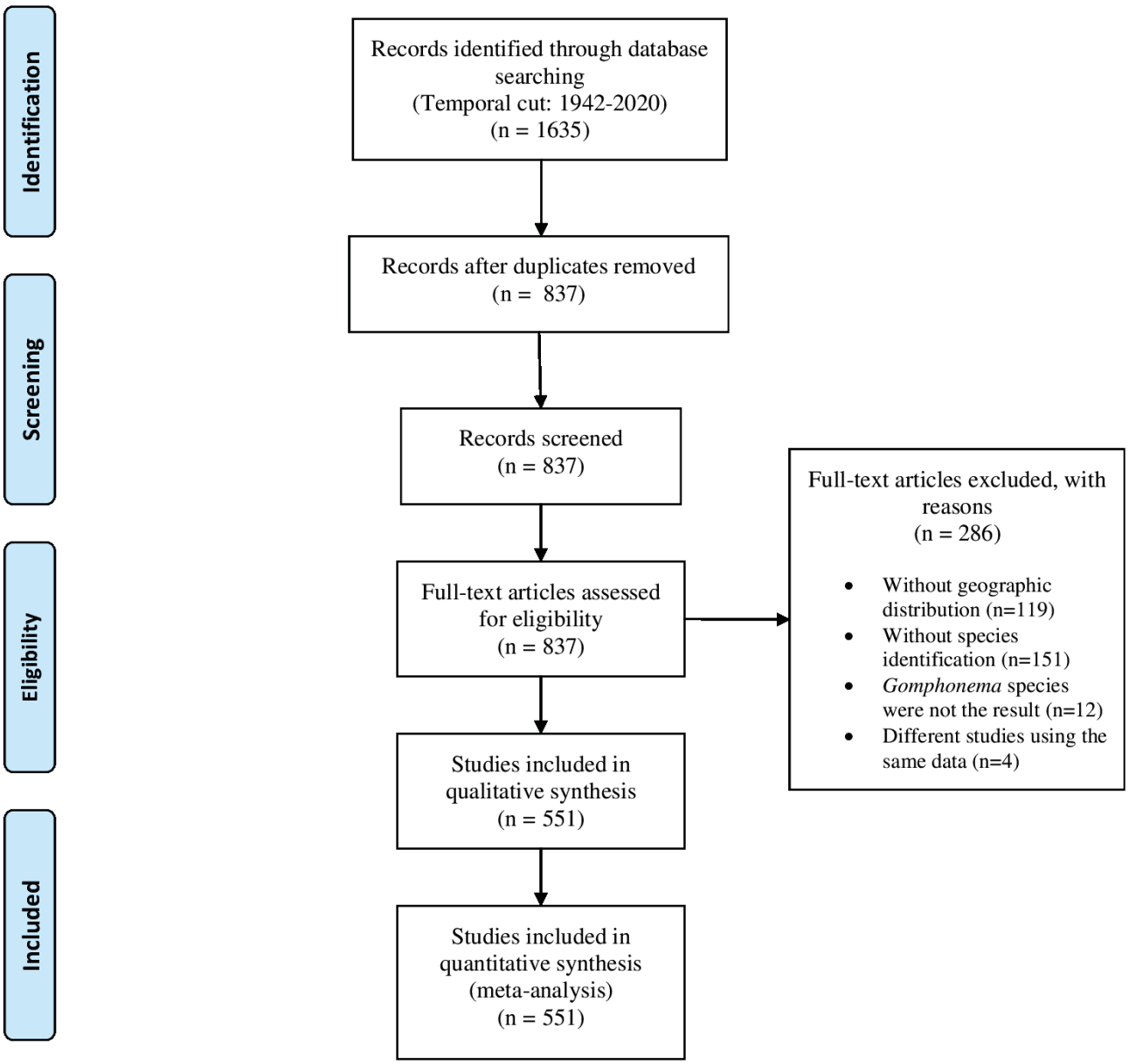

Figure 98. PRISMA flow chart showing selection of articles for review. Produced using a download able template available at http://www.prisma-statement.org/ (Moher et al, 2009)

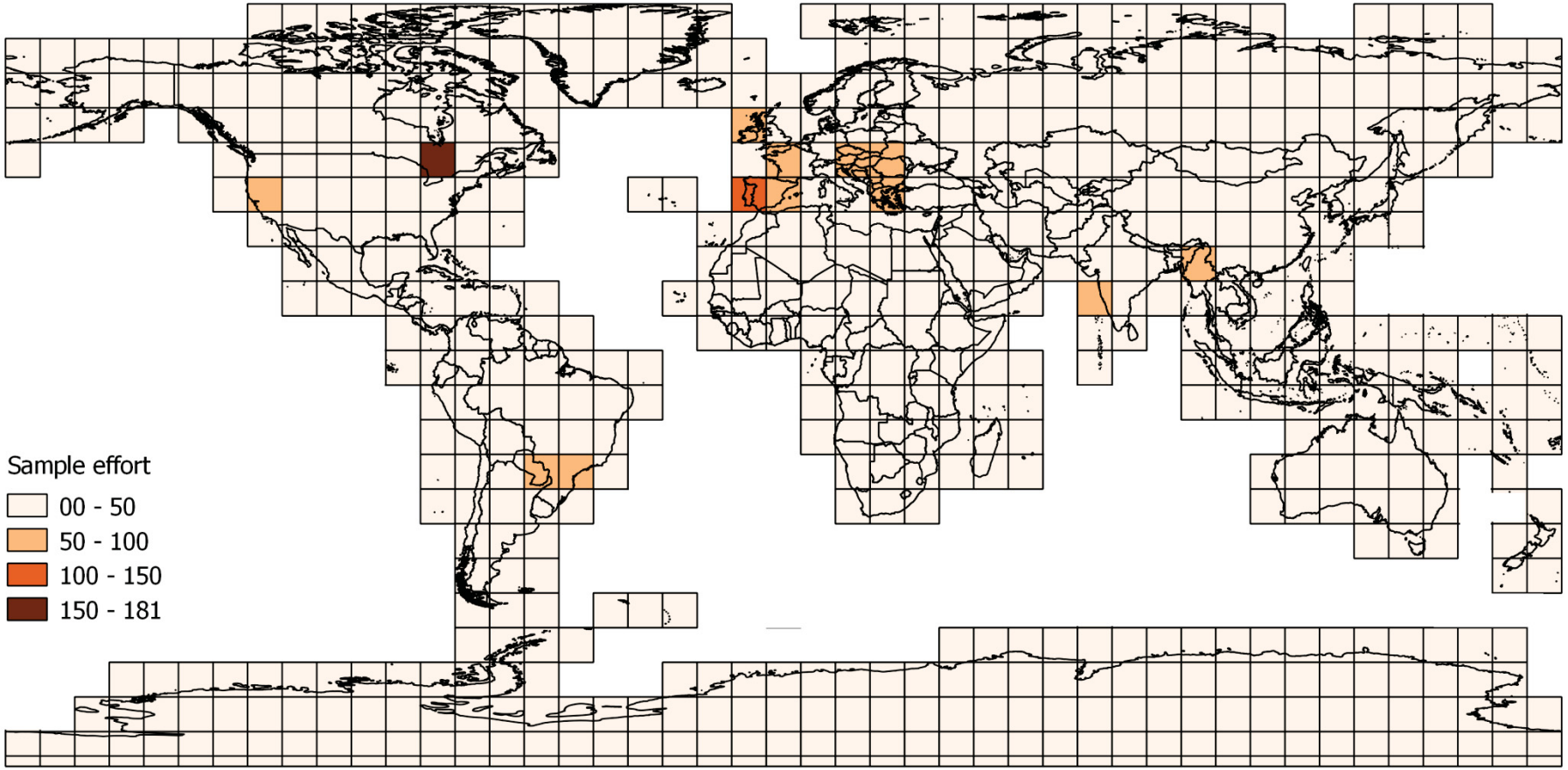

Fig 99. Sampling effort in the world. The darker squares have a higher number of species citations for the genus *Gomphonema* and lighter squares have a smaller number of citations.

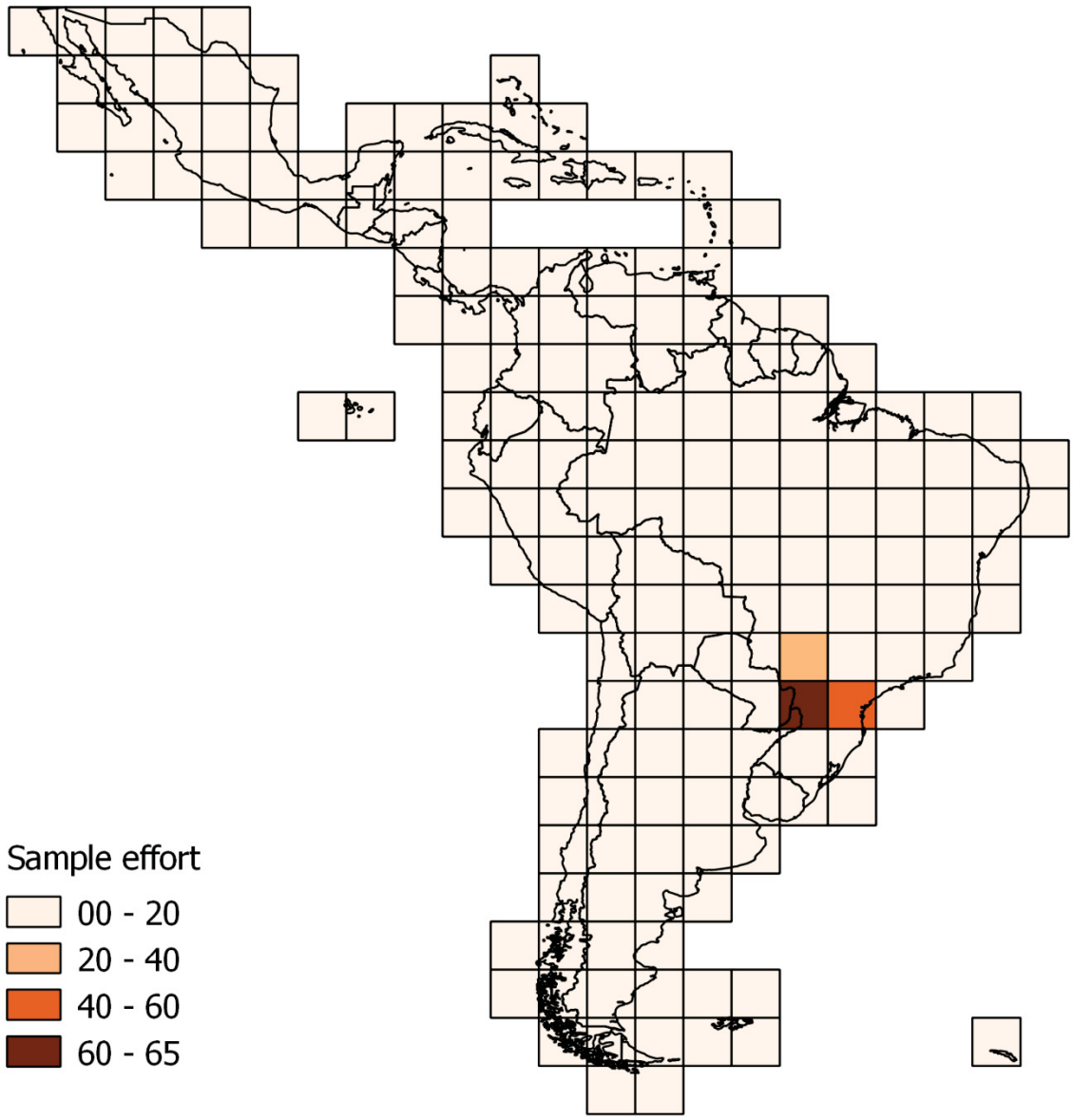

Fig 100. Sampling effort in the Neotropical region. The darker squares have a higher number of species citations for the genus *Gomphonema* and lighter squares have a smaller number of citations.

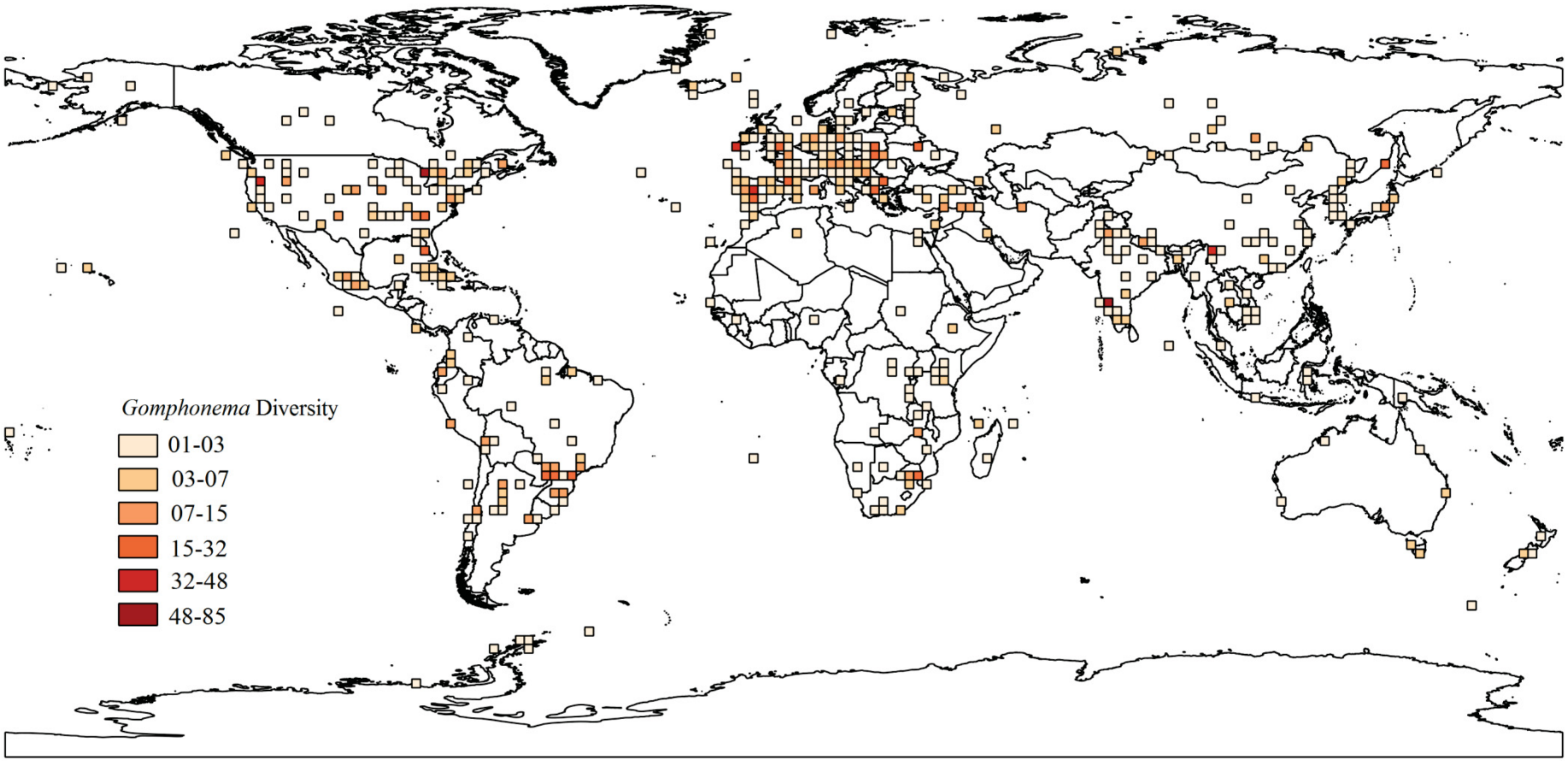

Fig 101. Diversity of species of genus Gomphonema in the world. The darker squares have a higher number of different species cited for the genus *Gomphonema* and lighter squares have a smaller number of citations.

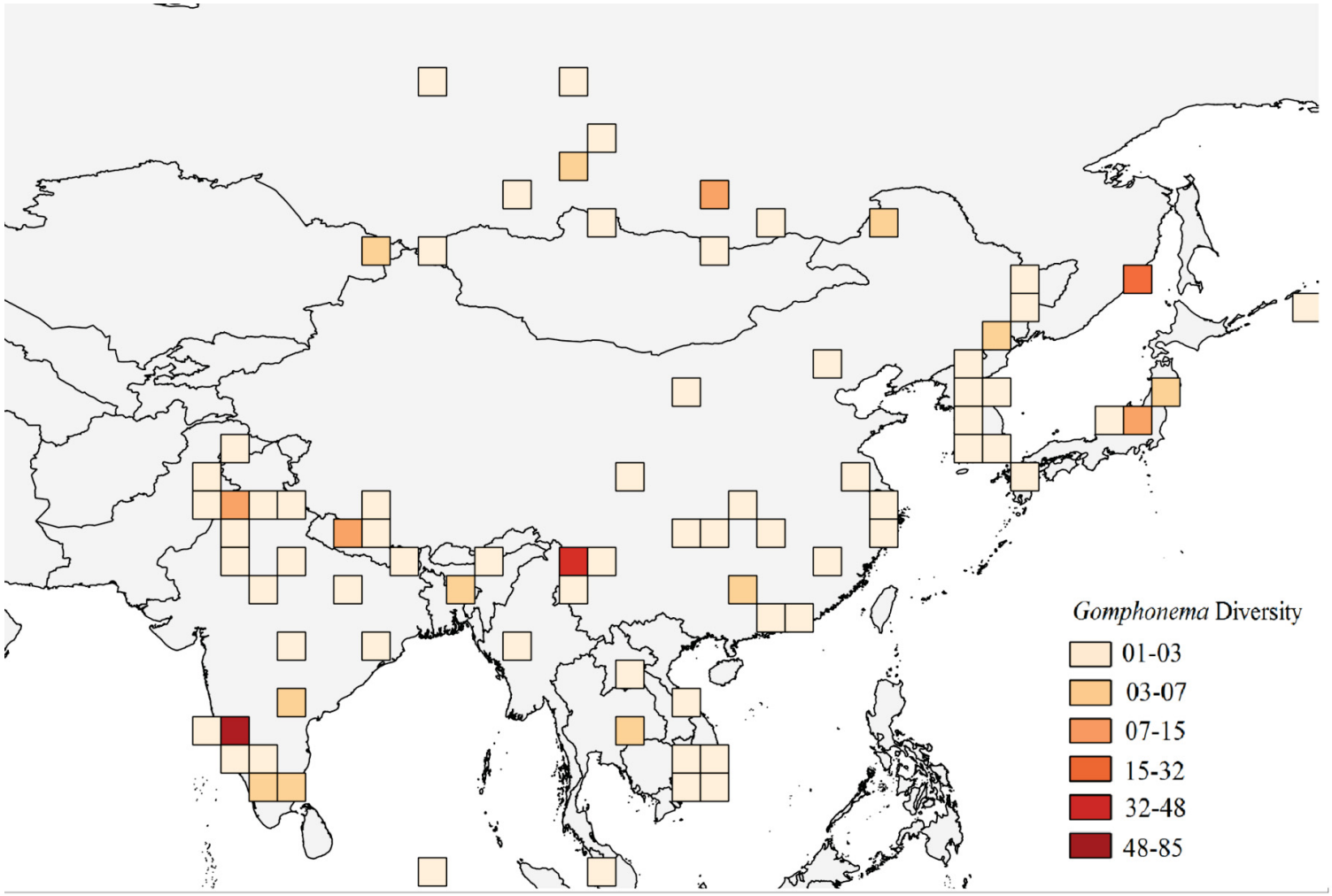

Fig 102. Diversity of species of genus *Gomphonema* in the India. The darker squares have a higher number of different species cited for the genus Gomphonema and lighter squares have a smaller number of citations

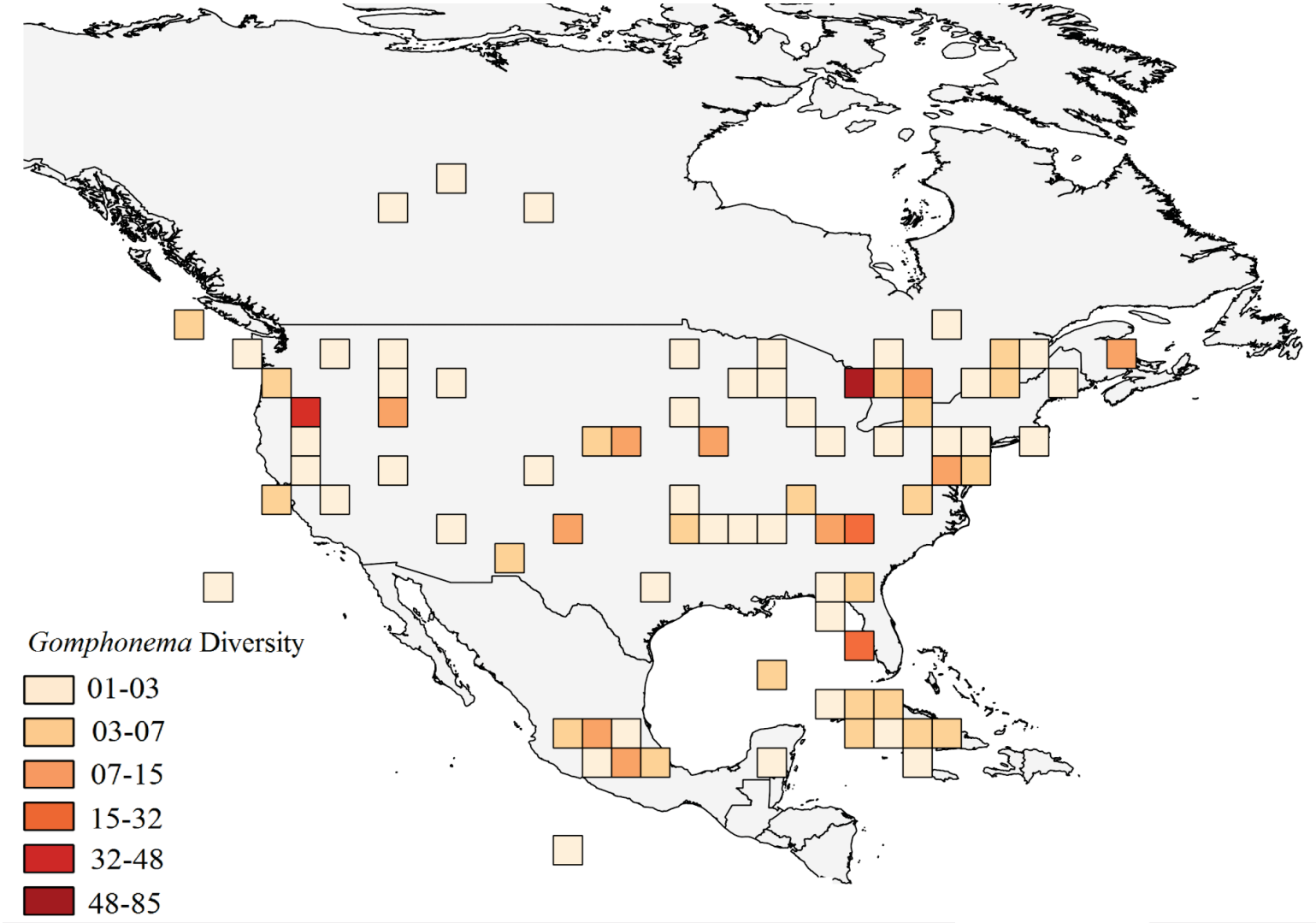

Fig 103. Diversity of species of genus *Gomphonema* in the world. The darker squares have a higher number of different species cited for the genus Gomphonema and lighter squares have a smaller number of citations

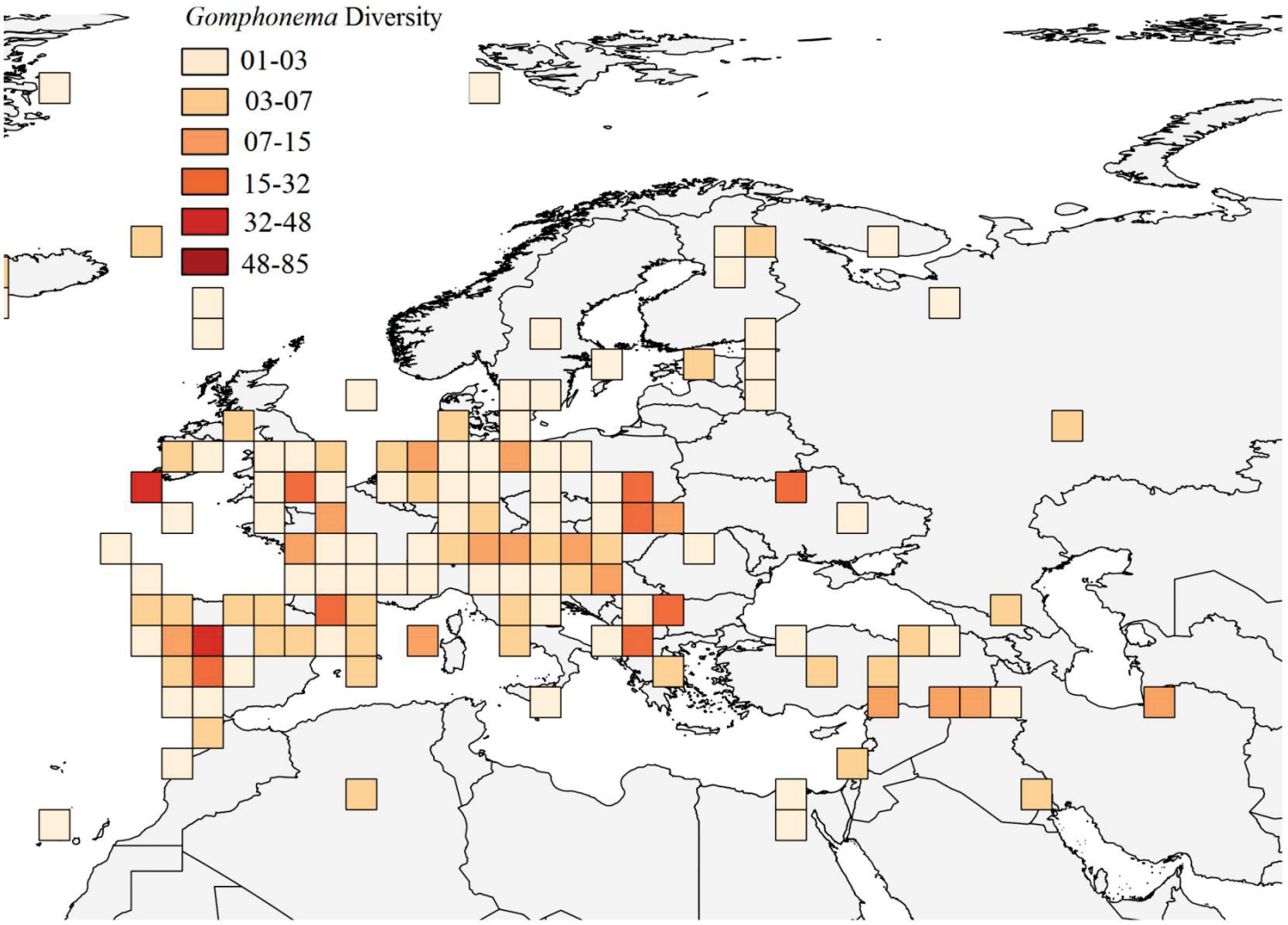

Fig 104. Diversity of species of genus *Gomphonema* in part of Europe. The darker squares have a higher number of different species cited for the genus Gomphonema and lighter squares have a smaller number of citations

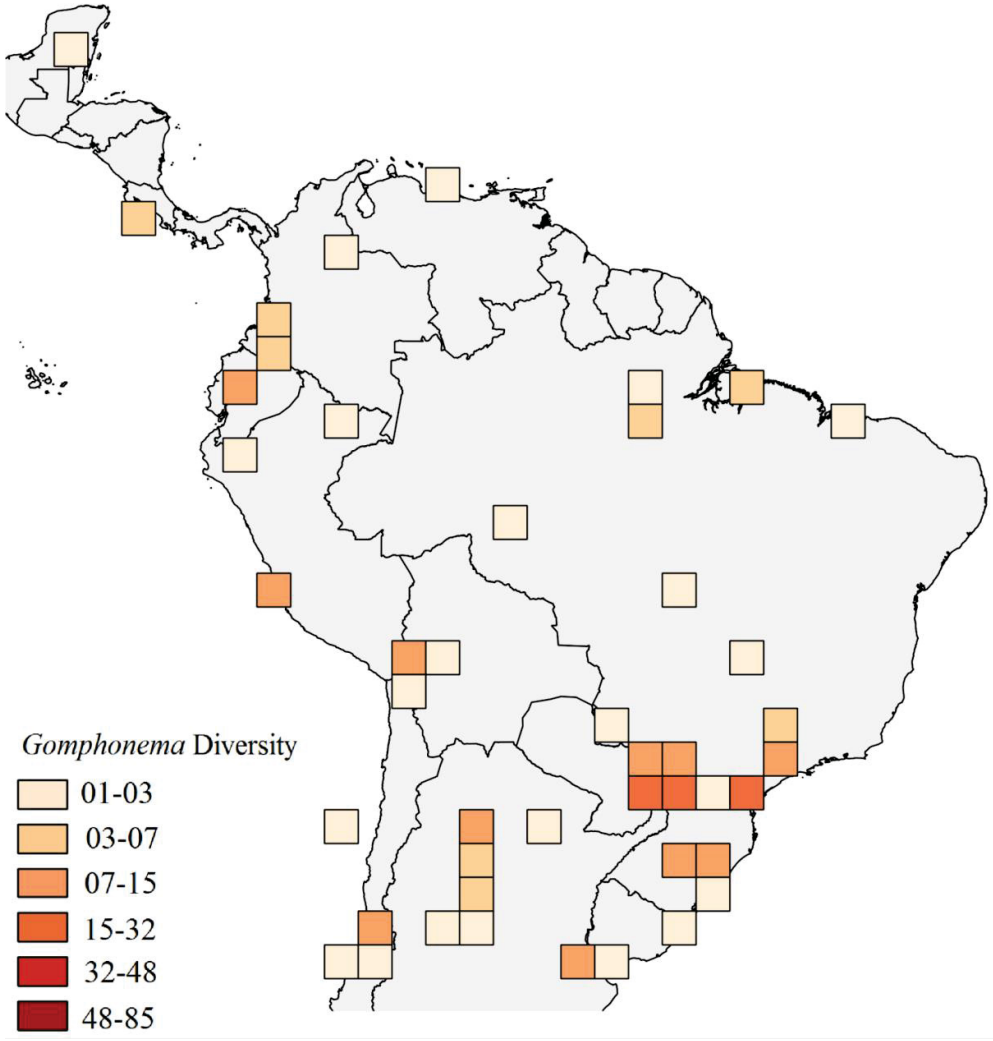

Fig 105. Diversity of species of genus *Gomphonema* in the Brazil. The darker squares have a higher number of different species cited for the 7. genus Gomphonema and lighter squares have a smaller number of citations

6. DIATOMS A BIOINDICATOR TO PERFLUOROOCTANOIC ACID (PFOA)

(Journal: Ecotoxicology and Environmental Safety)

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Abstract

Perfluorooctanoic acid (PFOA) is an emerging, stable contaminant, present in several aquatic habitats, with great capacity for bioaccumulation and difficult removal of the aquatic environment. This compound presents a great risk for living organisms, accumulating in organs such as kidneys and liver, as well as in the blood. Contamination in humans can occur by ingesting water, contaminated food, among others. Although PFOA bioaccumulates mainly in higher organisms in the food chain, microorganisms such as microalgae are also affected and can be used as bioindicators or in phytoremediation. Studies with diatoms and PFOA mainly relates the contaminant to the physiological responses of these microalgae. However, there are still no studies relating these physiological responses to the expression of different genes important for algal growth. The objective of this study was to verify the physiological reactions, the level of toxicity / tolerance to this pollutant and the expression of genes involved in the survival of some species of diatoms when exposed to different concentrations of PFOA. For this, in a culture of diatoms, a test was performed to verify the dose-response to PFOA (EC50), the content of the (Malondialdehyde) MDA was extracted to check the oxidative responses and the cells collected on the last day of exposure were stored in a freezer -80 °C for later analysis of gene expression in Real-time PCR (IN PROGRESS). It was observed that, at 96h, the value considered toxic to the community was 636.4 µg / L (0.64 mg / L) and at 72h, this rate was much higher than 38,417.6 µg / L (i.e. close to 38 mg / L). This result can be explained by a transient adaptation to PFOA. The highest content of MDA (7.17 x10-9 nmol g^{-1}) was in the concentration of 1 µg / L of PFOA, to which from the next PFOA concentration (10 µg / L) there was a gradual decrease in MDA. There was a negative correlation between MDA content and PFOA concentrations (-0.60). And in the regression analysis, the MDA x PFOA ratio was R2 0.62 and p value <0.0005. In general, the diatoms probably adapted to the contaminant, which activated its self-defense to decrease the content of MDA, since the content of MDA can critically affect cellular molecules. This hypothetical situation may have occurred in the flasks that has highest concentrations of PFOA. For it was observed a sudden increase in

MDA and subsequently a successive reduction in the vials with the highest concentrations of PFOA.

Keywords: growth algal, malondialdehyde, oxidative stress

1. Introduction

PFOA is an emerging contaminant of the class of perfluorinated compounds (PFCs) that has the capacity for bioaccumulation and a high toxic potential for living beings (Calafat et al., 2007; Ahrens, 2011; Arvaniti and Stasinakis, 2015). Contamination of the environment by PFOA occurs directly or indirectly, such as by wastewater, degradation of precursor substances in the product manufacturing process, among others (Becker, 2008). Its distribution is in a variety of habitats worldwide, such as surface water, air, silt, soils, sediments, and even polar caps (Lau et al., 2007).

This compound is thermally and chemically stable and is being used mainly as surfactants, in insecticides, emulsifiers, in the production of polytetrafluoroethylene, fluoropolymers and fluoroelastomers, in addition to teflon and tinctures to coat kitchen utensils (Betts, 2007; Lau et al. 2007) and it is also found in drinking water (Schwanz et al., 2016).

The main routes of human contamination occur through ingestion of contaminated water or food, since studies confirm the migration of this compound from food packaging (Begley et al., 2008; Trier et al., 2011; Xu et al., 2013).

The presence of PFOA in drinking water can be acceptable in low concentrations (0,07µg/L) (USEPA, 2016). However, it is still a problem, as exposure continues even at a low level can cause risk to human health (Xu et al., 2015). The presence of this compound in the human body can affect the biosynthesis of essential hormones, such as thyroid hormones, growth, estradiol, and sex hormones (Du et al., 2013, Lau et l., 2007, Wei et al., 2008). It also affects the components of the motor system (Goulding et al., 2017) and is being related to the high level of cholesterol and uric acid (Steenland et al., 2010). Other studies have shown that there are already detectable levels of PFOA in umbilical cord blood, indicating that this contaminant crosses the placenta (Apelberg et al., 2007; Inoue et al., 2004) and has been reported for several species that PFOA it is found mainly in muscle, blood and organs, such as liver and kidney (OECD, 2006).

The exposition of PFOA can cause changes like enlarged liver in rodents and primates, hepatocellular adenomas, alterations in the nervous system in rats (Goulding et al., 2016; Lau et al., 2007). The metabolism and transport of lipids, particularly fatty acids, can also be affected by PFOA in other organisms as such amphibians and rat (Guruge et al., 2006; Zhang et al., 2019).

In general, perfluorooctanoic acid accumulates in biota, in the upper organisms of the food chain as in amphibians (Zhang et al., 2019), birds (Kannan et al., 2002), fish (Peng et al., 2010), and mammals (Apelberg et al., 2007; Cui et al., 2009; Holzer et al., 2008; Luebker et al.,

2002). However, there are records that PFOA can also alter the density of aquatic species, such as the zooplanktonic community (Sanderson et al. 2003) and microalgae (Hu et al., 2014; Latala et al., 2009).

It is worth mentioning that when comparing PFOA concentrations in aquatic environments, considerable differences are being observed. In fresh water, PFOA concentrations in water range from 0.06 ng L^{-1} to 134 ng L^{-1} in countries such as China, Malaysia and Brazil (Nascimento et al., 2018; Pan et al., 2019; Xu et al., 2015; Zaibuddin et al., 2012). On the other hand, studies with marine waters show values ranging from 7-10 pg L^{-1} (Gulf of Guinea), from 50-60 pg L^{-1} (Bay of Biscay between Spain and France; west of Morocco and Western Sahara) and 500-600 pg L^{-1} (North Sea, between Norway and Denmark) (Stemmler and Lammel, 2010) in the Atlantic Ocean.

Changes in aquatic microorganisms are important, since according to Hu et al. (2014) the transfer of PFOA through food webs initiated from microalgae, can impact more significantly to higher organisms, since acute PFOA toxicity in microalgae can be evident from high milligram levels per liter. However, other studies show that microalgae can be affected from lower levels of PFOA (Latala et al., 2009), showing indicating that there is still no clear understanding of how microalgae respond to this contaminant and the possibility to functioning as a bioindicator tool.

Another issue is that microalgae can also function as phytoremediation agents such as in the absorption/adsorption or decomposition of PFOA (Albert et al., 2020; Hu et al., 2014).

There are indications that microalgae, such as green algae, may be more sensitive to PFOA when compared to higher plants (González-Naranjo and Boltes, 2014). Diatoms like *Skeletonema marinoi* were more responsive than green algae (*Chlorella vulgaris*) to pollutants of the class of PFOA (PFCs). Probably due to changes in the structure of the cell wall (Latala et al., 2009), since PFOA can modify membrane integrity and membrane potential of aquatic microorganisms (Rodea-Palomares et al., 2015; Feng et al., 2010; Xu et al., 2013).

The more frequent responses of microalgae (mainly chlorophytes) when exposed to PFOA is the inhibition of algal growth (Franqueira et al., 2000; Hu et al., 2014; Niu et al., 2019), due to changes in photosynthesis, which is already expected since this response to toxicity in microalgae by different pollutants was previously recorded (Franqueira et al., 2000; Zhou et al., 2013). In addition, compounds in the class of PFCs can cause damage related to the oxidizing activity of the cell-associated enzymes, catalase, and peroxidase (Niu et al., 2019).

Although there are studies on the effect of PFOA on diatoms and their physiological responses (Albert et al., 2020; Latala et al., 2009), however, there are still no studies to

understand the genetic mechanisms for these responses. Thus, it is not known yet how the expression of genes related to physiological responses occurs when the microalgae (*P. tricornutum*) is exposed to PFOA. This species is considered a model organism for toxicity tests, and this is the first study coupling physiological responses with gene expression.

Since other ways of assessing the toxicity of a pollutant directly would be through gene expression. There are several important genes related to the response of aquatic organisms under exposure to altered environments, such as: *psbA* gene - related to photosystem proteins that, under the effect of contaminants, can cause chlorophyll degradation (Chen et al., 2019; Franqueira et al ., 2000; Torres et al., 2008); gene *G3PDH5* - related to glyceraldehyde-3-phosphate dehydrogenase protein that plays an important role in the production of triacylglycerides (Zhang et al., 2014); gene *ß (1,3)*, related to the exo-1,3-ß-glucanase protein that is linked to the production of carbohydrates to which it can be influenced when the microorganism is under stress (Chauton et al., 2012; Mus et al. , 2013); *LHCF2* gene, related to fucoxanthin-chlorophyll a / c protein, which is associated with antioxidant activity (Bhaya and Grossman, 1993; Costa et al., 2013; Sahin et al., 2019; Wilhelm et al., 2013) being part of the biochemical route of oxidative stress that is commonly affected in environmental conditions unfavorable to microalgae, increasing the production of reactive oxygen species (ROS) (Niu et al., 2019) and the *FtsH* gene, related to the ATP-dependent zinc metalloprotease protein that is associated with the cell division of some organisms, like fish (Wei et al., 2014), but the expression activity of this gene related to cell division in diatoms has not yet been observed.

Therefore, the objective of this work is to verify physiological reactions, the level of toxicity/ tolerance to this pollutant, and the expression of genes involved in the survival of diatom when exposed to different concentrations of PFOA.

2. Material and Methods

2.1 Culture

The strain of diatom used in this experiment was isolated and provided by Center of Marine Studies from the Federal University of Paraná (CEM-UFPR). It was previously acclimatized for 8 days with f/2 culture medium (Guillard, 1975), prepared in distilled water and sea salt. The culture conditions were temperature at 21 °C , constant aeration, and lighting 25 µmol of photons m^{-2} s^{-1}. Afterward, the growth phases were determined (log, stationary and cell decline or death), through a growth curve, repeated three times. The growth curve was used for the growth inhibition test, described below (OECD 2006).

2.2 Growth inhibition test and determination of dose-response (EC50)

The growth inhibition test was performed with diatoms, following the 201 guidelines of the Organization for Economic Cooperation and Development (OECD, 2006), adjusting the time of exposure until the beginning of the cellular decline (96 h).

The experiment was conducted in 10 mL (tubes of 20 mL) and 350 mL (flasks of 500 mL) at the same time and under the same conditions. Eight concentrations (100 ng/ L, 500 ng/ L, 1 µg/ L, 10 µg/ L , 100 µg/ L, 500 µg/ L, 1 mg/ L, 5 mg/ L) of perfluorooctanoic acid (PFOA, CAS number: 335-67-1, 96% pure) were supplemented in f/2. The control group was growth in the absence of PFOA, at the same conditions described above (2.1). The initial cell concentration of diatoms was $1x10^6$ cells/ mL in all tubes and flasks. All treatments and the control were conducted in triplicates, totaling 27 tubes, and 27 flasks.

After 96 h of growth, the cultures were filtered (46 µm membranes) to obtain the biomasses, that were placed in microtubes (2 mL), and stored at -80 °C, for further analysis.

The dose-response analysis to cut cell production in half compared to the control (EC50) was based on data from the 96h of exposure to PFOA. The analysis was carried out according to Heever and Grobbelaar (1996).

2.3 MDA content determination at the different concentrations of PFOA (96 h)

Malondialdehyde (MDA) content was analyzed to assess oxidative responses in diatoms cells since MDA is the product of lipid peroxidation induced by reactive oxygen species (ROS) (Du et al. 2019).

Malonaldehyde (MDA) content was measured following the Heath and Packer 1968 protocol, using thiobarbituric acid (TBA) test for reactive substances. The membrane with the filtered cells was macerated with liquid nitrogen until it became a fine powder. Soon after, 1 mL of buffer solution (containing TBA) was added to the macerated membrane and kept stirring at 30 °C overnight. Then 1 mL of 0.1 % TCA was added to the sample, homogenized, and 1.4 mL was transferred to a tube and centrifuged at 10,000 rpm for 5 min. In a 0.5 mL aliquot of the supernatant, 2 mL of 0.5% (v/ v) TBA in 20% TCA was added. The mixture was heated to 95 ° C for 30 min and cooled for 10 minutes. Then centrifuged for 30 minutes at 4000 rpm, and immediately afterward, the absorbance of the supernatant was measured at 532 and 600 nm. The content of MDA was expressed in nmol / g^{-1} $x10^{-9}$ cells.

2.4 Real Time PCR (RTqPCR)

The genes for real-time PCR analysis are listed in the frame below (Frame 1).

3. Results

3.1 Algal Growth

The growth phases of specimen diatom in the stock culture were determined as a *log* phase between 3-4 days, a stationary phase 5 days and the beginning of the death phase 144 hours (6 days).

In the experiment with different concentrations of PFOA at the beginning of the growth curve, it was already possible to observe that there was no microalgae growth (Fig. 1).

In the regression analysis, to verify whether PFOA directly affected the growth microalgae cultivation, there was no statistical support to make this statement (p=0.18, R^2=0.20) (Table 1). However, there was a negative correlation (-0.50) between the concentration of PFOA and the number of cells (Table 2).

3.2 Dose-response EC 50

In the toxicity test (EC50), it was observed that, at 96h, the value considered toxic for the community was 636.4 µg/ L (0.64 mg/ L), whereas at 72h, this rate was much higher 38,417.6 µg/ L, that is, close to 38 mg/ L.

A negative correlation (-0.50) was observed between algae growth and PFOA concentration (Table 2). As previously described, the regression between PFOA and microalgal growth showed R^2 = 0.20 (20%) and p = 0.18, not showing a significant result to relate these two variables.

3.3 MDA content

There was an increase in MDA in PFOA concentrations from 0.1 to 10 µg/ L showing the highest MDA content (7.17 x10^{-9} nmol g^{-1}) in the concentration of 1 µg/ L PFOA, shortly after there was a gradual decrease reaching a level below the control group (1.84^{-9} $nmol^{-1}$ g^{-1}) (Table 3) (Fig. 2).

The correlation analysis between MDA content and microalgal growth was irrelevant (0.19), as well as the result of linear regression (R^2 = 0.61 and p = 0.4). However, when comparing the MDA content to the PFOA concentrations, the result showed a negative correlation (-0.60) (Table 2). And the regression analysis analyzing the MDA x PFOA ratio was R^2 0.62, and p value <0.0005 (Table 4) (Fig. 3).

3.4 RTqPCR – (In progress)

4. Discussion

4.1 Algal Growth

The lack of cell growth from the beginning to the end of the experiment (Fig. 1), may have had a response at the beginning of the exposure to which we would have the information if we had observed the microalgal growth in shorter intervals as in Deng et al. (2016). They obtained relevant information before 24h related to the exposure of another contaminant, ionic liquids ([C8mim]Br).

When we equated the concentrations of PFOA and the number of cells, we observed that there was a negative correlation (-0.50) (Table 2) showing that in the samples supplemented with a higher concentration of PFOA, there was a smaller number of cells. However, we cannot infer that the increase in the concentration of PFOA was directly related to the decrease in cell density, since in the regression analysis there was no support for this statement (Table 1). Even so, exposure to PFOA may have indirectly inhibited the growth of algae, as by oxidative stress, which can result in disturbances in the photosystem since the accumulation of MDA can degrade the chlorophyll present in microalgae (Chen et al., 2019)

Changes in photosynthesis and consequent decrease in the growth of microalgae can occur in different ways, and these changes are the main responses of photosynthetic microorganisms when exposed to a pollutant under stress conditions (Chen et al., 2019; Franqueira et al., 2000; Torres et al., 2008; Xu et al., 2013)

Therefore, we believe that exposure to PFOA may have inhibited the growth of microalgae (Fig. 1) indirectly through disturbances in the photosystem, as a consequence of changes in biochemical structures and physiology caused by PFOA, such as in the MDA content (Table 5).

4.2 Dose-response EC 50

Although diatoms survives in habitat with low concentrations of PFOA (7-600 pg/ L) (Stemmler and Lammel, 2010), it has been shown to be largely tolerant of this pollutant. Since it was observed the effective toxicity of the organism in 72 h - EC50 38,417.6 µg/ L (38 mg/ L) and in 96 h - EC50 in 634.4 µg/ L (0.64 mg/ L). However, there are still other microalgae, such as *Chlorella pyrenoidosa* and *Selenastrum capricornutum*, which showed greater tolerance to PFOA, presenting results of 217.46 mg/ L and 190.99 mg/ L respectively, for a 96 h dose-response - EC50 (Xu et al., 2013) .

The 72h response dose, which was higher than that at 96 h - EC50, can be explained by a transient adaptation to PFOA, since it is known that microalgae can absorb/adsorb the contaminant as well as decompose the PFOA (Albert et al., 2019; Hu et al., 2014). However, the fact that the acute toxicity of these microalgae is very high (mg/ L) can become a problem not for the microalgae themselves, but for other organisms such as fish and mammals that may have contact with the environment or directly with the microalgae (Hu et al., 2014).

The dose-response at 96 h-EC50 may have been lower since, due to the time of exposure to PFOA specimen of diatom, it could already be showing cellular impairment, such as oxidative reactions (ROS), for example (Niu et al., 2019). Even if, after a while, these oxidative activities decreased (Xu et al., 2013) there would still be an accumulation of products from intracellular reactions such as MDA content (Hu et al., 2014). Another factor that could also be related to the increased sensitivity to PFOA (compared to EC50 72h) is the impairment of the cell membrane (Latala et al., 2009; Rodea-Palomares et al., 2015; Xu et al., 2013).

4.3 MDA content

Malondialdehyde (MDA) is a secondary product of ROS-induced lipid peroxidation. The content of MDA reflects physiological stress and oxidative cell damage caused by different levels of lipid peroxidation. Therefore, the increase in MDA content has a positive correlation with the production of ROS.

In relation to PFOA concentrations, the MDA showed a rise from the lowest concentration of PFOA (100 ng/ L, 500 ng/ L and 1 µg/ L), presenting the highest value at the concentration 1 µg/ L. After this peak, there was a gradual decrease in which the highest concentration of PFOA (500 µg/ L) showed a lower MDA rate than the control group. It is possible to visualize a relationship between MDA and PFOA (Table 4)

It is evident that the initial peak of MDA increase when exposed to PFOA is due to lipid peroxidation, since this physiological response is commonly observed in different studies with different pollutants, both for the species *P. tricornutum* and for other species of diatoms (Manimaran et al., 2011; Qu et al., 2010; Wang et al., 2008).

The decrease in MDA content in subsequent concentrations of PFOA (10 µg/ L – 5000 µg/ L), leads us to believe that a higher content may have manifested hours earlier since our analysis of MDA content was done only at 96h. In Deng et al. (2006), which studied the effect of a Bromide compound on *Skeletonema costatum*, the highest content of MDA was observed in 20 h of exposure shortly after there was a decrease in the content ending the 96 h of experiment. The increase and then decrease in MDA content may also be the result of a cell

self-defense reaction, delaying lipid peroxidation which is caused by the accumulation of ROS (Reactive oxygen species) in the cell. This is due to the inhibition of algal growth affected by some contaminants (Deng et al., 2006; Du et al., 2019). In this way, self-defense also protects important molecules for organisms, such as proteins, chlrophyll and DNA (Deng et al., 2006). In our analysis we found a negative correlation between algal growth and PFOA concentration (Table 2), however, this correlation only shows us that the two variables behave oppositely, so another stressor variable or a set of variables may have affected the algal growth microalgae and thus causing a decrease in MDA.

4.4 RTq-PCR (In progress)

5. Conclusion

In summary, we believe that PFOA can affect microalgal growth indirectly since we have not proven its direct relationship with the decrease in growth of specimen of diatom (Table 1). However, this pollutant is negatively correlated with the number of cells present in the culture, which in a higher concentration of PFOA there are fewer cells. As well as it is also negatively correlated with the MDA content (Table 2).

However, we found that there is a relationship between PFOA and MDA (Table 3), which may vary according to the concentration of PFOA. Probably there is an adaptation of the microalgae to which it can activate its self-defense to decrease the MDA content, already that it can affect critical cellular molecules (Deng et al., 2006). This hypothetical situation may have occurred in the flasks that presented higher concentrations of PFOA. Since there was a sudden increase in MDA and subsequently there was a successive reduction in flasks with the highest concentrations of PFOA (Table 3).

Therefore, we believe that other factors may have affected algal growth, such as oxidative reactions, which in this work we observe through the content of MDA.

6. References

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Frame 1. Genes, Forward and Reverse sequences, identification of proteins related to genes, and references of the genes used for real-time PCR analysis.

Gene	Sequence 5'-3'	Protein ID	Reference
18S	F: TGCCCTTTGTACACACCGC R: AAGTTCTCGCAACCAACACCA	56377 (JGI)	Siaut et al., 2007
Act2	F: CGCCTCAATCAAGGTCAAGAT R: GCCAGATTCGTCGTATTCTTC	PHATRDRAF 29136 (JGI)	Siaut et al., 2007
LHCF2	F: GCCGATATCCCCAATGGATTT R: CTTGGTCGAAGGAGTCCCATC	PHATRDRAF 25172 (JGI)	Costa et al., 2013
psbA	F: TGATATCGATGGTATTCGTGAG R: TGGATACCGATAGCATTAGAAC	YP_874444 (NCBI)	Nymark et al 2009
G3PDH5	F: ACTGCCTGGCTACTTTGTTCC R: CCATACGACTTCGTCTTCCG	1129 (JGI)	Zhang et al 2014.
ß (1,3)	F: CTGGGGCGACGAATCATACT R: CTTCGGAGTCAATGCTGGTG	56506	Mus et al., 2013
ftsH	F: AAACGATGAGCGAGAACAAACT R: CCAACAACAATGACCCCTTTAT	4524632	Wei et al., 2014

Table 1. Regression analysis between PFOA and growth microalgal at 96h. $p<0.05$ ($p=0.18$)

Regression Statistics	
Multiple R	0,452525
R-Square	0,204779
Adjusted R-Square	0,072242
Standard Error	1660,19
Observations	8

Table 2. Correlation between PFOA concentration (ug/L), algal growth (number of cells/mL) and MDA (nmol g^{-1}) of diatoms

	PFOA	*n° cells*	*MDA ug/L*
PFOA	1		
n° cells	-0,50477	1	
MDA ug/L	-0,60838	0,19052	1

Table 3. MDA content and standard deviation at the different PFOA concentration

PFOA µg/L	MDA nmol g^{-1} ($x10^{-9}$)	Sd
0	1.85	1.15
0,1	6.98	1.00
0,5	4.88	0.79
1	**7.17**	1.03
10	5.43	1.15
100	3.78	1.14
500	3.71	0.74
1000	3.84	1.38
5000	1.17	0.61

Table 4. Regression analysis between MDA content and PFOA concentration at 96 h. ($p<0.0005$)

Regression Statistics	
Multiple R	0,79082
R-Square	0,625397
Adjusted R-Square	0,562963
Standard Error	1,28E-09
Observations	8

Table 5. Regression analysis between MDA content and growth algal at 96 h. (p=0.4)

Regression Statistics	
Multiple R	0,783508
R-Square	0,613884
Adjusted R-Square	0,549531
Standard Error	1,3E-09
Observations	8

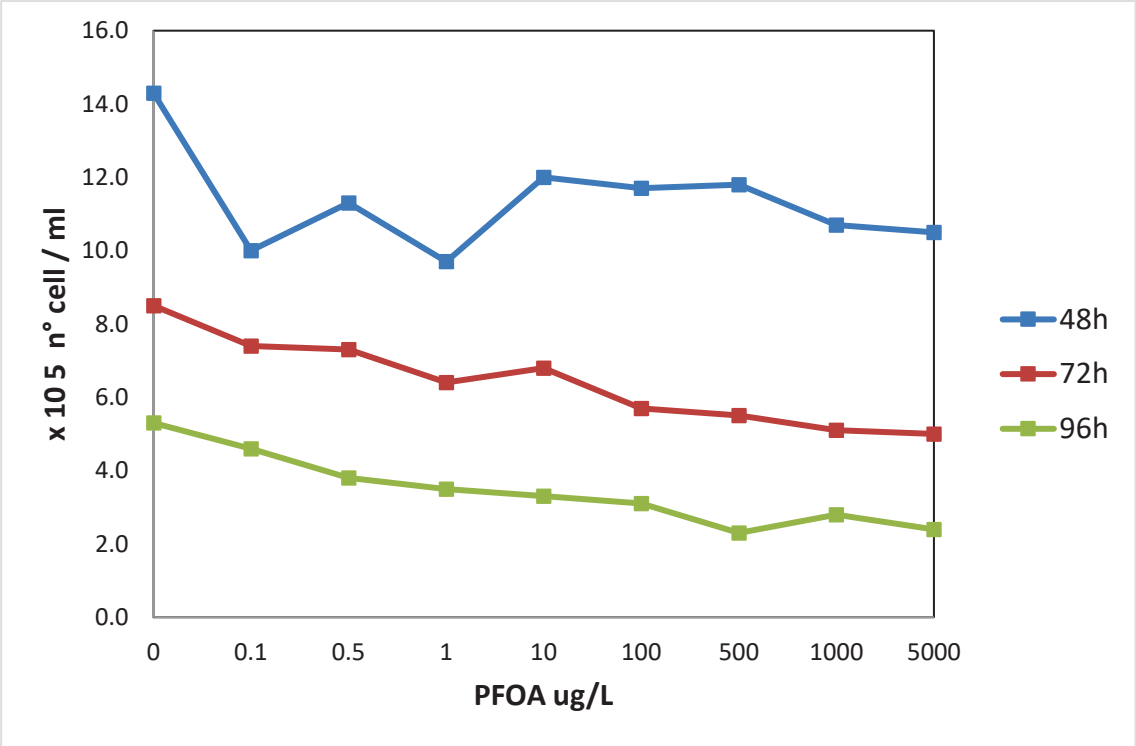

Figure 1. Growth algal at different PFOA concentration and different time (48h, 72h and 96h).

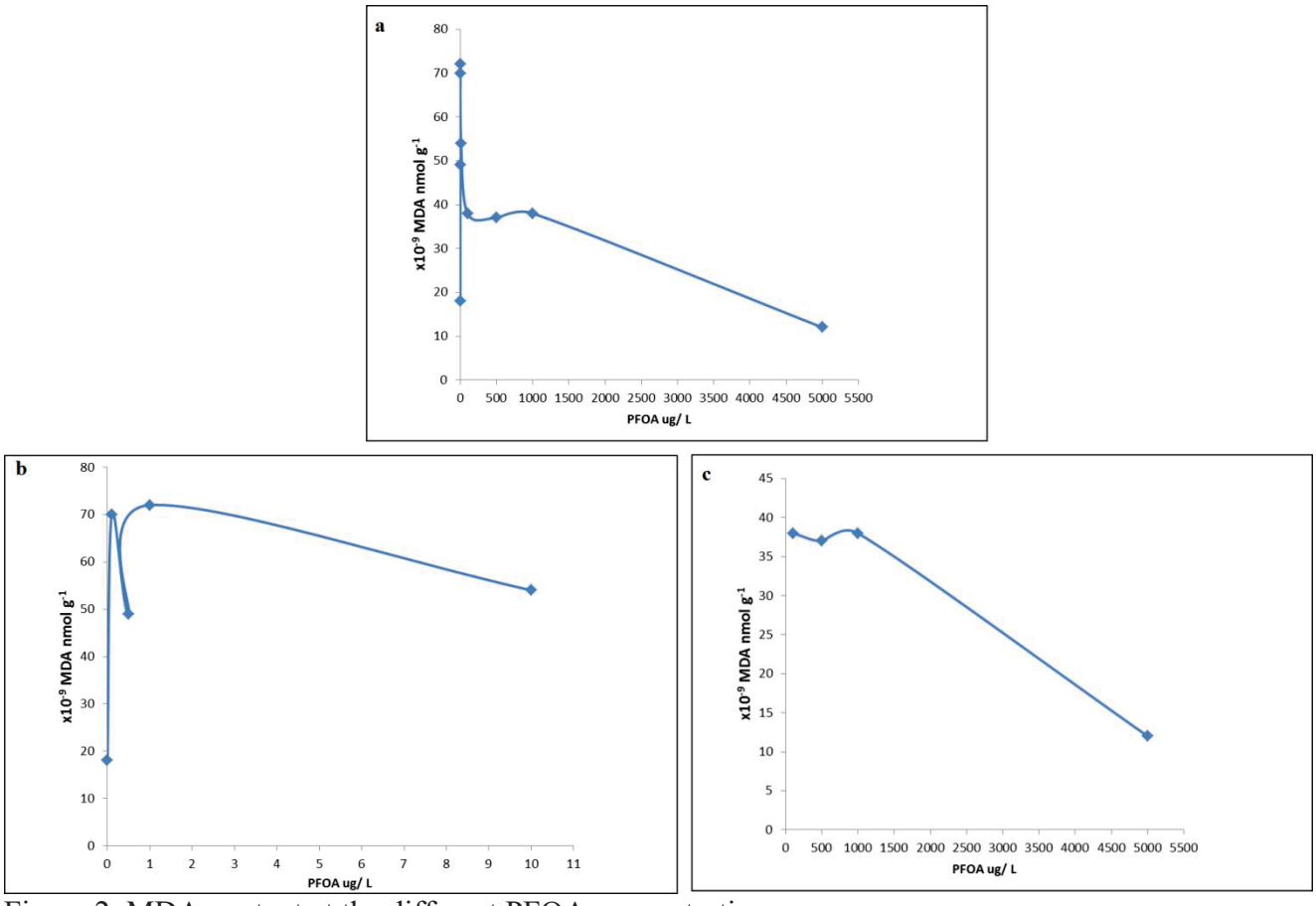

Figure 2. MDA content at the different PFOA concentrations

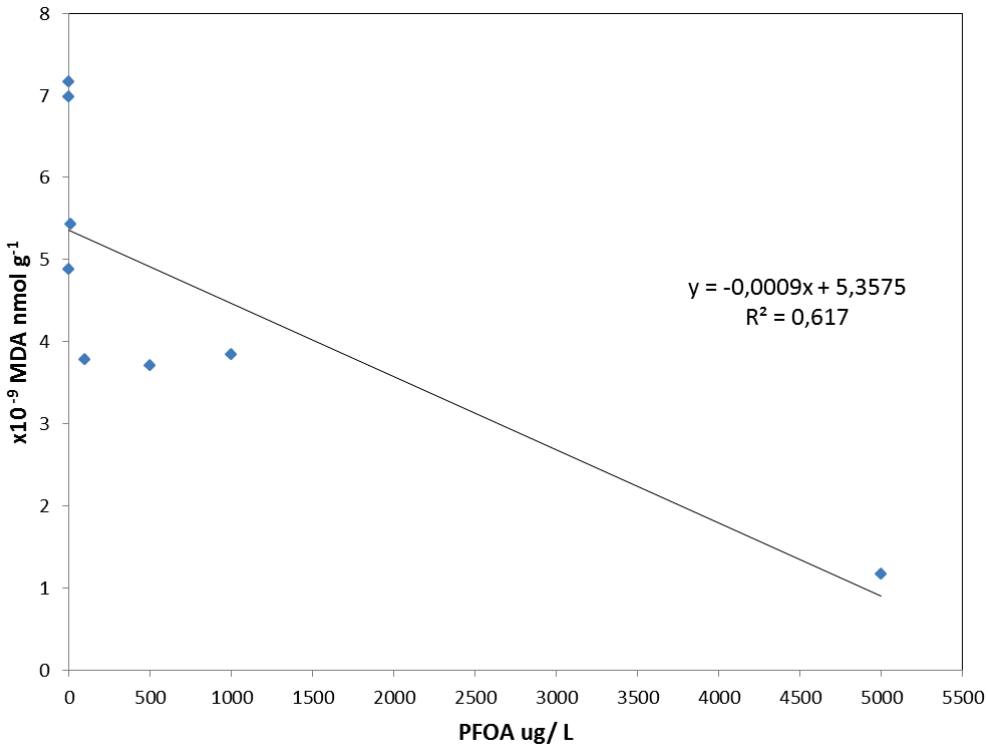

Figure 3. Regression analysis of MDA ($x10^{-9}$ nmol g^{-1}) content at the different PFOA (µg/L) concentration.

7. CONCLUSÕES E RECOMENDAÇÕES

O presente estudo mostrou que a correta identificação das espécies de diatomáceas é imprescindível, uma vez que, este é o ponto chave para diversos estudos, como distribuição de espécies endêmicas, espécies cosmopolitas, análise de diversidade, bioindicação do ambiente aquático e paleolimnologia e que a identificação equivocada poderia tendenciar resultados e levar a conclusões também equivocadas.

Entretanto, ainda há dificuldades para identificação de espécies de diatomáceas, uma vez que a morfologia da frústula, base na taxonomia, pode sofrer modificações resultantes de alterações ambientais, como na presença de poluentes.

A utilização de dados genéticos se mostra uma ferramenta promissora para auxiliar na identificação de espécies, porém ainda há muita dificuldade para estudos com diatomáceas, já que essas microalgas possuem um complexo genoma, um grande número de táxons e há constantes identificações de espécies novas. Além disso, há também a dificuldade do cultivo principalmente para espécies raras com baixa taxa de frequência, ao qual o esforço amostral poderia auxiliar nessa questão, amostrando de forma mais ampla abrangendo a diversidade de espécies da comunidade local.

Dessa forma, recomendamos que para identificações mais precisas sejam cruzados dados morfológicos com dados genéticos e que durante a amostragem de espécies o esforço amostral colete diversos substratos, eliminando o efeito da preferência de substratos, e que o número de amostra seja amplo, para que o esforço amostral represente a diversidade de comunidades do habitat.

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