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Różnorodność gatunkowa i autekologia okrzemek w ekosystemach wodnych pochodzenia antropogenicznego

Species diversity and autecology of diatoms
in aquatic ecosystems of anthropogenic origin

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DZIĘKUJĘ

Spis treści

I. Wstęp

1. Taksonomia okrzemek

Okrzemki (Bacillariophyta) po raz pierwszy zostały zaobserwowane na początku XVIII wieku, jako organizmy przytwierdzone do korzeni roślin wodnych z rodzaju *Lemna* Linnaeus (Leeuwenhoek 1703, Round i inni 1990). Następnie Müller w 1786 roku opisał pierwsze gatunki okrzemek, w tym *Vibrio paxillifer*, który w późniejszym czasie stał się typem pierwszego rodzaju okrzemek *Bacillaria* Gmelin*,* zaklasyfikowanym wówczas jednak do królestwa zwierząt (Gemelin 1791). Przez kolejne dekady obserwowano okrzemki jako ruchliwe organizmy wykazujące, jak się wtedy wydawało, cechy komórek zwierzęcych. Dlatego do połowy XIX wieku, w pracach Bory de Saint-Vincenta (1830, 1830a) oraz Ehrenberg'a (1828) nadal klasyfikowane były one do królestwa zwierząt. Przełomową pracą, w której okrzemki zostały zaklasyfikowane do organizmów autotroficznych – glonów, była *Die Kieselschaligen Bacillarien oder Diatomeen* z 1844 roku autorstwa F.T. Kützing'a. Przez kolejne stulecie, które obfitowało w liczne wyprawy naukowe (Grunow 1868, 1876, 1877, Cleve 1883, 1897, 1898, 1899) oraz doskonalenie techniki mikroskopii świetlnej (Masters 2008, Lancaster 2014), wzrosło zainteresowanie taksonomią wykorzystującą jako podstawę budowę morfologiczną ściany komórkowej okrzemek. Na przełomie XIX i XX wieku naukowcy zaczęli również bardziej szczegółowo badać inne aspekty ich morfologii i biologii (Round i inni 1990).

Ściana komórkowa okrzemek jest wysycona uwodnioną krzemionką, która powoduje jej usztywnienie i tworzy tak zwany pancerzyk, odporny na działanie czynników fizycznych i chemicznych. Budowa chemiczna ściany komórkowej okrzemek pozwala na poddanie komórki procedurze usunięcia organelli wewnątrzkomórkowych i pozyskanie samego pancerzyka do obserwacji cech morfologicznych z wykorzystaniem różnych technik mikroskopowych (Round i inni 1990). Od połowy ubiegłego wieku postęp technologiczny i dostępność mikroskopii elektronowej umożliwił bardziej szczegółową obserwację pancerzyków okrzemek (Kolbe 1948, 1951, Kolbe i Gölz 1943, Hendey i inni 1954). Przełomową pracą, w której dzięki wykorzystaniu transmisyjnej mikroskopii elektronowej (TEM) opisano poszczególne elementy pancerzyka okrzemek była *The fine structure of the frustule in centric diatoms: A suggested terminology* autorstwa Rossa i Sims z 1972 roku. W kolejnych dekadach wykorzystanie transmisyjnej i skaningowej mikroskopii elektronowej (TEM i SEM) stało się rutynową metodą badań struktur morfologicznych okrzemek (Round i inni 1990, John i Economou-Amilli 1991, Morales i inni 2001, Semina 2003, Witkowski i inni 2014, Genkal i Kulikovskiy 2016) oraz podstawą ich taksonomii (Falasco i inni 2009, Mertens i inni 2014, Thomas i Kociolek 2015).

Badania taksonomiczne oraz identyfikacja okrzemek z wykorzystaniem jedynie mikroskopii świetlnej, bazujące na cechach morfologicznych struktury pancerzyka, są trudne i skomplikowane. Główną przyczyną takiego stanu rzeczy jest fakt, że w przypadku zwłaszcza gatunków o niewielkich wymiarach, trudno jest prawidłowo rozpoznać wszystkie cechy morfologiczne różnicujące poszczególne taksony (Potapova i Hamilton 2007, Ponader i Potapova 2007, Wetzel i inni 2015, Blanco i inni 2017). Rozwiązaniem tego problemu jest wykonanie analizy struktur morfologicznych ściany komórkowej będących cechami taksonomicznymi z użyciem skaningowego mikroskopu elektronowego, które pozwalają odróżnić poszczególne gatunki (Lobo i inni 1990). Innym zagadnieniem wpływającym na jednoznaczność identyfikacji taksonomicznej jest wyróżnianie z jednego gatunku różnych form morfologicznych, określanych jako odmiany, formy lub morfotypy (Bahls i inni 1984). Wskazanie morfotypu wynika z obserwacji ”Janus cells” tego samego gatunku, które wyróżniają się odmienną budową morfologiczną. W wyniku zmian zachodzących w środowisku, morfologia ściany komórkowej okrzemek, wraz z kolejnymi podziałami komórkowymi może ulegać modyfikacji prowadząc do powstania ekomorfotypów (Stoermer 1967, Tuchman i inni 1984, Round i inni 1990, Stoermer i Julius 2003). Zgodnie z *International Code of Nomenclature for algae, fungi, and plants* (Turland i inni 2018), morfotyp lub też ekomorfotyp nie jest jednostką taksonomiczną, dlatego aby podkreślić odmienność morfotypu związanego z odmiennymi warunkami środowiskowymi, nadaje mu się rangę odmiany lub formy w obrębie gatunku na przykład *Stephanodiscus hantzschii* f. *tenuis* (Hustedt) Håkansson & Stoermer (Stoermer i Håkansson 1984, Häkansson 2002) lub *Mastogloia danseyi* f. *grevillei* (Smith) Edlund & Burge (Edlund i Burge 2019). Występujące w środowisku gatunki kryptyczne mogą również sprawić trudności w prawidłowej identyfikacji taksonomicznej. *Cyclotella meneghiniana* Kützing oraz *C. cryptica* Reimann, Lewin & Guillard różnią się od siebie pod względem molekularnym, jednak w określonych warunkach środowiska wodnego cechy morfologiczne *C. meneghiniana* i *C. cryptica* nie pozwalają na ich rozróżnienie (Beszteri i inni 2007, Hevia-Orube i inni 2016). Występowanie gatunków o niewielkich rozmiarach, odmian, form lub gatunków kryptycznych, ujmowanych w kompleksy gatunkowe, prowadzi do zubożenia informacji o strukturze taksonomicznej zbiorowiska (Potapova i Hamilton 2007, Ponader i Potapova 2007, Wetzel i inni 2015, Blanco i inni 2017, Pinseel i inni 2018). Niejednokrotnie do kompleksu gatunkowego włączone zostają taksony, które są notowane w różnych częściach świata, w podobnych ale również w skrajnie odmiennych warunkach środowiska. Pomimo, że poszczególne taksony w obrębie kompleksu gatunkowego mogą występować w odmiennych warunkach środowiskowych, również w wąskim zakresie ich zmienności, całemu kompleksowi przypisuje się szeroki zakres tolerancji ekologicznej. Konsekwencją takiego podejścia jest również błędne przypisanie wszystkim taksonom włączonym do kompleksu, takich samych ekologicznych wartości wskaźnikowych, co prowadzi do obarczonej błędem oceny stanu ekologicznego wód (Beszteri i inni 2007, Lundholm i inni 2012, Cantonati i inni 2016, Szczepocka i inni 2019).

Metody molekularne stosowane są coraz powszechniej również w badaniach taksonomicznych okrzemek (Medlin i inni 1996a, Medlin i inni 1996b, Medlin i inni 2000, Kulikovskiy i inni 2016, An i inni 2017, Pinseel i inni 2017, Pinseel i inni 2018, Kollár i inni 2019). Jednak, wykorzystanie zarówno technik molekularnych, jak i badań morfologii pancerzyka okrzemek, stanowi nowe, wieloaspektowe podejście w taksonomii tej grupy organizmów (Pinseel i inni 2017, Li i inni 2018, Kollár i inni 2019). Takie postępowanie pozwala na wyodrębnienie nowych taksonów, w tym również nowych gatunków z gatunków kryptycznych. Szczegółowy opis nowej jednostki taksonomicznej, zawierający zarówno dane molekularne, jak i morfologiczne, pozwala następnie na prawidłową identyfikację taksonów jedynie na podstawie ich cech morfologicznych (Lundholm i inni 2012, Li i inni 2018).

Metody molekularne, jako „narzędzie" w taksonomii, wciąż są rozwijane, poprzez stosowanie coraz większej liczby genów (Kollár i inni 2019), a uzyskane dane uzupełniają genetyczne bazy danych. Jednak, nie zawsze informacje genetyczne pochodzące z tych baz są na tyle dokładne aby otrzymywać prawidłową identyfikacje taksonomiczną. Obecnie nadal prowadzone są badania, które mają weryfikować jakość informacji genetycznych w kontekście badań nad identyfikacją gatunków (An i inni 2017). Analizy molekularne mogą również stanowić uzupełnienie dotychczas standardowych metod identyfikacji taksonomicznej okrzemek na podstawie struktury ściany komórkowej. Nakov i współautorzy (2017) przeprowadzili weryfikację molekularną grupy okrzemek centrycznych. Badania te doprowadziły do wyodrębnienia trzech różniących się molekularnie rodzajów, jednocześnie określili również, że każdy z nich charakteryzował się innymi cechami morfologicznymi. Wyniki tych badań umożliwiają przypisanie nowych taksonów do konkretnego rodzaju wykorzystując następnie jedynie budowę morfologiczną pancerzyka.

Wieloaspektowe podejście do badań nad taksonomią glonów oprócz analizy molekularnej i morfologicznej powinno zawierać również informacje o ich ekologii, autekologii (Komárek 2016). Występowanie odmian, form i gatunków kryptycznych jest związane z warunkami środowiskowymi, w których występuje dana populacja, dlatego dane o ekologii danego taksonu są istotne z punktu widzenia poprawnej identyfikacji gatunkowej (Bicudo i inni 2016, Pinseel i inni 2018).

W opisie nowych gatunków istotne jest aby dane dotyczące ich autekologii były jak najszersze. Gatunek – *Amphora ohridana* Levkov został opisany z jeziora Ohrid w Macedonii. Opis dotyczący warunków środowiskowych panujących w tym jeziorze zawierał jedynie informacje o tym, że źródłem fosforu jest jezioro Prespa, którego wody wpływają do jeziora Ohrid (Levkov i inni 2007). Z innych materiałów źródłowych można uzyskać informację, że wody jeziora Ohrid klasyfikowane są do I klasy jakości (Program of monitoring of surface waters in the Republic of Macedonia) (Janevski i inni 2006). Kilka lat później występowanie *A. ohridana* zostało stwierdzone w zdegradowanej, miejskiej rzece, w której parametry fizyczne i chemiczne wody świadczyły o jej wysokim zanieczyszczeniu (Żelazna-Wieczorek i inni 2010). Obserwacje występowania tego gatunku w skrajnie odmiennych warunkach środowiska dały podstawy aby stwierdzić, iż ma on szerokie spektrum ekologiczne oraz występuje w wodach o różnym rodzaju i stopniu zanieczyszczenia. Obecnie coraz częściej opisy nowych gatunków zawierają szczegółowe dane o warunkach środowiska, w którym były notowane (Żelazna-Wieczorek 2011, Kennedy i inni 2019).

2. Okrzemki jako organizmy wskaźnikowe

Organizmy wskaźnikowe, bioindykatory, są to pojedyncze organizmy lub grupy organizmów, których obserwowane reakcje na czynniki biotyczne i abiotyczne panujące w ekosystemie, w którym występują, pozwalają ocenić stan tego ekosystemu. Wśród organizmów wskaźnikowych wyróżnia się te, które reagują na obecność zanieczyszczeń – bioindykatory zanieczyszczeń, na przykład organizmy wrażliwe na zanieczyszczenie powietrza pyłami; na zmiany zachodzące w siedlisku – ekologiczne bioindykatory, na przykład na okresowe ale naturalne wysychanie cieków wodnych; na trwałe zmiany lub zmiany trwale zmieniające warunki środowiska – bioindykatory środowiskowe, na przykład w wyniku budowy zbiorników zaporowych na rzekach;

na zmiany w strukturze zbiorowiska organizmów – bioindykatory różnorodności biologicznej, na przykład gatunki endemiczne (Gerhardt 2011, Parmar i inni 2016).

Na początku XX wieku Kolkwitz i Marsson (1909) stworzyli pierwszy system klasyfikacji ekologicznej – system saprobów. Podstawą tego systemu są organizmy wskaźnikowe, zaklasyfikowane do jednej z klas: polisaprobowej, mezosaprobowej (α i β) i oligosaprobowej, w zależności od ich reakcji na obciążenie wód materią organiczną. Wraz z rozwojem badań w zakresie autekologii organizmów wskaźnikowych system ten był zmieniany i doskonalony (Butcher 1947, Kolkwitz 1950, Liebmann 1951, Fjerdingstad 1964, Sládeček 1965, Sládeček 1973). Obok systemu saprobów pojawiały się kolejne systemy ekologiczne, a wśród nich te, które uwzględniały okrzemki o określonym zakresie tolerancji na czynniki środowiska wodnego, takie jak zanieczyszczenie środowiska materią organiczną (Lange-Bertalot 1979), odczyn wody (Hustedt 1938, 1957), zasolenie (Kolbe 1927, Hustedt 1953, Denys i inni 1983), zawartość tlenu (Hustedt 1937, 1938, 1957, Cholnoky 1968) i żyzność wód (Rott i inni 1999). Denys (1991a, 1991b) opracował system klasyfikacji ekologicznej dla 14 cech środowiska wodnego oraz form ekologicznych, do których przyporządkował 980 taksonów okrzemek. Klasyfikacja zaproponowana przez Denysa została opracowana na podstawie 800 prób pochodzących z rdzeni z holocenu, uzyskanych głównie ze złóż odkrywkowych z obszaru wschodniego wybrzeża Belgii.

Przełomową pracą o możliwości wykorzystania wiedzy o autekologii współcześnie występujących okrzemek oraz klasyfikacji ekologicznej tych mikroorganizmów jest *A coded checklist and ecological inicator values of freshwater diatoms from the Netherland* autorstwa Van Dama, Mertens i Sinkeldama z 1994 roku. Autorzy opracowali system, w którym przypisali wartości wskaźnikowe 948 taksonom okrzemek wobec odczynu wody, zasolenia, metabolizmu azotu, stężenia tlenu, saprobii, trofii oraz warunków wilgotnościowych środowiska. Odczyn wody został określony w 6 stopniowej skali w oparciu o prace Hustedta (1938, 1939). Podstawą klasyfikacji w odniesieniu do zasolenia wody było stężenie jonów chlorkowych wyrażone 4-stopniową skalą. Pierwotnie klasyfikacja okrzemek wobec zasolenia wody została opracowana przez Van der Werffa i Hula (1957–1974), którzy przyjęli 7-stopniową skalę włączając w nią również gatunki ze środowiska brakicznego i morskiego. Van Dam i współautorzy zdecydowali się pominąć klasy od 5 do 7, w których stężenia jonów chlorkowych (od 5000 do ponad 17000 mg l^{-1} Cl^{-}) są charakterystyczne dla ekosystemów morskich. Metabolizm azotu, w systemie klasyfikacji Van Dama i współautorów, jest określony w 4-stopniowej skali, w której gatunki sklasyfikowane są względem przyswajalności azotu z wody od takich, które tolerują go w niewielkim stężeniu do takich, które wymagają jego stałego dopływu w wysokim stężeniu. Klasyfikacja metabolizmu azotu została opracowana na podstawie prac Cholnokygo (1968) oraz Van Dama (1975). Pięciostopniowa skala opisująca optymalną zawartość tlenu rozpuszczonego w wodzie dla poszczególnych taksonów okrzemek została opracowana na podstawie prac Hustedta (1938, 1939, 1957), Cholnokyego (1968) oraz Van Dama (1975). Klasyfikacja ta wyróżnia taksony okrzemek, począwszy od tych, które wymagają 100% wysycenia tlenem w środowisku wodnym do takich, które żyją w warunkach 10% wysycenia tlenem. Saprobowość wód, według klasyfikacji Van Dama i współautorów, została opracowana w 5-stopniowej skali w oparciu o zawartość materii organicznej oraz tlenu rozpuszczonego w wodzie. Podstawą tej klasyfikacji jest system saprobów opracowanym przez Kolkwitza i Marssona z 1908 roku, który następnie został zmodyfikowany. Siedmiostopniowa skala klasyfikująca gatunki okrzemek względem trofii została opracowana biorąc pod uwagę stężenie biogenicznych związków nieorganicznych, takich jak: fosforany, azot, węgiel i krzemionka. Jednak, tworząc klasyfikację troficzną Van Dam i współautorzy, nie dysponując danymi eksperymentalnymi, opierali się na niepełnych danych zamieszczonych w publikacjach innych autorów (Sommer 1989, Whitmore 1989, Willén 1991, Agbeti 1992, Anderson i inni 1993, Hofmann 1993). Ostatnią cechą środowiska, ujętą w systemie klasyfikacji Van Dama i współautorów, są warunki wilgotnościowe. Klasyfikacja ta została wprowadzona na podstawie danych z pracy Denysa (1991a) z niewielkimi modyfikacjami.

Od lat 80. XX wieku w Europie zaczęto konstruować pierwsze indeksy okrzemkowe opierające się na systemach klasyfikacji ekologicznej, są to SPI-Specific Pollution sensitivity Index i GDI-Generic Diatom Index. Indeksy te są wykorzystywane w ocenie stopnia zanieczyszczenia organicznego wody (CEMAGREF 1982, Rumeau i Coste 1988). W kolejnych latach stworzono kolejne indeksy okrzemkowe: TDI-Trophic Diatom Index (Kelly i Whitton 1995), BDI-Biological Diatom Index (Lenoir i Coste 1996) i EPI-D-Eutrophication Pollution Diatom Index (Dell'Uomo 1996). W związku z koniecznością dostosowania polskiego prawa do wymagań Ramowej Dyrektywy Wodnej (Dyrektywa 2000/60/WE Parlamentu Europejskiego i Rady z dnia 23 października 2000 roku), zaproponowano wprowadzenie do oceny wód powierzchniowych multimetrycznego indeksu okrzemkowego dla rzek (IO) i jezior (IOJ). Indeksy te opisują stan ekologiczny wód powierzchniowych w 5-cio stopniowej skali (Picińska-Fałtynowicz i Błachuta 2010).

Okrzemki, na podstawie przypisanym im ekologicznym wartościom wskaźnikowym, stały się jednym z najlepszych narzędzi do oceny jakości wód powierzchniowych. Mnogość systemów klasyfikacji ekologicznej oraz liczne indeksy okrzemkowe zrodziły potrzebę uporządkowania i zgromadzenia tych informacji. W 1993 roku powstał program OMNIDIA (Lecointe i inni 1993), który zawiera 18 indeksów okrzemkowych oraz 11 systemów klasyfikacji ekologicznej w tym system Van Dama i współautorów (1994) (https://omnidia.fr/en/features/). Program ten zawiera bazę danych, do której wprowadzono informacje o taksonach okrzemek wraz z przypisanymi im ekologicznymi wartościami wskaźnikowymi i wrażliwością na wybrane warunki środowiska wodnego. Baza danych jest stale modyfikowana i obecnie zawiera charakterystykę ekologiczną ponad 24 tysięcy taksonów okrzemek. Drugą składową programu jest arkusz kalkulacyjny, który na podstawie danych dotyczących liczebności poszczególnych taksonów w próbie, a następnie wyników obliczeń indeksów okrzemkowych oraz interpretacji systemów klasyfikacji ekologicznych daje informację o stanie jakości wód (Lecointe i inni 1993). Obecnie dane pozyskiwane z programu OMNIDIA są powszechnie wykorzystywane do oceny jakości wód powierzchniowych oraz informacji ekologicznych o okrzemkach, w krajach europejskich, ale coraz częściej w różnych regionach świata (Szczepocka i Żelazna-Wieczorek 2018).

3. Zbiorniki pokopalniane

Ekosystemy wodne powstałe na skutek górniczej działalności człowieka charakteryzują się specyficznymi warunkami hydrologicznymi, geologicznymi i hydrochemicznymi. Budowa geologiczna podłoża stanowiącego misę zbiorników pochodzenia antropogenicznego kształtuje warunki środowiskowe i przyczynia się do występowania specyficznych zbiorowisk fauny i flory preferującej określone czynniki abiotyczne (Johnston i inni 2009; Krawczuk i inni 2016).

Na skład chemiczny wód, poza procesami hydrogeochemicznymi, zachodzącymi pomiędzy wodami zbiorników i podłożem skalnym budującym misę zbiornika mają również wpływ wody podziemne i głębinowe, które zostały wprowadzone do obiegu wód powierzchniowych, na przykład w wyniku pogłębiania odkrywki kopalni (Konsencjusz i inni 2012; Sienkiewicz i Gąsiorowski 2017).

Najliczniej eksploatowanymi surowicami w Polsce są surowce energetyczne (49%) w tym największy udział stanowi węgiel kamienny (75%) i węgiel brunatny (13%) (Burkowicz i inni 2014). Przy eksploatacji złóż węgla brunatnego obserwuje się tak zwany kwaśny drenaż, który prowadzi do zmian fizycznych i chemicznych, w tym zakwaszenia wód gruntowych lub naturalnych cieków wodnych, do których woda z kopalni jest odprowadzana (Dwiki i inni 2015; Sienkiewicz i Gąsiorowski 2017). W kopalniach węgla brunatnego często obserwuje się obecność wysadów solnych lub wypływ zasolonych wód podziemnych. W wyniku procesu wydobywania złóż węgla, którym towarzyszą pokłady soli, konieczne jest odprowadzanie zasolonej wody podziemnej z miejsc eksploatacyjnych poza obszar odkrywki. Po zaprzestaniu działalności kopalni, obserwuje się również wypłukiwanie skał zawierających sole chlorkowe. W wyniku tych procesów, wody zbiorników powstałych po odkrywkowych kopalniach węgla brunatnego charakteryzują się podwyższonym stężeniem jonów chlorkowych (Yudovich i Ketris 2006, Zgórska i inni 2016).

Wyróżniającym się typem śródlądowych wód powierzchniowych są ekosystemy słonowodne (ang. athalassic habitats), które mogą mieć różną genezę. Jedne z nich mogą być naturalnego pochodzenia, takie jak jeziora, na których stopień zasolenie ma wpływ niewielka odległość od morza (Veres i inni 1995) lub antropogenicznego które powstały w wyniku poszukiwania soli metodą odwiertów. Odwierty wykonywane były w celu potwierdzenia obecności słonej wody, która wskazuje na obecność pokładów soli kamiennej. Obszarem bogatym w pokłady soli cechtyńskiej jest antyklina Kłodawsko-Łęczycka, która rozciąga się od miejscowości Solec Wielki, przez Łęczycę aż do Kłodawy. W Kłodawie obecnie eksploatowane są pokłady soli kamiennej metodą górniczą. Pod koniec XVIII wieku w okolicy Łęczycy wykonano próbne odwierty w celu określenia głębokości pokładów soli kamiennej. Odwierty te stanowią dzisiaj samoistne wypływy słonych wód podziemnych, tworząc unikalne ekosystemy wodne i lądowe (Olaczek, nieopublikowana rozprawa doktorska 1963).

Wpływ działalności odkrywkowych kopalni rud żelaza na środowisko naturalne jest silniejszy niż w przypadku kopalni węgla brunatnego. Wynika to z obecności podwyższonej zawartości związków metali, w tym metali ciężkich w wydobywanym surowcu. Wody odprowadzane z odkrywki rudy żelaza lub wody wypełniające zbiornik poeksploatacyjny tych złóż, mogą zawierać podwyższone stężenie jonów metali ciężkich oraz kwasowy odczyn wody (Gleekia i inni 2016). W szczególnych warunkach można zaobserwować proces prowadzący do alkalizacji wód zbiornika w wyniku obecności związków węglanowych i wodorowęglanowych. Proces ten prowadzi do zobojętnia lub podwyższenia odczynu w wodach zbiorników powstałych po kopalni rud żelaza (Metesh i inni 1998). W wyniku zmian odczynu wody na zasadowy jony metali ciężkich są wiązane w osadach i nie są wykrywalne w toni wodnej (Wright i inni 2007).

Głównym sposobem rekultywacji odkrywkowych kopalni minerałów jest ich przekształcenie w zbiorniki wodne. Kopalnia węgla brunatnego „Konin” jako pierwsza w Polsce, wdrożyła próby rekultywacji terenów pogórniczych na przełomie lat 50. i 60. ubiegłego wieku (Kasztelewicz i inni 2007). Jest to ważny etap procesu wydobycia surowców, tym bardziej jeśli eksploatacji węgla brunatnego towarzyszą zanieczyszczenia mogące powodować kwaśny drenaż (Dwiki i inni 2015, Sienkiewicz i Gąsiorowski 2017).

4. Autekologia i taksonomia okrzemek w zbiornikach pokopalnianych

Zbiorniki powstałe w wyniku zalania odkrywkowych kopalni surowców mineralnych reprezentują ekosystemy o unikalnych i często ekstremalnych warunkach środowiskowych. Zbiorowiska okrzemek występujące w tego typu ekosystemach były obiektem badań od kilkudziesięciu lat. Jednak, większość badań dotyczyła prześledzenia zmian klimatu w czasie, w oparciu o badania paleoekologiczne wykorzystując bioindykacyjne właściwości okrzemek (De Haan i inni 1993, Rakowska 1996, Thomas i John 2006, Sienkiewicz i Gąsiorowski 2016). Wiedza dotycząca autekologii okrzemek występujących w zbiornikach pokopalnianych jest niepełna, większość prowadzonych badań dotyczyła głównie określenia różnorodności gatunkowej w tego typu siedliskach (Van Landingham 1968, De Haan i inni 1993, Rakowska 1996, Ferreira da Silva i inni 2009, Luís i inni 2009, 2016, Sienkiewicz i Gąsiorowski 2016).

II. Hipoteza badawcza

W pracy postawiono następującą hipotezę, którą poddano weryfikacji:

Unikatowe warunki środowiskowe panujące w ekosystemach wodnych powstałych w wyniku eksploatacji kopalin determinują różnorodność taksonomiczną okrzemek w nich występujących umożliwiając weryfikację istniejących i ustalenie nowych ekologicznych wartości wskaźnikowych tej grupy mikroorganizmów.

III. Cel pracy

Weryfikacja postawionej hipotezy wymagała określenia celu pracy, którym było zbadanie różnorodności gatunkowej oraz autekologii okrzemek bentosowych występujących w ekosystemach wodnych pochodzenia antropogenicznego jakimi są zbiorniki pokopalniane.

Do realizacji celu badań sformułowano następujące zadania badawcze:

- zbadanie i określenie warunków środowiskowych wyznaczonych ekosystemów wodnych;
- określenie różnorodności taksonomicznej okrzemek w wybranych ekosystemach wodnych;
- zbadanie zmienności wewnątrzgatunkowej wybranych okrzemek bentosowych, a następnie ich weryfikacja taksonomiczna;
- wyznaczenie gatunków charakterystycznych okrzemek dla danego typu ekosystemu wodnego;
- zbadanie zależności pomiędzy występowaniem gatunków charakterystycznych a wybranymi parametrami fizycznymi i chemicznymi wody badanych ekosystemów;
- zbadanie i zweryfikowanie autekologii gatunków charakterystycznych okrzemek bentosowych dla wybranych ekosystemów wodnych;
- określenie lub weryfikacja ekologicznych wartości wskaźnikowych okrzemek w klasyfikacji Van Dama i współautorów (1994).

IV. Publikacje stanowiące rozprawę doktorską

Zgodnie z ustawą *Prawo o szkolnictwie wyższym i nauce z dnia 20 lipca 2018 art. 187 punkt 3* na niniejszą rozprawę doktorską składa się zbiór opublikowanych i powiązanych tematycznie artykułów naukowych:

- Żelazna-Wieczorek J., **Olszyński R.M.** i Nowicka-Krawczyk P. 2015. Half a century of research on diatoms in athalassic habitats in central Poland. *Oceanological and Hydrobiological Studies* 44(1): 51–67.
  doi.org/10.1515/ohs-2015-0006 (**Załącznik 1**)
  Impact Factor: 0.519
  5-letni Impact Factor: 0.854
  Lista czasopism A MNiSW: 15pkt/40 pkt*
- Żelazna-Wieczorek J. i **Olszyński R.M.** 2016. Taxonomic revision of *Chamaepinnularia krookiformis* Lange–Bertalot et Krammer with a description of *Chamaepinnularia plinskii* sp. nov. *Fottea* 16(1): 112–121.
  doi.org/10.5507/fot.2016.001 (**Załącznik 2**)
  Impact Factor: 1.350
  5-letni Impact Factor: 2.11
  Lista czasopism A MNiSW: 30pkt/40 pkt*
- **Olszyński R.M.** i Żelazna-Wieczorek J. 2018. *Aulacoseira pseudomuzzanensis* sp. nov. and other centric diatoms from post iron ore mining reservoirs in Poland. *Diatom Research* 33(2): 155–185.
  doi.org/10.1080/0269249X.2018.1509886 (**Załącznik 3**)
  Impact Factor: 1.169
  5-letni Impact Factor: 1.25
  Lista czasopism A MNiSW: 25pkt/70 pkt*
- **Olszyński R.M.**, Szczepocka E. i Żelazna-Wieczorek J. 2019. Critical multi-stranded approach for determining the ecological values of diatoms in unique aquatic ecosystems of anthropogenic origin. *PeerJ* 7:e8117.
  doi.org/10.7717/peerj.8117 (**Załącznik 4**)
  Impact Factor: 2.353
  5-letni Impact Factor: 2.70
  Lista czasopism A MNiSW: 100 pkt

***(punkty przyznane do 31 lipca 2019/punkty po 31 lipca 2019 roku)**

- Materiały uzupełniające: Percentage share of diatoms in individual classes of prevalence according to the Tümpling & Friedrich factor (1999).
  doi.org/10.7717/peerj.8117/supp-1 (Załącznik 4.1).
- Materiały uzupełniające: Average percentages of diatom species in individual classes of ecological values according to Van Dam, Mertens & Sinkeldam (1994) based on OMNIDIA 6.0.6 software.
  doi.org/10.7717/peerj.8117/supp-2 (Załącznik 4.2).
- Materiały uzupełniające: Classes of ecological indicators values by Van Dam, Mertens & Sinkeldam (1994).
  doi.org/10.7717/peerj.8117/supp-3 (Załącznik 4.3).
- Materiały uzupełniające: Percentage of characteristicc species in the unknown (total) class according to Van Dam, Mertens & Sinkeldam (1994) at each sampling point.
  doi.org/10.7717/peerj.8117/supp-4 (Załącznik 4.4).
- Dane surowe: Raw data.
  doi.org/10.7717/peerj.8117/supp-5 (Załącznik 4.5).

V. Teren badań, materiały i metody pracy

W celu weryfikacji postawionej hipotezy badawczej wytypowano trzy obiekty hydrologiczne powstałe w wyniku przekształceń antropogenicznych związanych z poszukiwaniem kopalin – soli kamiennej lub zalaniem odkrywkowych kopalni surowców mineralnych: węgla brunatnego i rud żelaza. Wytypowane obiekty znajdują się w granicach województw łódzkiego i wielkopolskiego (Ryc. 1).

Rycina 1. Lokalizacja obiektów hydrologicznych wyznaczonych do przeprowadzenia badań: A. Kompleks hydrologiczny Pełczyska. B. Kompleks zbiorników w Łęczycy. C. Zbiornik Bogdałów (Olszyński et al. 2019).

Szczegółowy opis terenu badań, metod poboru prób i analizy warunków środowiskowych, przygotowania materiałów do analizy jakościowej i półilościowej okrzemek oraz metod analizy danych znajduję się w publikacjach stanowiących rozprawę doktorską (**Załącznik 1:** Żelazna-Wieczorek i inni 2015, **Załącznik 2:** Żelazna-Wieczorek i Olszyński 2016, **Załącznik 3:** Olszyński i Żelazna-Wieczorek 2018, **Załącznik 4:** Olszyński i inni 2019). Niniejszy rozdział oraz zawarte w nim podrozdziały przedstawiają skrócony opis terenu badań, wykorzystanych materiałów, w tym metodyki poboru prób oraz metod opracowania materiału.

1. Teren badań

Pierwszym badanym obiektem jest kompleks hydrologiczny znajdujący się w Pełczyskach (PE) (51°58’35.68”N, 19°14’17.02”E) w gminie Ozorków, w województwie łódzkim (Ryc. 1). Kompleks ten składa się z wypływu słonej wody, rowu odprowadzającego wodę oraz stawu, do którego odprowadzana jest słona woda. W obrębie kompleksu Pełczyska wyznaczono 3 miejsca poboru prób: wypływ wody (D.PESB), rów (D.PEDB) oraz staw (D.PEPB). Powstanie kompleksu hydrologicznego w Pełczyskach sięga końca XVIII wieku i związane jest z wykonaniem odwiertów w celu poszukiwania solanki. Okoliczni mieszkańcy wykopali niewielki staw i kanały, w celu odprowadzania i gromadzenia wypływającej słonej wody, która zalewała pola uprawne. Następnie odwiert został otoczony betonową cembrowiną, a przed kilkoma laty podjęto próbę zasypania go. Obecnie części nasypu została zredukowana, a słona woda wypływa nadal na powierzchnię ziemi w miejscu dawnego odwiertu (**Załącznik 1:** Żelazna-Wieczorek i inni 2015, **Załącznik 2:** Żelazna-Wieczorek i Olszyński 2016, **Załącznik 4:** Olszyński i inni 2019).

Drugim obiektem hydrologicznym wytypowanym do przeprowadzania badań jest kompleks trzech zbiorników miejskich w Łęczycy (LE) (52°3′5.30″N; 19°11′50.24″E) (województwo łódzkie) (Ryc. 1). W obrębie kompleksu w Łęczycy wyznaczono 3 miejsca poboru prób: zbiornik 1 (D.LEP1), zbiornik 2 (D.LEP2) oraz zbiornik 3 (D.LEP3). Zbiorniki powstały w wyniku zalania odkrywkowej kopalni rud żelaza na początku 90. lat XX wieku. Zbiorniki 1 i 2 są ze sobą połączone wąskim przesmykiem, a zbiornik 2 i 3 – kanałem (**Załącznik 3:** Olszyński i Żelazna-Wieczorek 2018, **Załącznik 4:** Olszyński i inni 2019).

Trzecim obiektem hydrologicznym jest zbiornik w miejscowości Bogdałów (BO) (52°2′51.29″N; 18°35′51.49″E) (województwo wielkopolskie) (Ryc. 1). Do badań wyznaczono jeden punkt poboru prób (D.BOZB). Zbiornik powstał w wyniku zalania odkrywkowej kopalni węgla brunatnego na początku lat 90. ubiegłego wieku. Odkrywka została częściowo zasypana nakładami z kopalni Koźmin, w celu uszczelnienia dna przyszłego zbiornika, a następnie wypełniona wodą podziemną, gruntową oraz odprowadzaną z pobliskiej eksploatowanej okrywki (**Załącznik 4:** Olszyński i inni 2019).

2. Pobór i analiza prób

Z objętych badaniem obiektów hydrologicznych zebrano 44 próby fitobentosu okrzemkowego oraz 31 prób wody do analizy parametrów fizycznych i chemicznych od lipca 2013 do grudnia 2016 roku.

Do analizy jakościowej wykorzystano łącznie 66 prób fitobentosu okrzemkowego, w tym 44 zebrane we wskazanym wyżej czasie, 15 zebranych przez Profesora Marcina Plińskiego w latach 1964/1965 oraz trzy zebrane przez Profesor Joannę Żelazną-Wieczorek w latach 1992/1994 z kompleksu hydrologicznego w Pełczyskach. Podczas analiz taksonomicznych wykorzystano cztery próby z materiałem typowym wybranych gatunków okrzemek uzyskanych z *Friedrich–Hustedt–Zentrum für Diatomeenforschung Institute for Polar and Marine Research*, w Bremerhaven:

- *Pinnularia krookiformis* Krammer z zasolonych mokradeł w Nordrhein–Westfalen (Salzgebiet südlich von Salzkotten, Nordrhein–Westfalen Germany, leg. Wygasch, 17.04.1982 – KR604B,
- *Melosira muzzanensis* Meister z Lago di Muzzano, Italy E1289,
- *Cyclotella pseudostelligera* Hustedt z Ems, Station 197, Profil L, nahe Neue Schleuse bei Papenburg E524,
- *Cyclotella woltereckii* Hustedt z Java, Butenzorg C. 98 Teich in Motanischen Garten, Wallacea-Expedition AS1329.

Do analizy porównawczej mającej na celu zbadanie struktury zbiorowisk okrzemek występujących w trzech różniących się od siebie ekosystemach, wykorzystano 44 próby zebrane w latach 2013–2016, w tym 12 prób z kompleksu Pełczyska, 24 próby z kompleksu zbiorników w Łęczycy oraz 8 ze zbiornika Bogdałów. Próby z wyznaczonych obiektów hydrologicznych zbierane były raz na kwartał (**Załącznik 4**, 4.5: Olszyński i inni 2019).

Kompleks hydrologiczny Pełczyska był podany poszerzonej analizie z wykorzystaniem prób archiwalnych, która miała na celu zbadanie potencjalnych zmian w strukturze zbiorowisk okrzemek w okresie 50 lat. Analizę jakościową i półilościową okrzemek przeprowadzono na podstawie 30 prób fitobentosu okrzemkowego, w tym 12 zebranych w latach 2013/2014, 3 zebranych w latach 1992/1994 oraz 15 zebranych w latach 1964/1965 (**Załącznik 1:** Żelazna-Wieczorek i inni 2015).

Analizę okrzemek przeprowadzono na podstawie oczyszczonego materiału/osadu zatopionego w syntetycznej żywicy Naphrax® w postaci trwałych preparatów z wykorzystaniem technik mikroskopii świetlnej (Nikon Eclipse 50i, 1000× powiększenie, z wykorzystaniem obiektywu impresyjnego 100×/1.25) oraz oczyszczonego osadu techniką skaningowej mikroskopii elektronowej (Phenom ProX, 8 i 20 nm warstwy złota, przy 10 kV).

Warunki środowiska wodnego badanych ekosystemów scharakteryzowano badając 19 parametrów fizycznych i chemicznych wody. Wykonano analizę *in situ* następujących parametrów: odczynu wody, przewodnictwa elektrolitycznego i temperatury wody. Pomiary zostały wykonane przyrządami do badań w terenie firmy Elmetron: pehametrem CP-401 i konduktometrem CC-401. Analiza pozostałych parametrów fizycznych i chemicznych została wykonana w Laboratorium Pracowni Geologii, na Wydziale Nauk Geograficznych Uniwersytetu Łódzkiego.

W przypadku kompleksu hydrologicznego Pełczyska do analizy zmian warunków środowiskowych w ciągu 50 lat wykorzystano dostępne dane o parametrach fizycznych i chemicznych z piśmiennictwa.

W pracy wykorzystano następujące analizy matematyczne analiza MDS, nMDS, HCA, Shade Plot, SIMPER oraz PCA. W pracy wykorzystano programy OMNIDIA 6.0.6, PRIMER 7.0.13 i STATISTICA 13.

VI. Omówienie wyników

1. Warunki środowiskowe w badanych ekosystemach wodnych

Skład chemiczny wody w kompleksie hydrologicznym Pełczyska ze względu na budowę geologiczną terenu charakteryzował się wysokim stężeniem jonów chlorkowych (powyżej 3000 mg l^{-1}). Na podstawie przeprowadzonej analizy wykazano zmianę stężania jonów chlorkowych, których stężenie zmniejszało się od wypływu do stawu. Wykorzystując archiwalne dane chemiczne wody, stwierdzono że stężenie jonów chlorkowych obniżyło się w ciągu 50 lat (**Załącznik 1:** Żelazna-Wieczorek i inni 2015). Poszczególne miejsca poboru prób w kompleksie hydrologicznym Pełczyska charakteryzowały się wysokim przewodnictwem elektrolitycznym przekraczającym 9000 µS cm^{-1} oraz najwyższym stężeniem jonów Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, HCO_3^{-}, PO_4^{3-} i SO_4^{2-} w stosunku do pozostałych wybranych do badań ekosystemów wodnych (**Załącznik 2:** Żelazna-Wieczorek i Olszyński 2016, **Załącznik 4:** Olszyński i inni 2019).

W kompleksie zbiorników w Łęczycy, które powstały po zalaniu wyrobisk kopalni rud żelaza, woda charakteryzowała się wysokim odczynem oraz najniższym stężeniem jonów wodorowęglanowych w stosunku do pozostałych badanych ekosystemów. Geneza powstania zbiorników w Łęczycy wskazuje, że warunki w nich panujące powinny charakteryzować się zakwaszeniem wody oraz podwyższonym stężeniem jonów żelaza i manganu (Metesh i inni 1998). Jednak badane parametry wody, pobieranej do analizy z głębokości nie większej niż 0,5 metra, wykazały alkaliczny odczyn wody przy jednoczesnym niskim stężeniu jonów $Fe^{2+/3+}$ i Mn^{3+}(**Załącznik 3:** Olszyński i Żelazna-Wieczorek 2018, **Załącznik 4:** Olszyński i inni 2019).

W zbiorniku Bogdałów, który powstał po rekultywacji wyrobiska, po kopalni węgla brunatnego, badane parametry chemiczne i fizyczny wykazały alkaiczny odczyn wody przy jednoczesnym, najniższym stężeniu jonów K^{+}, Cl^{-} i NH_4^{+}, w odniesieniu do wszystkich badanych ekosystemów (**Załącznik 4:** Olszyński i inni 2019).

2. Różnorodność taksonomiczna okrzemek w badanych ekosystemach wodnych

Analiza jakościowa zbiorowisk okrzemek trzech wybranych obiektów hydrologicznych pochodzenia antropogenicznego przeprowadzona na podstawie 66 prób potwierdziła występowanie w nich łącznie 401 taksonów okrzemek należących do 80 rodzajów. Najliczniej reprezentowanymi rodzajami były *Nitzschia* Hassall (49 taksonów), *Navicula* Bory (44), *Caloneis* Cleve (25), *Gomphonema* Ehrenberg (20), *Fragilaria* Lyngbye (18), *Amphora* Ehrenberg ex Kützing (16), *Cymbella* Agardh (13), *Halamphora* (Cleve) Mereschkowsky (13) oraz *Surirella* Turpin (11).

Analiza struktury jakościowej zbiorowisk okrzemek na podstawie współcześnie zebranych 44 prób pozwoliła na identyfikację 381 taksonów, przy czym w kompleksie hydrologicznym Pełczyska – 139 taksonów, w trzech zbiornikach w Łęczycy – 192 taksonów, natomiast w zbiorniku w Bogdałowie – 188 taksonów. Gatunkami odnotowanymi w ponad 75% prób (Załącznik 4.1: Olszyński i inni 2019) a jednocześnie dominującymi w kompleksie hydrologicznym Pełczyska były *Navicula veneta* Kützing, którego udział procentowy w próbach wynosił od 11 do 41% i *Nitzschia frustulum* (Kützing) Grunow z maksymalnym udziałem 16%. W zbiornikach w Łęczycy gatunkami stałymi i dominującymi były *Cyclostephanos dubius* (Hustedt) Round (od 3 do 68% udziału w próbie) i *Stephanodiscus hantzschii* Grunow (do 40% udziału w próbie), a w zbiorniku w Bogdałowie największy udział procentowy w próbach miał *Achnanthidium minutissimum* (Kützing) Czarnecki (*sensu lato*) (do 61% udziału w próbie), *Pantocsekiella ocellata* (Pantocsek) Kiss & Ács (do 34% udziału w próbie) i *Mastogloia smithii* Thwaites ex Smith (do 22% udziału w próbie) (**Załącznik 4**, 4.5: Olszyński i inni 2019).

Przeprowadzona analiza porównawcza struktury jakościowej i półilościowej zbiorowisk okrzemek wszystkich wybranych obiektów badań, wykorzystująca metodę skalowania wielowymiarowego (nMDS), której podstawą jest obliczenie współczynnika podobieństwa Bray-Curtisa wykazała, że zbiorowiska okrzemek trzech badanych ekosystemów wyraźnie różnią się od siebie. Na istotność tej analizy wskazuje wartość stresu 0,07 dla diagramu 3-D, a poszczególne próby są ze sobą zgrupowane odpowiadając obiektom hydrologicznym, z których zostały zebrane (**Załącznik 4:** Olszyński i inni 2019). Ponadto analiza skalowania wielowymiarowego wykazała, że próby z kompleksu hydrologicznego Pełczyska są wyraźnie zróżnicowane ze wskazaniem odrębności prób zebranych ze stawu (wartość stresu 0,1 dla diagramu 3-D) (**Załącznik 1:** Żelazna-Wieczorek i inni 2015, **Załącznik 4:** Olszyński i inni 2019).

Kompleks hydrologiczny Pełczyska w czasie 50 lat ulegał przekształceniom wynikającym z działalności człowieka. W celu wskazania zróżnicowania struktury zbiorowisk okrzemek w czasie wykonano hierarchiczną analizę klasterową na podstawie współczynnika jakościowego i ilościowego podobieństwa Bray-Curtisa dla prób zgromadzonych w zbiorach Katedry Algologii i Mykologii oraz zebranych współcześnie. Dendrogram analizy klasterowej wykazał, że zbiorowiska okrzemek z prób zebranych w latach 2013/2014 stanowią odrębną grupę/klaster i różniły się od zbiorowisk okrzemek z prób z lat 1964/1965 i 1992/1994, co związane jest głównie ze spadkiem koncentracji jonów chlorkowych w czasie (**Załącznik 1:** Żelazna-Wieczorek i inni 2015).

W celu wskazania gatunków, które najsilniej wpłynęły na podobieństwo i niepodobieństwo zbiorowisk okrzemek pomiędzy poszczególnymi próbami w badanych ekosystemach, zastosowano transformację analizy MDS typu „Shade Plot”. Analiza wykazała 11 gatunków okrzemek, które najsilniej wpłynęły na podobieństwo między próbami w co najmniej dwóch badanych ekosystemach, były to: *Navicula veneta* Kützing, *Navicula cincta* (Ehrenberg) Ralfs, *Navicula gregaria* Donkin, *Nitzschia frustulum* (Kützing) Grunow, *Nitzschia inconspicua* Grunow, *Nitzschia palea* (Kützing) Smith, *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot, *Amphora pediculus* (Kützing) Grunow, *Cyclotella meneghiniana* Kützing, *Fragilaria radians* (Kützing) Williams & Round i *Achnanthidium minutissimum* (Kützing) Czarnecki (*s.l.*). W kompleksie hydrologicznym Pełczyska, gatunkami najsilniej wpływającymi na odrębność tego ekosystemu były: *Chamaepinnularia krookiformis* (Krammer) Lange-Bertalot & Krammer, *C. plinskii* Żelazna-Wieczorek & Olszyński, *Craticula buderi* (Hustedt) Lange-Bertalot, *C. halophila* (Grunow) Mann, *Gomphonema parvulum* (Kützing) Kützing, *Nitzschia liebethruthii* Rabenhorst, dla kompleksu zbiorników w Łęczycy były to: *Cyclostephanos dubius* (Hustedt) Round, *C. invisitatus* (Hohn i Hellermann) Theriot, Stoermer & Håkasson, *Stephanodiscus binatus* Håkansson & Kling, *S. parvus* Stoermer & Håkansson, *Discostella woltereckii* (Hustedt) Houk & Klee oraz dla zbiornika Bogdałów: *Mastogloia smithii* Thwaites ex Smith, *Pantocsekiella ocellata* (Pantocsek) Kiss & Ács, *P. pseudocomensis* (Scheffler) Kiss & Ács, *Encyonopsis subminuta* Krammer & Reichardt, *Nitzschia dissipata* var. *media* (Hantzsch) Grunow (**Załącznik 4:** Olszyński i inni 2019).

3. Zmienności międzygatunkowa i wewnątrzgatunkowa wybranych taksonów okrzemek

Specyficzne warunki środowiskowe panujące w obiektach hydrologicznych powstałych w wyniku działalności człowieka i wysoki udział w zbiorowiskach gatunków rzadko notowanych w innych typach ekosystemów, umożliwiły obserwację zmienności międzygatunkowej, jak i wewnątrzgatunkowej okrzemek.

Warunki środowiska wodnego wyróżniające się wysokim stężeniem jonów chlorkowych w kompleksie hydrologicznym Pełczyska i frekwencja *Chamaepinnularia krookiformis sensu lato* sięgająca 12% udziału w próbie, umożliwiły zbadanie jego zmienności wewnątrzgatunkowej. Analiza zmienności została przeprowadzona na podstawie ośmiu szczegółowo dobranych cechach morfologicznych tego taksonu z wykorzystaniem skalowania wielowymiarowego (MDS). Analiza wykazała podobieństwo i niepodobieństwo między poszczególnymi okazami grupując je w trzy odrębne zbiory, które następnie zostały określone jako morfotypy. Wytypowane cechy morfologiczne wyodrębnionych morfotypów zostały porównane z cechami obserwowanymi u okazów pochodzących z materiału typowego *Pinnularia krookiformis* (≡*Chamaepinnularia krookiformis*) Krammer 1992 z zasolonych mokradeł w Nordrhein–Westfalen (Salzgebiet südlich von Salzkotten, Nordrhein–Westfalen Germany, leg. Wygasch, 17.04.1982 – KR604B). Jeden z morfotypów wykazywał największą odmienność morfologiczną zarówno względem dwóch pozostałych morfotypów, jak i okazów pochodzących z materiału typowego. Na podstawie zmienności cech morfologicznych obserwowanych w mikroskopie świetlnym (LM) i skaningowego mikroskopu elektronowego (SEM), danych literaturowych, oraz szczegółowej analizy danych archiwalnych dotyczącej klasyfikacji taksonomicznej *C. krookiformis s.l.* opisano nowy dla nauki gatunek – *Chamaepinnularia plinskii* Żelazna-Wieczorek & Olszyński (**Załącznik 2:** Żelazna-Wieczorek i Olszyński 2016).

Kompleks hydrologiczny w Łęczycy, obejmujący trzy nieprzepływowe zbiorniki, położone w zabudowanej strefie miejskiej, charakteryzujące się zasadowym odczynem wody i warunkami podwyższonej żyzności i saprobowości, umożliwiły obserwację stałych i licznie w nich reprezentowanych okrzemek centrycznych, ich zmienność wewnątrzgatunkową i międzygatunkową (**Załącznik 3:** Olszyński i Żelazna-Wieczorek 2018).

Frekwencja do 7% udziału w próbie *Aulacoseira muzzanensis sensu lato* (Meister) Krammer umożliwiła szczegółową analizę zmienności cech morfologicznych z wykorzystaniem skaningowego mikroskopu elektronowego (SEM). Praca Krammera z 1991 roku, w której został opisany *A. muzzanensis,* wskazuje na istnienie dwóch morfotypów tego gatunku. Pierwszy to „status α", któremu odpowiada morfologicznie populacja *A. muzzanensis s.l.* ze zbiorników w Łęczycy oraz drugi „status τ", którego nie odnotowano w badanych zbiornikach. Przeprowadzona szczegółowa analiza morfologiczna materiału typowego *Melosira muzzanensis* (≡*A. muzzanensis*) Meister z jeziora we Włoszech (Lago di Muzzano, Italy E1289) wykazała obecność w nim tylko jednego morfotypu – „status τ". Na podstawie przeprowadzonej analizy morfologicznej oraz danych literaturowych opisano nowy dla nauki gatunek – *Aulacoseira pseudomuzzanensis*

Olszyński & Żelazna-Wieczorek. Ponadto ze względu na odmienną budowę morfologiczną okryw łączących i separacyjnych u okrzemek z rodzaju *Aulacoseira* Thwaites, zaproponowano aby kolejne nowo opisywane gatunki z tego rodzaju zawierały pełny opis cech morfologicznych obu typów okryw z wyraźnym podkreśleniem ich różnic (**Załącznik 3:** Olszyński i Żelazna-Wieczorek 2018).

W próbach z kompleksu hydrologicznego w Łęczycy odnotowano występowanie dwóch gatunków z rodzaju *Discostella* Houk & Klee: *D. woltereckii* (Hustedt) Houk & Klee oraz *D. pseudostelligera* (Hustedt) Houk & Klee. Szczegółowa analiza cech morfologicznych z wykorzystaniem skaningowego mikroskopu elektronowego (SEM) potwierdziła, iż zidentyfikowane taksony różnią się od siebie. Jednakże stwierdzenie występowania obu gatunków w jednym środowisku/zbiorniku falsyfikuje opublikowane dotychczas informacje wskazujące na ich odmienną autekologię. *D. woltereckii* został opisany z obszaru tropikalnego natomiast *D. pseudostelligera* ze strefy klimatu umiarkowanego. Weryfikacja występowania obu gatunków w zależności od środowiska została wykonana na podstawie szczegółowej analizy cech morfologicznych okazów z materiałów typowych obu gatunków *Cyclotella pseudostelligera* (≡*D. pseudostelligera*) Hustedt z Ems (Station 197, Profil L, nahe Neue Schleuse bei Papenburg E524) i *Cyclotella woltereckii* (≡*D. woltereckii*) Hustedt z Indonezji (Java, Butenzorg C. 98 Teich in Motanischen Garten, Wallacea-Expedition AS1329). Analiza cech morfologicznych okazów z materiałów typowych stanowiących podstawę do wyróżnienia omawianych gatunków wykazała, iż oba gatunki występują w każdym z materiałów typowych (**Załącznik 3:** Olszyński i Żelazna-Wieczorek 2018).

Cyclotella meneghiniana Kützing był notowany w próbach z kompleksu hydrologicznego w Łęczycy, w ponad 95% prób z maksymalnym udziałem w próbie – 10%. Umożliwiło to obserwację szerokiej zmienności budowy morfologicznej pancerzyków tego gatunku. Na podstawie obserwacji okazów *C. meneghiniana* w mikroskopie świetlnym (LM) odnotowano występowanie dwóch morfotypów różniących się budową morfologiczną okrywy. Pierwszy morfotyp charakteryzował się płaską okrywą, drugi natomiast pofalowaną. Szczegółowa analiza cech morfologicznych tego gatunku z wykorzystaniem skaningowego mikroskopu elektronowego (SEM) potwierdziła cechy morfologiczne zaobserwowane w mikroskopie świetlnym oraz wykazała obecność dodatkowych cech umożliwiając wyodrębnienie submorfotypów. Wysoka zmienność morfologiczna w obrębie jednego gatunku, obserwowana w jednym typie siedlisk potwierdza, że *C. meneghiniana* może być uważana jako gatunek kryptyczny (**Załącznik 3:** Olszyński i Żelazna-Wieczorek 2018).

4. Wyznaczenie gatunków charakterystycznych okrzemek oraz zbadanie zależności pomiędzy ich występowaniem a parametrami środowiska

Odrębność wytypowanych do badań obiektów hydrologicznych wynika zarówno z budowy geologicznej podłoża, jak i zależnych od niej warunków chemicznych i fizycznych wody, które zdeterminowały strukturę zbiorowisk okrzemek bentosowych w nich występujących (**Załącznik 4:** Olszyński i inni 2019). W celu wskazania gatunków charakterystycznych dla poszczególnych obiektów hydrologicznych różniących się warunkami środowiskowymi wykonano analizę SIMPER. Analiza ta polega na określeniu, która zmienna w danej próbie, w tym przypadku gatunek okrzemki, najsilniej wpływa na podobieństwo lub niepodobieństwo do każdej następnej próby. W wyniku przeprowadzonej analizy określono 38 taksonów charakterystycznych dla trzech badanych obiektów hydrologicznych, w tym dla kompleksu hydrologicznego Pełczyska – 20 gatunków: *Chamaepinnularia krookiformis* (Krammer) Lange-Bertalot & Krammer, *C. plinskii* Żelazna-Wieczorek & Olszyński, *Cocconeis placentula* Ehrenberg, *Craticula buderi* (Hustedt) Lange-Bertalot, *C. halophila* (Grunow) Mann, *Fragilaria famelica* (Kützing) Lange-Bertalot, *F. sopotensis* Witkowski & Lange-Bertalot, *Gomphonema parvulum* (Kützing) Kützing, *Hippodonta hungarica (Grunow)* Lange-Bertalot, Metzeltin & Witkowski, *Navicula cincta* (Ehrenberg) Ralfs, *N. veneta* Kützing, *Nitzschia frustulum* (Kützing) Grunow, *N. inconspicua* Grunow, *N. liebethruthii* Rabenhorst, *N. palea* (Kützing) Smith, *N. perminuta* Grunow, *N. tubicola* Grunow, *Planothidium delicatulum* (Kützing) Round & Bukhtiyarova, *P. frequentissimum* (Lange-Bertalot) Lange-Bertalot i *Tabularia fasciculata* (Agardh) Williams & Round; dla trzech zbiorników w Łęczycy – 11 gatunków: *Achnanthidium minutissimum* (Kützing) Czarnecki (*s.l.*), *Amphora pediculus* (Kützing) Grunow, *Cyclostephanos dubius* (Hustedt) Round, *C. invisitatus* (Hohn i Hellermann) Theriot, Stoermer & Håkasson, *Cyclotella meneghiniana* Kützing, *Navicula gregaria* Donkin, *N. moskalii* Metzeltin, Witkowski & Lange-Bertalot, *Nitzschia palea* (Kützing) Smith, *Stephanodiscus binatus* Håkansson & Kling, *S. hantzschii* Grunow i *S. parvus* Stoermer & Håkansson; oraz dla zbiornika Bogdałów – 7 taksonów: *Achnanthidium minutissimum* (Kützing) Czarnecki (*s.l.*), *Diatoma moniliformis* (Kützing) Williams, *Encyonopsis subminuta* Krammer & Reichardt, *Mastogloia smithii* Krammer & Reichardt, *Nitzschia dissipata* var. *media* (Hantzsch) Grunow, *Pantocsekiella ocellata* (Pantocsek) Kiss & Ács i *P. pseudocomensis* (Scheffler) Kiss & Ács. Ze względu na to, iż dwa gatunki: *Achnanthidium minutissimum* (*s.l.*) oraz *Nitzschia palea,* były charakterystyczne dla co najmniej dwóch ekosystemów zostały one wyłączone z analizy mającej na celu wskazanie parametrów środowiska wodnego determinujących ich występowanie. W rezultacie dalszej analizie poddano 36 taksonów charakterystycznych dla trzech badanych kompleksów hydrologicznych (**Załącznik 4:** Olszyński i inni 2019). Na podstawie analizy korelacji wybrano 15 z 19 parametrów chemicznych i fizycznych odzwierciedlających warunki środowiska. W celu wykazania zależności pomiędzy występowaniem gatunków charakterystycznych a parametrami środowiska wykonano analizę głównych składowych (PCA). Analiza wyróżniła 3 grupy gatunków charakterystycznych. Przynależność gatunków do pierwszej grupy (grupa A) zdeterminował odczyn wody oraz stężenie jonów HCO_3^-, Ca^{2+} i $Fe^{2+/3+}$. Druga grupa (grupa B) nie wykazała istotnej korelacji z badanymi parametrami środowiskowymi. Na obecność okrzemek wyróżnionych w trzeciej grupie (grupa C) miało wpływ stężenie jonów K^+, Mg^{2+}, Na^+, SO_4^{2-}, Cl^-, PO_4^{3-}, Mn^{3+}, przewodnictwo elektrolityczne oraz barwa wody (**Załącznik 4:** Olszyński i inni 2019).

5. Autekologia gatunków charakterystycznych okrzemek oraz określenie lub weryfikacja ekologicznych wartości wskaźnikowych

Dla 36 gatunków charakterystycznym okrzemek, wyznaczonych dla badanych obiektów hydrologicznych, zestawiono na podstawie informacji zawartych w bazie danych programu OMNIDIA, ekologiczne wartości wskaźnikowe w systemie klasyfikacji Van Dama i współautorów (1994). Następnie, do dalszej analizy wybrano 16 taksonów, które nie miały przypisanej co najmniej jednej ekologicznej wartości wskaźnikowej w bazie programu OMNIDIA (**Załącznik 4**, 4.4: Olszyński i inni 2019).

W celu weryfikacji ekologicznych wartości wskaźnikowych dla poszczególnych wybranych taksonów charakterystycznych zostało przeprowadzone wielowątkowe wnioskowanie. Obejmowało ono wykazanie zależności pomiędzy występowaniem danego gatunku a analizowanymi parametrami środowiska, następnie analizę bibliografii w odniesieniu do danych dotyczących warunków środowiskowych, w których występował dany takson. Kolejnym wątkiem było określenie warunków środowiskowych charakteryzujących badane ekosystemy wodne na podstawie gatunków, które mają już przypisane ekologiczne wartości wskaźnikowe według klasyfikacji

Van Dama i współautorów (1994) zamieszczone w bazie danych programu OMNIDIA (**Załącznik 4**, 4.2: Olszyński i inni 2019).

Zgodność wnioskowania na podstawie wszystkich trzech przesłanek wynikających z analizy warunków środowiskowych panujących w badanym ekosystemie, analizy ekologii gatunków w oparciu o piśmiennictwo oraz określenie warunków środowiskowych na podstawie znanej już autekologii gatunków współwystępujących, dała możliwość ustanowienia lub zaproponowania zmiany dla 16 taksonów okrzemek charakterystycznych 36 ekologicznych wartości wskaźnikowych w klasyfikacji Van Dama i współautorów (1994) (**Załącznik 4:** Olszyński i inni 2019). W tabeli 1 zestawiono zaproponowane zmiany i propozycje ustanowienia nowych ekologicznych wartości wskaźnikowych.

Tabela 1. Propozycja zmian lub ustanowionych nowych ekologicznych wartości wskaźnikowych według klasyfikacji Van Dama i współautorzy (1994). Zielonym kolorem wyróżnione zostały zaproponowane zmiany. Opis skali znajduje się w załączniku 4.3.

Takson	Warunki wilgotnościowe	Metabolizm azotu	Odczyn wody	Steżenie tlenu	Zasolenie	Saprobia	Trofia
Chamaepinnularia krookiformis	~~3~~ 4	0	3	0	~~3~~ 4	~~1~~ 4	~~0~~ 4
Chamaepinnularia plinskii	~~0~~ 4	0	~~0~~ 3	0	~~0~~ 4	~~0~~ 4	~~0~~ 4
Craticula buderi	~~0~~ 3	0	~~0~~ 4	0	~~0~~ 4	~~0~~ 3	~~0~~ 4
Cyclostephanos invisitatus	~~0~~ 1	0	~~0~~ 4	0	2	0	5
Diatoma moniliformis	0	0	0	0	0	0	0
Encyonopsis subminuta	0	0	3	1	1	1	1
Mastogloia smithii	3	0	4	0	4	2	0
Navicula cincta	~~0~~ 3	0	0	0	2	0	7
Navicula moskalii	0	0	~~0~~ 4	0	~~0~~ 2	0	~~0~~ 5
Nitzschia dissipata var. *media*	0	0	4	0	2	~~0~~ 2	0
Nitzschia liebethruthii	~~0~~ 4	0	~~5~~ 3	0	4	~~0~~ 4	~~0~~ 4
Pantocsekiella pseudocomensis	0	0	0	0	0	0	0
Planothidium delicatulum	3	1	~~5~~ 3	0	~~4~~ 5	5	3
Planothidium frequentissimum	0	2	4	3	2	4	7
Stephanodiscus binatus	0	0	~~0~~ 4	0	~~0~~ 2	0	0
Stephanodiscus parvus	0	0	~~5~~ 4	0	2	0	6

Wysokie stężenie jonów chlorkowych w śródlądowym kompleksie hydrologicznym Pełczyska, wykraczające poza skale klasyfikacji przyjętej przez Van Dama i współautorów (1994), spowodowało konieczność zastosowania 7-stopniowej skali halobów Van der Werffa i Hula (1957–1974) obejmującą również gatunki występujące w wodach o stężeniu jonów chlorkowych odpowiadającym środowisku brakicznemu i morskiemu. Zastosowanie 7-stopniowej skali było spowodowane faktem, iż w klasyfikacji Van Dama i współautorów (1994) wykorzystanej w programie OMNIDIA 5.3 (wersja aktualna w 2015) dla wód śródlądowych nie występowały kategorię wyższe niż 4. Na tej podstawie zaproponowano rozszerzenie klasyfikacji halobów wykorzystywanej w programie OMNIDIA o dodatkowe klasy według klasyfikacji Van der Werffa i Hula (1957–1974) oraz zaproponowano ustanowienie w niej, ekologicznych wartości wskaźnikowych dla wybranych gatunków: *Halamphora tenerrima* (Aleem & Hustedt) Levkov – 7 klasa (morskie), *Parlibellus crucicula* (Smith) Witkowski,Lange-Bertalot & Metzeltin, *Staurophora salina* (Smith) Mereschkowsky, *Halamphora dominici* Ács & Levkov i *H. subsalina* Levkov – 6 klasa (morsko-brakiczne), *Achnanthes brevipes* Agardh, *Navicula perminuta* Grunow i *Opephora mutabilis* Sabbe & Wyverman – 5 klasa (brakiczno-morskie) (**Załącznik 1:** Żelazna-Wieczorek i inni 2015).

VII. Wnioski i weryfikacja hipotezy badawczej

Zbiorniki wodne, które powstały w wyniku przekształceń antropogenicznych związanych z eksploatację kopalin surowców mineralnych, charakteryzują się warunkami środowiskowymi, na które wpływ ma specyficzna budowa geologiczna danego zbiornika.

Warunki środowiskowe determinują strukturę jakościową i ilościową zbiorowisk okrzemek oraz występowanie gatunków charakterystycznych dla danego ekosystemu.

Daje to możliwości obserwacji międzygatunkowej oraz wewnątrzgatunkowej zmienności cech morfologicznych, prowadząc do wzbogacenia wiedzy na temat taksonomii oraz budowy morfologicznej tej grupy mikroorganizmów.

Specyficzne warunki środowiskowe panujące w kompleksie hydrologicznym Pełczyska zdeterminowały zróżnicowanie taksonomiczne zbiorowisk okrzemek, pozwalając na obserwację zmienności budowy morfologicznej *Chamaepinnularia krookiformis sensu lato*. Na podstawie specyficznych cech morfologicznych tego gatunku oraz różnic w budowie morfologicznej okazów z materiału typowego *Pinnularia krookiformis* (≡*Chamaepinnularia krookiformis*) obserwowanych w mikroskopie świetlnym (LM) oraz skaningowym mikroskopie elektronowym (SEM), **został opisany nowy dla nauki gatunek okrzemki *Chamaepinnularia plinskii*.**

Kompleks hydrologiczny w Łęczycy charakteryzował się podwyższonym odczynem wody, warunkami podwyższonej żyzności i saprobowości. Tak specyficzne warunku środowiskowe zdeterminowały skład gatunkowy okrzemek, w którym licznie występował *Aulacoseira muzzanensis sensu lato*. Na podstawie szczegółowo przeprowadzonej analizy budowy morfologicznej *A. muzzanensis s.l.* w badanych zbiornikach oraz okazów z materiału typowego *Melosira muzzanensis* (≡*A. muzzanensis*), z wykorzystaniem technik mikroskopii świetlnej oraz skaningowej mikroskopii elektronowej, stwierdzono odmienność budowy morfologicznej okazów tego taksonu występującego w kompleksie hydrologicznym w Łęczycy a okazami z materiału typowego. **Pozwoliło to wyodrębnić nowy dla nauki gatunek okrzemki *Aulacoseira pseudomuzzanensis*.**

Wewnątrzgatunkowa zmienność morfologiczna była również zaobserwowano w populacji *Cyclotella meneghiniana* występującego w kompleksie hydrologicznym w Łęczycy. Na podstawie przeprowadzonej analizy budowy morfologicznej tego taksonu, z wykorzystaniem technik mikroskopii świetlnej oraz skaningowej mikroskopii elektronowej stwierdzono występowanie dwóch morfotypów. **Występowanie w jednej populacji, w tych samych warunkach środowiskowych, odmiennych morfologicznie form może wskazywać na to, iż *Cyclotella meneghiniana* jest gatunkiem kryptycznym.**

W kompleksie hydrologicznym w Łęczycy warunki środowiskowe pozwoliły na obserwację dużej zmienności taksonomicznej w szczególności wśród okrzemek centrycznych. **Pozwoliło to na weryfikację międzygatunkowej zmienności morfologicznej pomiędzy dwoma gatunkami z rodzaju *Discostella: D. wolterecki* i *D. pseudostelligera*.** Szczegółowa analiza morfologiczna z wykorzystaniem skaningowej mikroskopii elektronowej potwierdziła odrębność budowy morfologicznej tych gatunków. **Ponadto wykazano, że warunki środowiskowe nie stanowią kryterium w ich rozróżnieniu, co zostało również wykazane na podstawie analizy materiału typowego obu gatunków.**

Warunki środowiskowe panujące w badanych zbiornikach pokopalnianych umożliwiają weryfikacje lub ustalenie nowych zakresów tolerancji gatunków okrzemek na wybrane parametry środowiskowe, określenie gatunków charakterystycznych oraz weryfikację istniejących i ustalenie nowych ekologicznych wartości wskaźnikowych. Specyficzne warunki środowiskowe panujące w ekosystemach wodnych pochodzenia antropogenicznego, jakimi są zbiorniki powstałe po górniczej działalności człowieka, prowadzą do tworzenia się specyficznych dla nich zbiorowisk okrzemek. Badając strukturę tych zbiorowisk w odniesieniu do czynników fizycznych i chemicznych panujących w określonym ekosystemie, możliwe jest wskazanie gatunków dla nich charakterystycznych. Pozwala to na weryfikację zakresu tolerancji tych gatunków na warunki środowiskowe i ustanowienie ekologicznych wartości wskaźnikowych. **Unikalne warunki środowiskowe panujące w kompleksie hydrologicznym Pełczyska, kompleksie zbiorników w Łęczycy oraz w zbiorniku Bogdałów, umożliwiły dla 16 gatunków charakterystycznych zaproponowanie ustanowienia nowych lub zmianę obecnych ekologicznych wartości wskaźnikowych.**

Badania przeprowadzone w toku niniejszej rozprawie doktorskiej umożliwiły weryfikację hipotezy badawczej. **Wyniki badań oraz wnioski z nich płynące potwierdzają, że postawiona hipoteza badawcza jest prawdziwa.**

VIII. Załączniki

Załącznik 1: Żelazna-Wieczorek J., Olszyński R.M. i Nowicka-Krawczyk P. 2015. Half a century of research on diatoms in athalassic habitats in central Poland. *Oceanological and Hydrobiological Studies* 44(1): 51–67.

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Oświadczenie o współautorstwie

Oświadczam, że mój wkład w przygotowanie oryginalniej pracy polegał na: przygotowaniu koncepcji pracy, zaplanowaniu prac w terenie, identyfikacji taksonomicznej części materiału diatomologicznego, opracowaniu wyników z wykorzystaniem metod matematycznych. Udział w dyskusji i interpretacji uzyskanych wyników i w przygotowaniu ostatecznej wersji manuskryptu. Swój udział w przygotowanie pracy oceniam na 60%.

dr hab. Joanna Żelazna-Wieczorek prof. UŁ
Katedra Algologii i Mykologii

Oświadczam, że mój wkład w przygotowanie oryginalniej pracy polegał na: zaplanowaniu i realizacji prac w terenie, identyfikacji taksonomicznej części materiału diatomologicznego, przygotowanie dokumentacji fotograficznej dokumentującej zróżnicowanie taksonomiczne okrzemek. Udział w analizie i interpretacji uzyskanych wyników. Pracowałem również na ostateczną wersją manuskryptu. Swój udział w przygotowanie pracy oceniam na 30%.

mgr Rafał M. Olszyński
Katedra Algologii i Mykologii

Oświadczam, że mój wkład w przygotowanie oryginalnej pracy polegał na: przygotowaniu i opracowaniu wyników analiz warunków środowiskowych oraz udział w dyskusji i interpretacji uzyskanych wyników. Swój udział w przygotowanie pracy oceniam na 10%

dr Paulina Nowicka-Krawczyk
Katedra Algologii i Mykologii

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Half a century of research on diatoms in athalassic habitats in central Poland

by

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Abstract

Part of the geology in the Łódź province was formed during the Upper Permian period when rich Zechstein salt was deposited. Groundwater drains the deposits and flows out in the village of Pełczyska, creating a unique hydrogeological site in Central Poland. An inland, athalassic ecosystem can be a reference site for halophile microflora. The outflow with surrounding marshes has been an algological research site since 1964.

The research reveals changes recorded in diatom assemblages from athalassic habitats, characterized by a wide range of salinity levels, and verifies the tolerance of taxa to salinity. The comparative analysis was based on the diatom material sampled in 1964-1965, 1992-1994 and on recently collected samples.

The analysis revealed the temporal change in assemblages caused by a change in the chloride concentration, and the spatial change from one to another habitat type, characterized by varying salinity levels. The halophilic species in the studied habitats included e.g. *Halamphora dominici*, *H. tenerrima*, *Navicula digitoconvergens*, *N. meulemansii*, *Staurophora salina*. The analysis of changes allowed the verification of the species' requirements and tolerance range to the salinity factor. Therefore, in the case of *Fragilaria famelica* and *Halamphora sydowii*, we propose a change in the halobion system classification.

Key words: Bacillariophyta, halobion system, salinity, temporal and spatial changes

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Introduction

The salt marshes near Łęczyca (Central Poland) are, on a global scale, unique natural brackish water habitats, where diatoms, typical of saline waters, are present. Inland habitats characterized by a high concentration of chloride ions have hardly been investigated. There are only a few locations in the world where diatom assemblages occur in inland salt waters. Saltwater inland ecosystems (athalassic ecosystems) are vulnerable to climate change, therefore the presence of halophilic diatom taxa at these sites may be exposed to dynamic changes (Veres et al. 1995). The hot springs in Kenya and New Zealand are an example of habitats also characterized by an increased concentration of chloride ions, however, a very high temperature was the factor that had the greatest influence on the dynamics of diatom assemblages in these habitats (Owen et al. 2008). Saltwater ecosystems, such as lakes and springs, located in coastal zones are significantly different from those located inland, because the water chemistry at these sites is strongly associated with the sea or ocean (Starmach 1969; Aboal et al. 1998; Novelo et al. 2007; Lutyńska 2008; Antón-Garrido et al. 2013).

The first study of macro- and microflora at the salt marshes near Łęczyca was performed by Olaczek, and the results of these observations were presented in Olaczek (unpublished PhD thesis, 1963). However, the data on the algal communities, and in particular the diatoms, were very scanty. A few years later, Pliński presented (unpublished MS thesis, 1966) an analysis of algological material from a spring and a pond located in the Pełczyska village. Part of the analysis concerning the diatom assemblages was published (Pliński 1969).

The algological material collected by Pliński, deposited at the University of Łódź, Department of Algology and Mycology, and the data from Pliński (1969) were used for the verification and comparative analysis of the recently collected material in 2013 and 2014.

In the 1970s, a few papers concerning algal communities from the salt marshes near Łęczyca were published (Pliński 1971a, Pliński 1971b, Pliński 1971c; Pliński 1973). Twenty years later, the outflow of salt waters in the village of Pełczyska became a study site of Żelazna-Wieczorek who presented the results of algological analysis in Żelazna-Wieczorek (unpublished PhD thesis, 1996). Żelazna-Wieczorek focused on the *Vaucheria* De Candolle (2002) genus, however, diatoms simultaneously collected with the *Vaucheria* samples have been recently verified according to modern taxonomy. The studies of diatoms from the outflow and the pond in Pełczyska were also performed by Rakowska (1997) in 1972 and 1995. The results of Rakowska (1997) provide comparative material for the observation of changes in diatom assemblages occurring over 50 years in salt water habitats of Central Poland.

Long-term research on the diatom microflora from the athalassic habitats allowed the determination of qualitative and quantitative changes in diatom assemblages, on both temporal and spatial scales. The changes were associated with various levels of salinity, as well as anthropogenic transformations.

This paper presents the long-term changes which have been recorded in diatom assemblages from the athalassic habitats characterized by various levels of salinity, and verifies the tolerance of particular diatom taxa to salinity.

Study area

The study area is located in Pełczyska, a village next to the town of Łęczyca in the Łódź province (51°58'35.68"N, 19°14'17.02"E) (Fig. 1). This area is located on the border of two large geological structures: the Kujawy anticlinorium and the Łódz synclinorium. The Kłodawsko-Łęczycka anticline – part of the Kujawy anticlinorium, extends from the village of Solec Wielki, through the towns of Łęczyca and Błonie, towards the town of Kłodawa.

The Zechstein salt occurs in the core of the Kłodawsko-Łęczycka anticline. The structure of the anticline is asymmetric. Its eastern part has a gentle decline, while the western part is steep and

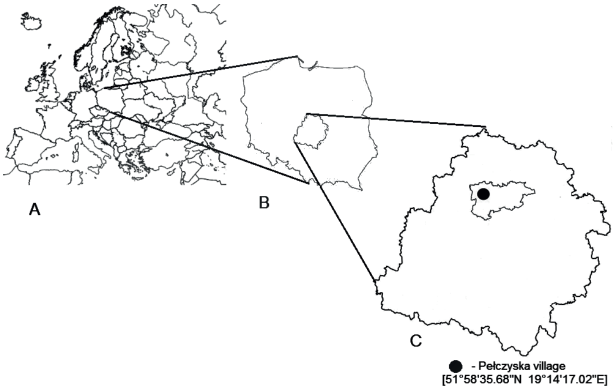

Fig. 1

Location of Pełczyska. A. Location of Poland. B. Location of Łódź province. C. Location of Pełczyska intersected by faults. The Zechstein salt near Łęczyca is deposited at a depth of 1500 m, however, due to its location at the crossing of the Pełczyska faults, the water may drain the deposits and flow out in the Pełczyska area (Olaczek, unpublished PhD thesis, 1963).

The genesis of the salt water outflows and the salt water marshes, which were the study area of many algological research projects, dates back to the late 18th century. During this period, many boreholes were made in search of brine, and consequently a spontaneous salt water outflow in Pełczyska can be observed to this day. Next to the outflow, a pond was made, which accumulated the water discharged from the drilling (Olaczek, unpublished PhD thesis, 1963). The morphology of the outflow in Pełczyska evolved over the decades. Since the 1960s, when the first study of the microflora of this habitat was carried out, the outflow was surrounded by a concrete slabs (Fig. 2). In the 1990s, the concrete gradually degraded (Fig. 3) until the first decade of the 21st century when the outflow was backfilled with a material of unknown composition (Fig. 4) (Pliński, unpublished MS thesis, 1966; Żelazna-Wieczorek, unpublished PhD thesis, 1996).

The study area included three sampling sites. The first site was located at the backfilled outflow, the second one at the ditch through which the water flows from the outflow to the third sampling site, i.e. the pond (Fig. 5).

Data on physical and chemical water analysis of the study area were obtained from Olaczek (unpublished PhD thesis, 1963), and Żelazna-Wieczorek (unpublished PhD thesis, 1996). In recent studies (2013/2014), the physical and chemical conditions of the water were analyzed at all sampling sites. All data are compiled in Table 1.

Fig. 2

Sampling performed by Marcin Pliński in the outflow in 1964/1965

Fig. 3

Degraded concrete slabs surrounding the outflow in 1992/1994

Fig. 4

Backfilled outflow in 2013/2014

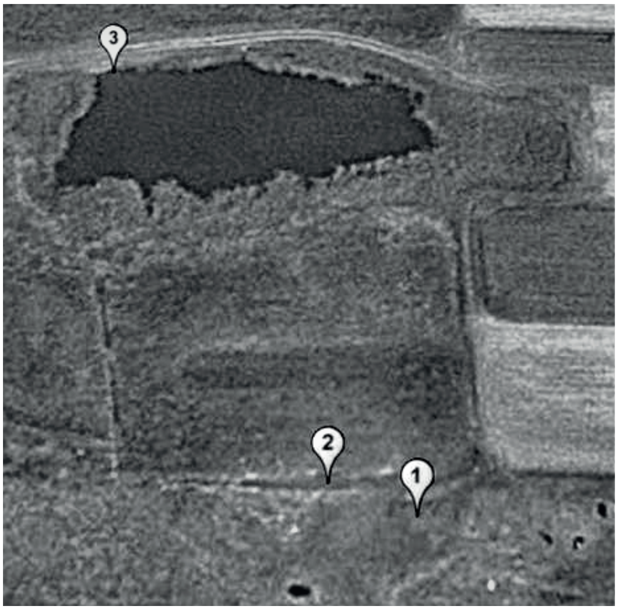

Fig. 5

Location of the sampling sites: 1 – the outflow; 2 – the ditch; 3 – the pond (source: maps.google.com)

Table 1

Physical and chemical parameters of water at the studied sites analyzed in: 1962 (Olaczek, unpublished PhD thesis, 1963), 1994 (Żelazna-Wieczorek 2002) and 2014

Unit		1962	1994		2014		
		X - OUTFLOW	IV - OUTFLOW	X - OUTFLOW	III - OUTFLOW	III - DITCH	III - POND
T	(°C)	7.00	7.00	8.00	6.70	7.80	8.20
pH		7.40	7.20	7.22	7.99	6.40	7.82
Cond	(µS)	n/d	7764.00	7681.00	4450.00	5170.00	2930.00
T. Hard	(mval l^{-1})	37.60	10.84	11.85	n/d	n/d	n/d
Cl^-	(mg l^{-1})	2560.00	2910.86	3116.19	1585.00	1006.00	685.00
SO_4^{2-}		225.00	154.44	214.44	187.60	165.00	166.20
NH_4^+		2.00	2.38	2.20	2.09	0.19	0.46
NO_2^-		n/d	0.02	0.01	n/d	n/d	n/d
NO_3^-		n/d	0.51	1.50	n/d	n/d	n/d
PO_4^{3-}		n/d	0.18	0.05	12.46	8.57	9.10
Na^+		n/d	1528.00	1413.00	500.70	453.30	277.30
K^+		n/d	19.65	22.60	n/d	n/d	n/d
Ca^{2+}		480.00	132.10	144.80	171.30	165.00	75.80
Mg^{2+}		195.00	51.68	56.16	43.8	35.90	25.6

Materials and methods

A total of 15 samples collected by Pliński in 1964/1965, 3 samples collected by Żelazna-Wieczorek in 1992/1994, and 12 samples collected in 2013/2014 were used in the diatomological analysis (Table 2). Diatoms from 1964/1965 were sampled from the outflow (4 samples), the area surrounding the outflow (6 samples), the ditch (2 samples) and the pond (3 samples). The material from 1992/1994 was sampled only from the outflow, while the recent samples, i.e. from 2013/2014, were collected from the outflow, the ditch and the pond (4 samples were collected from each site).

Benthic samples were collected by a pipette from the surface layer of the sediment. Samples were transferred into 125 ml containers and transported to a laboratory. Every sample was preserved with 4% formaldehyde solution. To obtain pure diatom frustules, the collected material was exposed to chemical purification according to Żelazna-Wieczorek (2011). The purified diatom suspension was pipetted onto cover slips and left overnight at room temperature. Permanent microscopic slides were mounted in Naphrax® synthetic resin (refractive index 1.73). All samples and slides are deposited in the collection of the Department of Algology and Mycology, the University of Łódź.

Taxonomical analysis of the benthic diatoms was made by examining permanent slides under a light microscope (Nikon YS 100 and Nikon Eclipse E400) with 1000× magnification (planachromatic oil-immersion objective 100×/1.25). Five hundred valves were identified and counted in consecutive visible areas over half the width of the 20 mm × 20 mm cover glass. In addition, the slides were re-examined and taxa previously unidentified were added to the list. The percentage of taxa in each sample was calculated. Dominant taxa, with relative abundance above 10%, were determined.

The tolerance of particular taxa to salinity was determined according to the data obtained from OMNIDIA 5.3 computer software, and the following references: Krammer, Lange-Bertalot 1986; Krammer, Lange-Bertalot 1991a; Krammer, Lange-Bertalot 1991b; Krammer, Lange-Bertalot 1997; Krammer 1997a; Krammer 1997b; Krammer 2000; Witkowski et al. 2000; Lange-Bertalot 2001; Krammer 2003; Lange-Bertalot et al. 2003; Levkov 2009, Hofmann et al. 2011. Diatom taxa tolerance to salinity was classified on the basis of the seven-grade halobion system, introduced by Van der Werff and Hulls 1957-1974 (Denys et al. 1983). This system was later used by Van Dam et al. 1994 to establish a reduced, four-grade system. However, the reduced halobion system does not include brackish-marine, marine-brackish and marine species (Denys et al. 1983, Van Dam et al. 1994) (Table 3).

Mathematical methods were used to compare the diatom assemblages, and to analyze both the diatom diversity and the relationship between diatom taxa distribution. The Bray-Curtis similarity index was used to determine the similarity between samples from different study periods and sampling sites (Żelazna-Wieczorek 2011). Hierarchical Cluster analysis based on the Bray-Curtis similarity was

Table 2

The total number of samples and the number of samples for each study period and study site; in addition, data from references are provided

	Samples	Taxa	Taxa outflow	Taxa around outflow	Taxa ditch	Taxa pond
Total	30	179	132	87	94	103
1964/1965	15	129	47	87	65	68
1992/1994	3	79	79	n/d	n/d	n/d
2013/2014	12	141	125	n/d	64	75
Pliński 1969	76	62	39	37	20	27
Rakowska 1997	n/d	63	40	n/d	n/d	59

Table 3

The halobion system according to Van Dam et al. 1994 and Van der Werff and Huls 1957-1974 (Denys et al. 1983) and corresponding ranges of chloride ions and salinity

Van Dam et al. 1994		Van der Werff and Huls 1957-1974		Cl$^-$ (mg l^{-1})	Salinity (mg l^{-1})
1	fresh	1	fresh	< 100	<180
2	fresh brackish	2	fresh-brackish	100-500	180-900
3	brackish fresh	3	brackish-fresh	500-1000	900-1800
4	brackish	4	brackish	1000-5000	1800-9000
		5	brackish-marine	5000-10000	9000-18000
		6	marine brackish	10000-17000	18000-30000
		7	marine	>17000	>30000

used to determine whether the groupings are more dependent on the temporal relationships between diatom taxa in particular periods of time (Żelazna-Wieczorek 2011). To analyze the spatial and temporal relationships between diatom taxa in particular habitats, the multidimensional scaling procedure (Multi-Dimensional-Scaling – MDS) based on the Bray-Curtis similarity (Żelazna-Wieczorek 2011) was used. The PRIMER 6.1.10 software was used for the calculations.

Results

The qualitative analysis of 30 samples revealed the presence of 179 diatom taxa from 49 genera. The most numerous genera were *Navicula* (30 taxa) and *Nitzschia* (26 taxa). Species characteristic of saline waters were identified within *Navicula*: *Navicula cincta* (Ehrenberg) Ralfs (Fig. 6 D1-D5), *N. digitoconvergens* Lange-Bertalot (Fig. 6 C1-C5), *N. meulemansii* Mertens, Witkowski & Lange-Bertalot (Fig. 6 E1-E5), *N. peregrina* (Ehrenberg) Kützing, *N. rhynchotella* Lange-Bertalot (Fig. 6 G1-G2), *N. salinarum* Grunow (Fig. 6 H1-H3), *N. salinicola* Hustedt (Fig. 6 B1-B4). *Nitzschia* was represented by: *Nitzschia commutata* Grunow, *N. constricta* (Kützing) Ralfs, *N. liebetruthii* Rabenhorst, *N. nana* Grunow (Fig. 7 G), *N. vitrea* Norman, *N. vitrea* var. *salinarum* Grunow (Fig. 7 F). *Halamphora* was also taxonomically rich and represented by 10 taxa, including: *Halamphora acutiuscula* (Kützing) Levkov (Fig. 8 B1-B3), *H. borealis* (Kützing) Levkov (Fig. 8 F1-F7), *H. dominici* Ács & Levkov (Fig. 8 E1-E5), *H. paraveneta* (Lange-Bertalot, Cvacini, Tagliaventi & Alfinito) Levkov (Fig. 8 C1-C5), *H. sydowii* (Cholnoky) Levkov (Fig. 8 A1-A7), *H. tenerrima* (Aleem & Hustedt) Levkov (Fig. 8 G1-G6), *H. veneta* (Kützing) Levkov (Fig. 8 D1-D4).

Of the nine *Fragilaria* taxa, *Fragilaria famelica* (Kützing) Lange-Bertalot (Fig. 9 B1-B9), and *F. sopotensis* Witkowski & Lange-Bertalot were most abundant (Fig. 9 A1-A7). The *Gomphonema* was represented by 8 taxa, including *Gomphonema italicum* Kützing and *G. utae* Lange-Bertalot & Reichardt. *Surirella* was represented by *Surirella ovalis* Brébisson (Fig. 7 M), *S. striatula* Turpin, and *S. venusta* Østrup (Fig. 7 I1-I2). *Planothidium* was represented by 5 species, including e.g. *Planothidium delicatulum* (Kützing) Round & Bukhtiyarova (Fig. 9 G1-G4), *P. engelbrechtii* (Cholnoky) Round & Bukhtiyarova and *P. pericavum* (Carter) Lange-Bertalot (Fig. 9 F1-F6). Five *Rhopalodia* taxa were present i.a. *Rhopalodia constricta* (W. Smith) Krammer in Lange-Bertalot & Krammer and *Rhopalodia gibberula* (Ehrenberg) Müller (Fig. 7

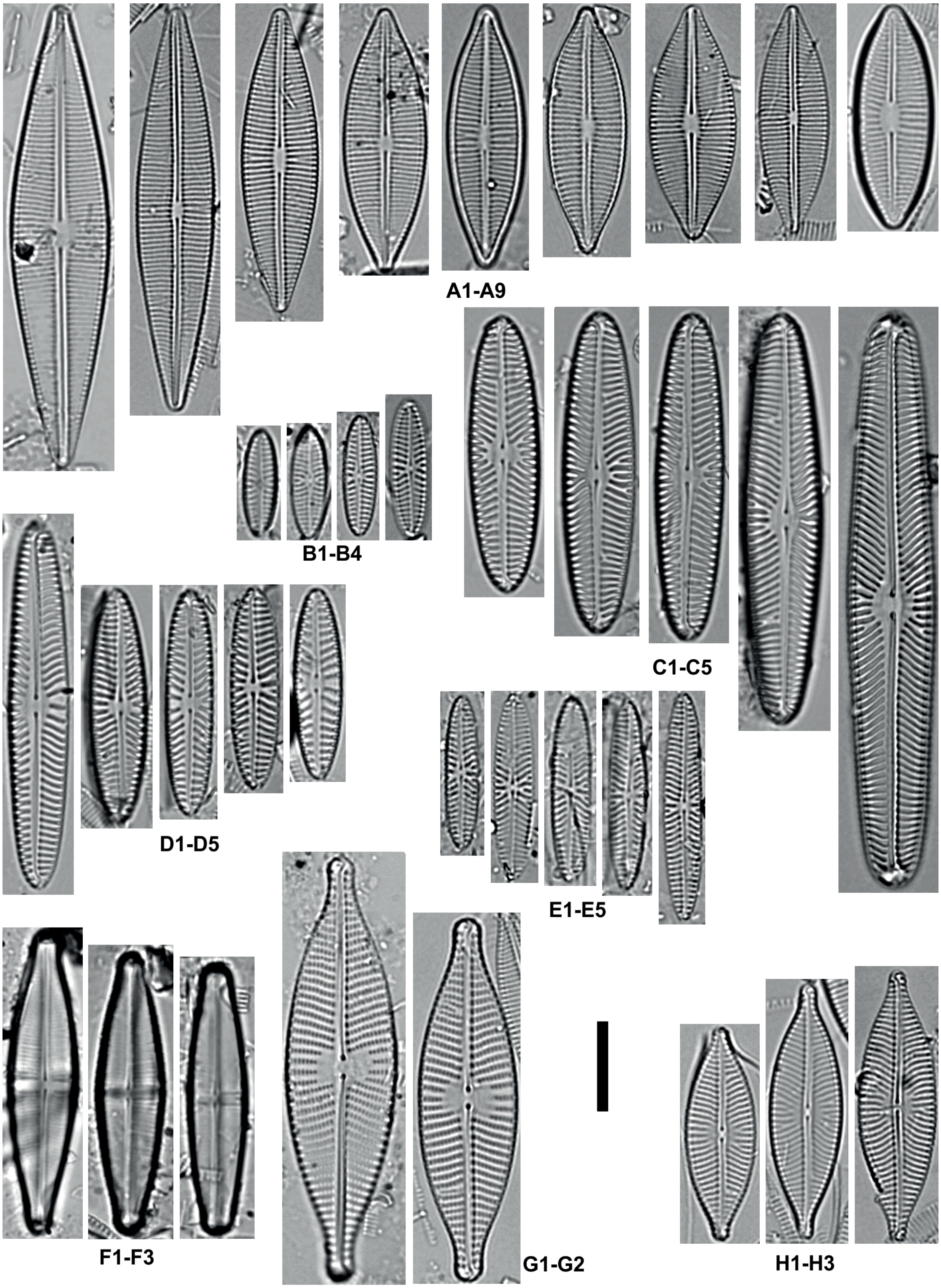

Fig. 6

A1-A9. *Craticula halophila*, B1-B4. *Navicula salinicola*, C1- C5. *Navicula digitoconvergens*, D1-D5. *Navicula cincta*, E1-E5. *Navicula meulemansii*, F1-F3. *Staurophora salina*, G1-G2. *Navicula rhynchotella*, H1-H3. *Navicula salinarum*. Scale bar = 10 μm

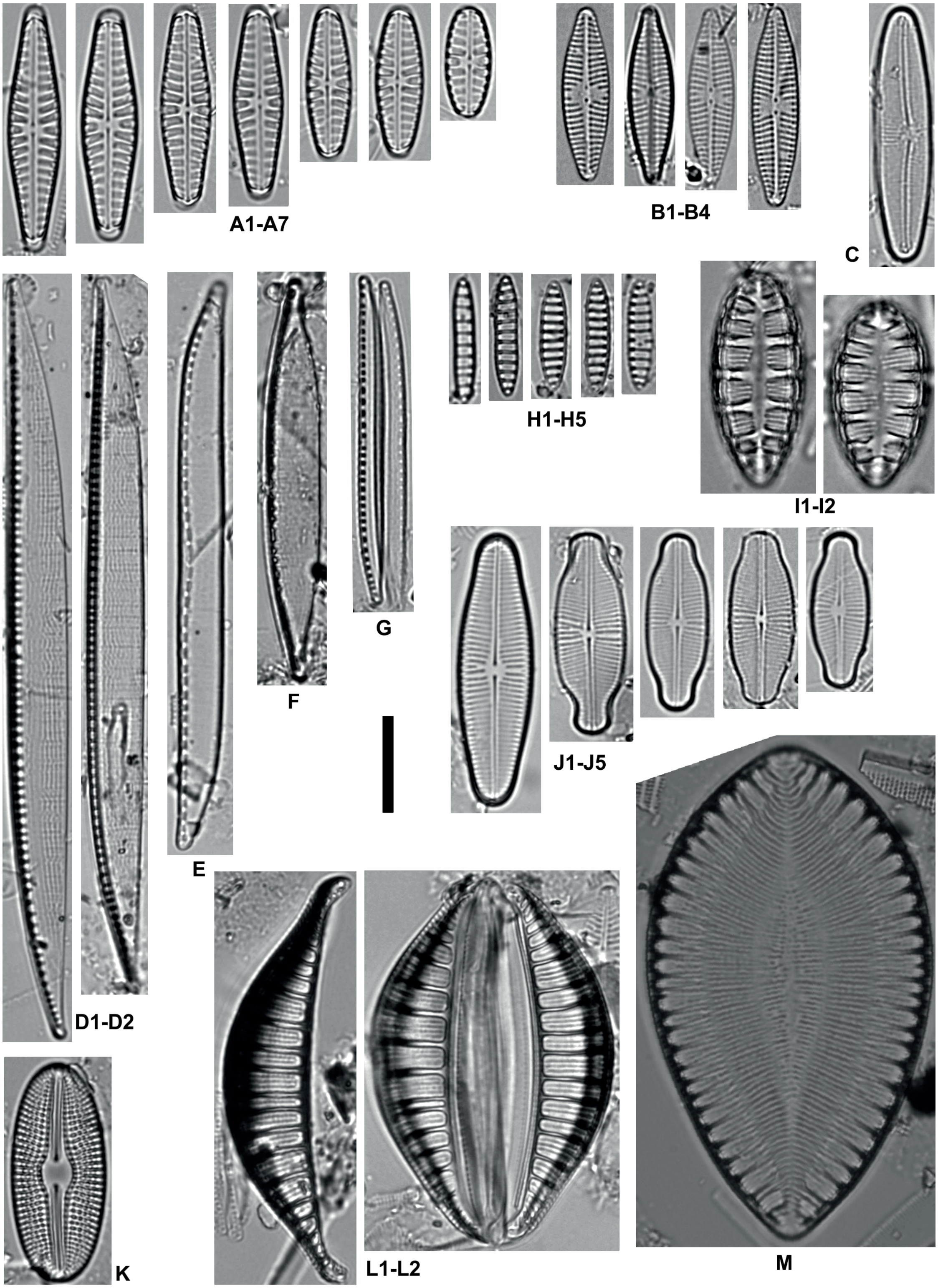

Fig. 7

A1-A7. *Hippodonta hungarica*, B1-B4. *Navicula veneta*, C. *Frustulia creuzburgensis*, D1-D2. *Nitzschia sigma*, E. *Nitzschia scalpelliformis*, F. *Nitzschia vitrea* var. *salinarum*, G. *Nitzschia nana*, H1-H5. *Denticula subtilis*, I1-I2. *Surirella venusta*, J1-J5. *Parlibellus protracta*, K. *Diploneis* cf. *krammeri*, L1-L2. *Rhopalodia gibberula*, M. *Surirella ovalis.* Scale bar = 10 µm

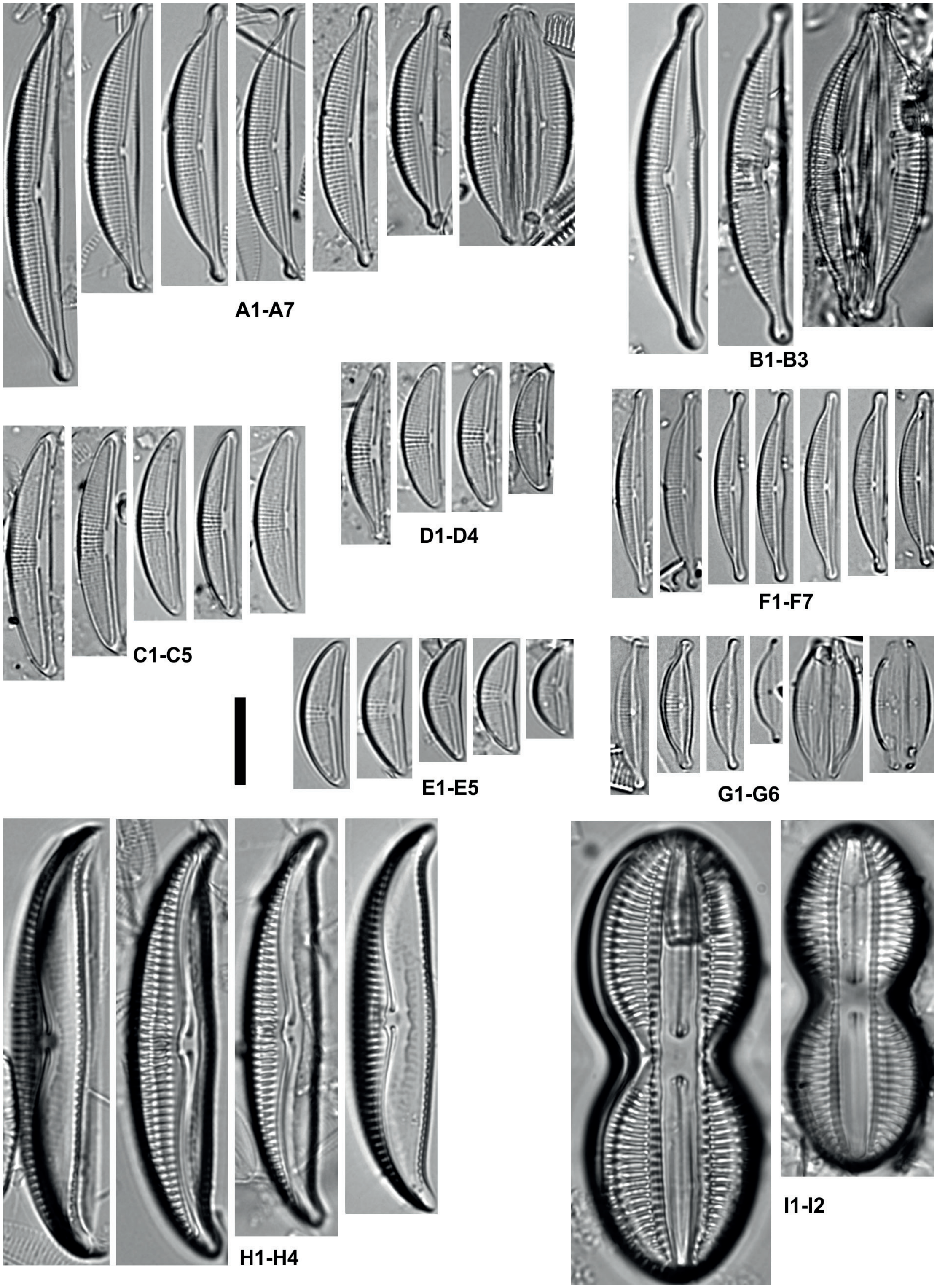

Fig. 8

A1-A7. *Halamphora sydowii*, B1-B3. *Halamphora acutiuscula*, C1-C5. *Halamphora paraveneta*, D1-D4. *Halamphora veneta*, E1-E5. *Halamphora dominici*, F1-F7. *Halamphora borealis*, G1-G6. *Halamphora tenerrima*, H1-H4. *Amphora commutata*, I1-I2. *Diploneis interrupta*. Scale bar = 10 µm

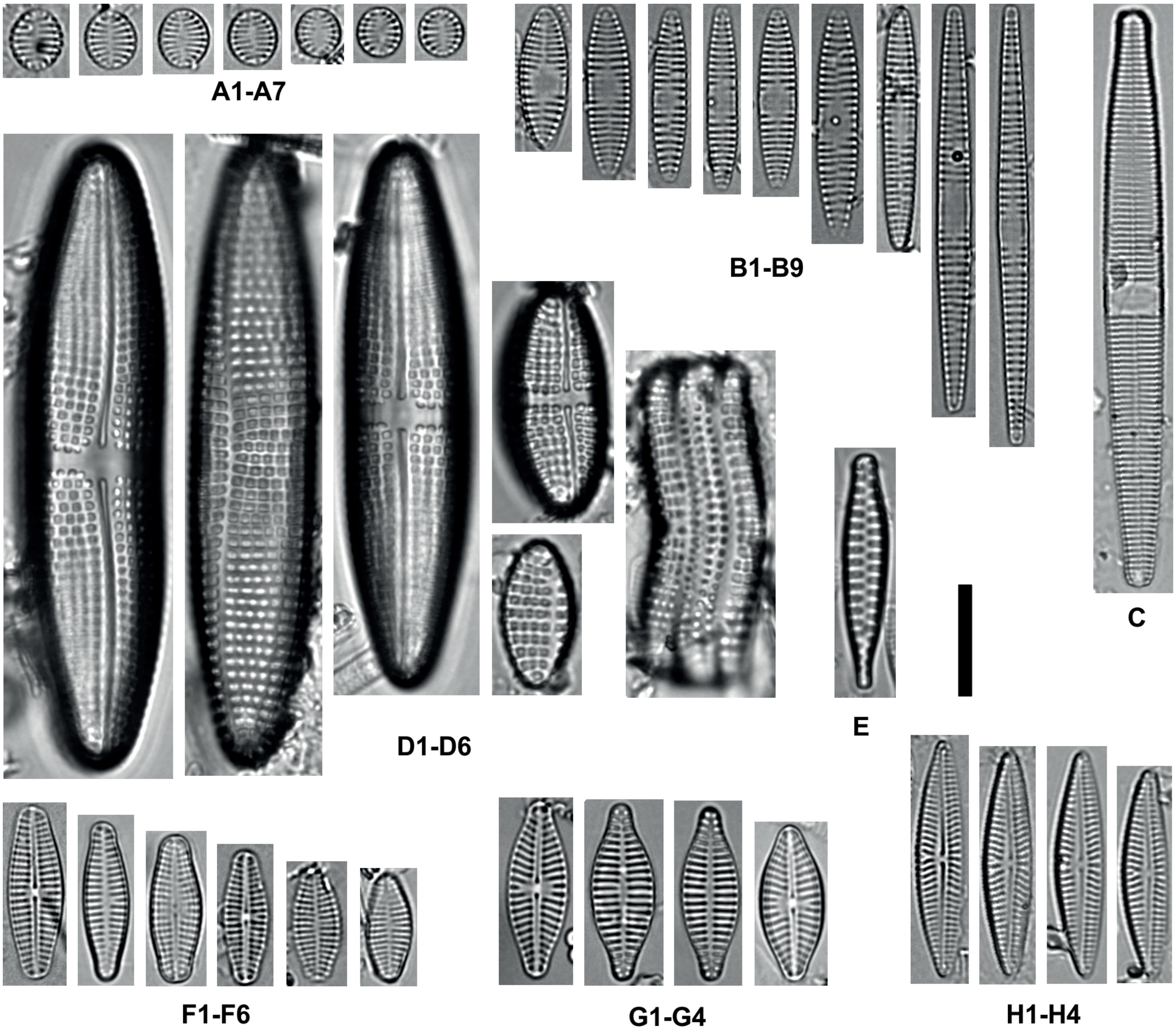

Fig. 9

A1-A7. *Fragilaria sopotensis*, B1-B9. *Fragilaria famelica*, C. *Ctenophora pulchella*, D1-D6. *Achnanthes brevipes*, E. *Opephora mutabilis*, F1-F6. *Planothidium pericavum*, G1-G4. *Planothidium delicatulum*, H1-H4. *Navicymbula pusilla*. Scale bar = 10 µm

L1-L2). Among those identified ones, some taxa apparently represented brackish water forms, e.g. *Achnanthes brevipes* Agardh (Fig. 9 D1-D6), *Amphora commutata* Grunow (Fig. 8 H1-H4), *Anomoeoneis sphaerophora* f. *sculpta* (Ehrenberg) A. Schmidt, *Denticula subtilis* Grunow (Fig. 7 H1-H5), *Diploneis interrupta* (Kützing) Cleve (Fig. 8 I1-I2), *D.* cf. *krammeri* (Fig. 7 K), *Entomoneis paludosa* (W. Smith) Reimer, *Frustulia creuzburgensis* (Krasske) Hustedt (Fig. 7 C), *Mastogloia elliptica* (Agardh) Cleve, *M. smithii* Thwaites ex W. Smith, *Navicymbula pusilla* (Grunow) Krammer (Fig. 9 H1-H4), *Navicula kefvingensis* (Ehrenberg) Kützing, *Nitzschia scalpelliformis* Grunow (Fig. 7 E), *N. sigma* (Kützing) W. Smith (Fig. 7 D1-D2), *Opephora mutabilis* (Grunow) Sabbe & Wyverman (Fig. 9 E), *Parlibellus protracta* (Grunow) Witkowski, Lange-Bertalot & Metzeltin (Fig. 7 J1-J5) and *Staurophora salina* (W. Smith) Mereschkowsky (Fig. 6 F1-F3).

A total of 129 taxa were identified in the samples collected in 1964/1965, only 79 taxa in 1992/1994, and 141 taxa in the samples collected recently (in 2013/2014) (Table 2).

Over the 50 years, the number of species at the studied sites increased from 129 to 141, and the biggest change was observed in the outflow area. The ditch was the least dynamic site in terms of the number of diatom taxa (Table 2).

The contribution of certain diatom species in assemblages significantly changed over the years. Among the dominant species, the percentage of *Craticula halophila* (Grunow) D.G. Mann (Fig. 6 A1-A9), i.e. 50% in the outflow in 1964/1965, clearly decreased in 1992/1994 and 2013/2014 (Fig. 10 A). *Ctenophora pulchella* (Ralfs ex Kützing) Williams & Round (Fig. 9 C) was present in only a few samples, however, the percentage of this species exceeded 20% (Fig. 10 B). *Fragilaria famelica* was a typical species of the outflow and the area surrounding the outflow. This species was recorded at the study sites, both in 1964/1965 (85% contribution) and in 2013/2014 (Fig. 10 C). *Fragilaria sopotensis* (33% relative abundance) was the characteristic species of the pond. It also occurred in the ditch, but with a low percentage – below 5% (Fig. 10 D). In the case of *Navicula veneta* Kützing (Fig. 7 B1-B4), the percentage of this species in the outflow and in the ditch samples gradually increased from 1964/1965 to 2013/2014 (Fig. 10 E). *Planothidium delicatulum* occurred mainly in the outflow in 1992/1994 with a relative abundance exceeding 40% (Fig. 10 F). *Chamaepinnularia krookiformis* (Krammer) Lange-Bertalot & Krammer occurred mainly in the ditch. In the samples from 1964/1965, its abundance was low, while in the samples from 2013/2014, the percentage of this species clearly increased (Fig. 11 A). *Halamphora sydowii* was recorded mainly in the outflow and in the area surrounding the outflow. During the study period, the percentage of the species gradually decreased. In 1964/1965, the contribution exceeded 10%, while in 1992/1994 it decreased below 3%. Recently the species was not recorded (Fig. 11 B). *Hippodonta hungarica* (Grunow) Lange-Bertalot, Metzeltin & Witkowski (Fig. 7 A1-A7) was recorded in the samples from 1964/1965 at all sites except the ditch. In 2013/2014, the percentage of the species decreased in the outflow, and increased in the pond (Fig. 11 C). *Navicula cincta* was recorded mainly in the area surrounding the outflow in the samples from 1964/1965, with the relative abundance not exceeding 10%. In 2013/2014, this species was recorded only in the pond, with relative abundance exceeding 10% (Fig. 11 D). The change in the percentage at the study sites was also evident in the case of *Navicula salinarum*. This species was recorded mainly in the outflow and in the area surrounding the outflow in 1964/1965. In 2013/2014, the percentage of *N. salinarum* decreased in the outflow and increased in the pond (Fig. 11 E). *Tabularia fasciculata* (Agardh) Williams & Round was recorded mainly in the outflow and in the area surrounding the outflow. The highest abundance of the species (28%) was recorded in the outflow samples from 1994 (Fig. 11 F).

The Bray-Curtis similarity index was used to determine the similarity between diatom assemblages in samples from different study periods, and the results are presented in Figure 12. The recently collected samples (2013/2014) were all grouped as a separate cluster (Cl. II). The samples from 1964/1965 and 1992/1994 did not reveal a clear diversification (Cl. III), with the exception of the samples collected from the pond in 1964/1965 (Cl. I). The diatom assemblages from the pond samples collected in 1964/1965 were very specific; the similarity of these samples to others was less than 15%. The separate position of samples from the pond was also confirmed by the MDS analysis, which was conducted to determine the similarity of samples based on the study sites (Fig. 13). The samples collected from the pond were a group separated from the others, while samples collected from the outflow and the ditch in 2013/2014 were very similar.

On the basis of the diatom classification with respect to the salinity, according to the seven-grade halobion system of Van der Werff and Hulls 1957-1974 (Table 3), the following taxa were most abundant in terms of the number of taxa: 2 – fresh-brackish, 3 – brackish-fresh and 4 – brackish. The least numerous were: 5 – brackish-marine, 6 – marine brackish, and 7 – marine (Fig. 14). However, taking into consideration the percentage of particular taxa at each study site, taxa from the 4th category were most abundant in the outflow, and taxa from the 3rd and the 2nd category were less abundant (Fig. 15 A); in the pond – taxa from the 2nd category were most abundant and taxa from the 3rd category were less abundant (Fig. 15 B).

Discussion

The spatial and temporal variability of diatom assemblages observed in the athalassic habitats is associated with different concentrations of chlorides and direct/indirect human impact, such as agricultural use of the surrounding area and backfilling of the depression. The differences in the similarity of diatom assemblages, observed in samples collected from the pond in all study periods,

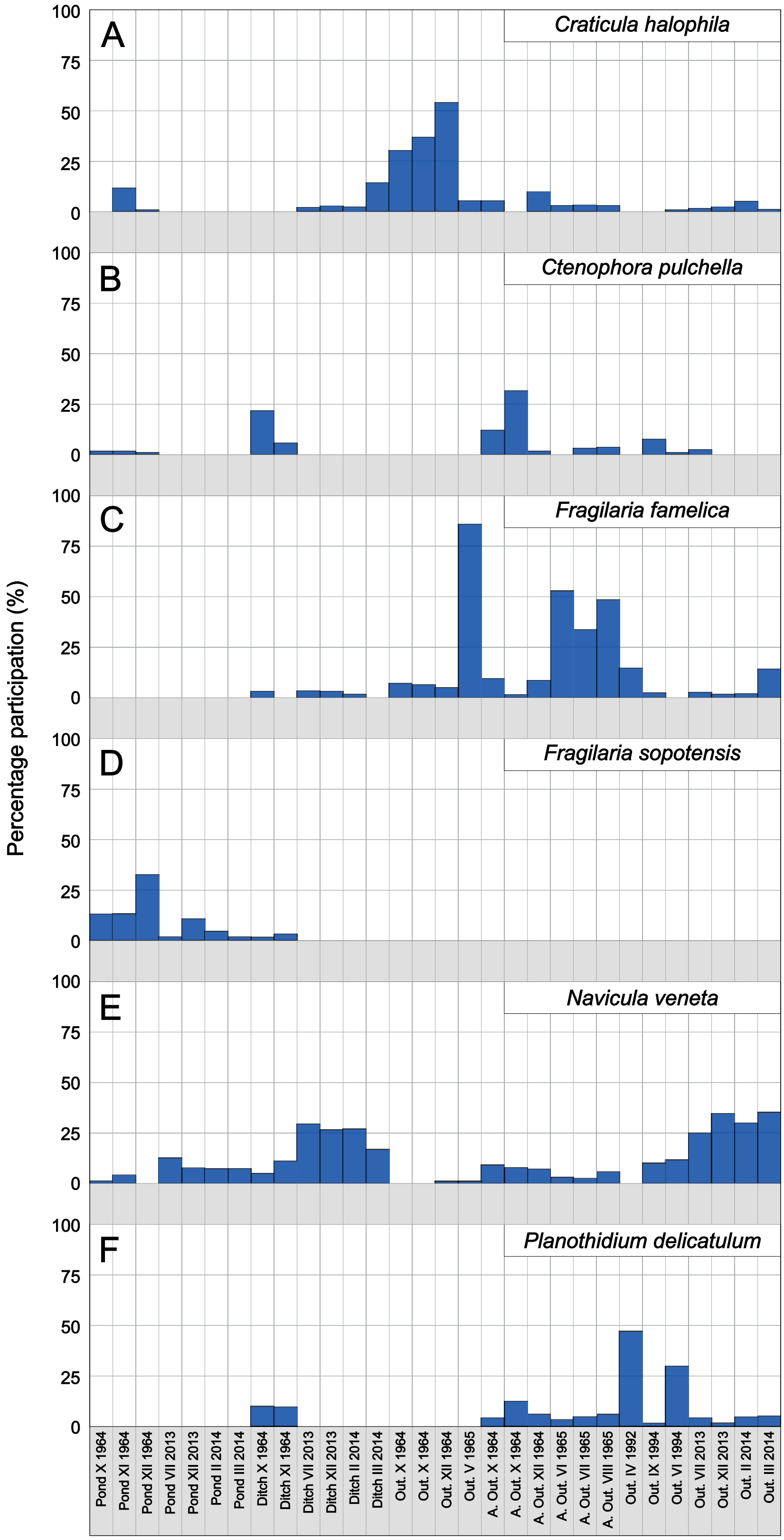

Fig. 10

The relative abundance (%) of diatom species in the studied samples

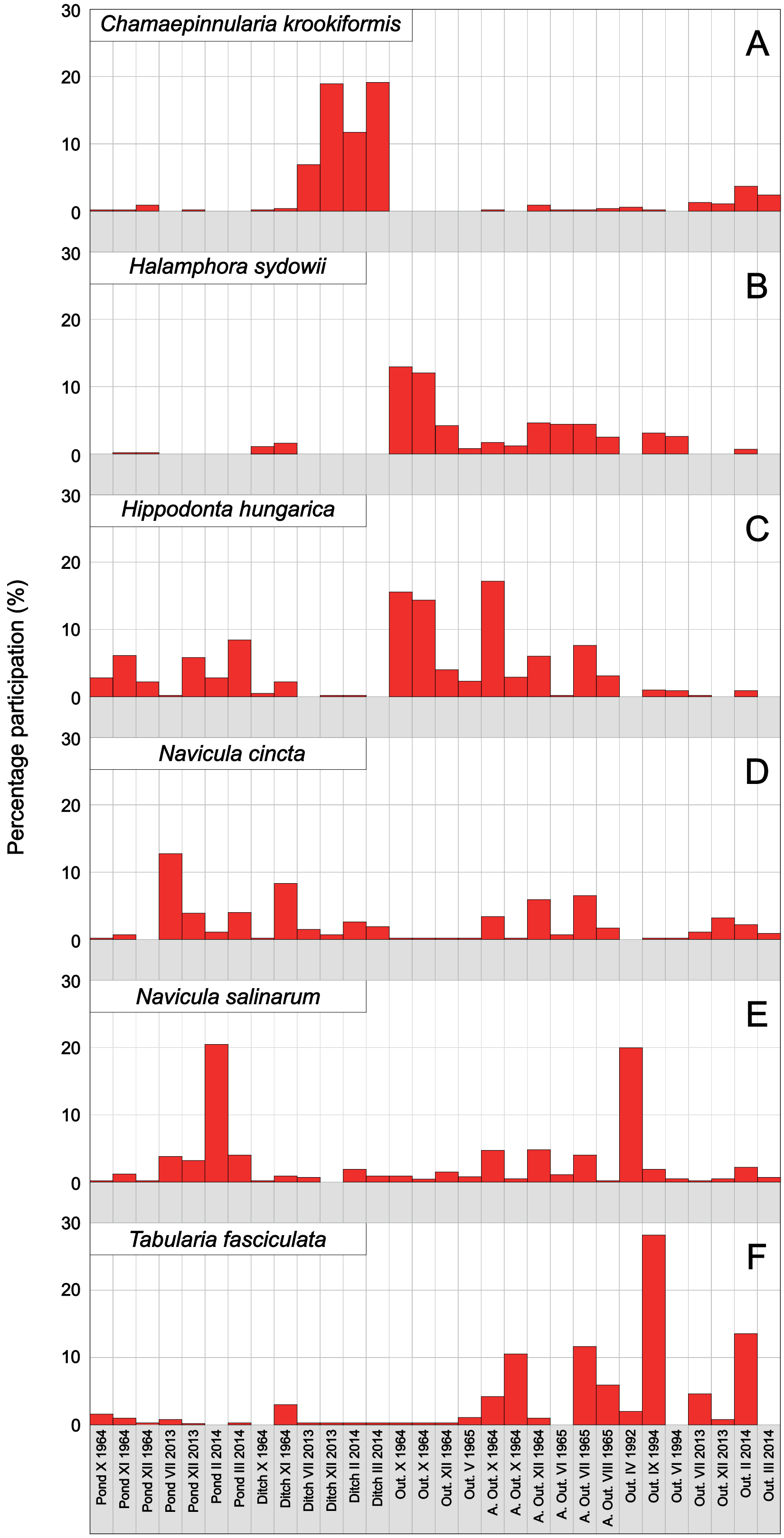

Fig. 11

The relative abundance (%) of diatom species in the studied samples

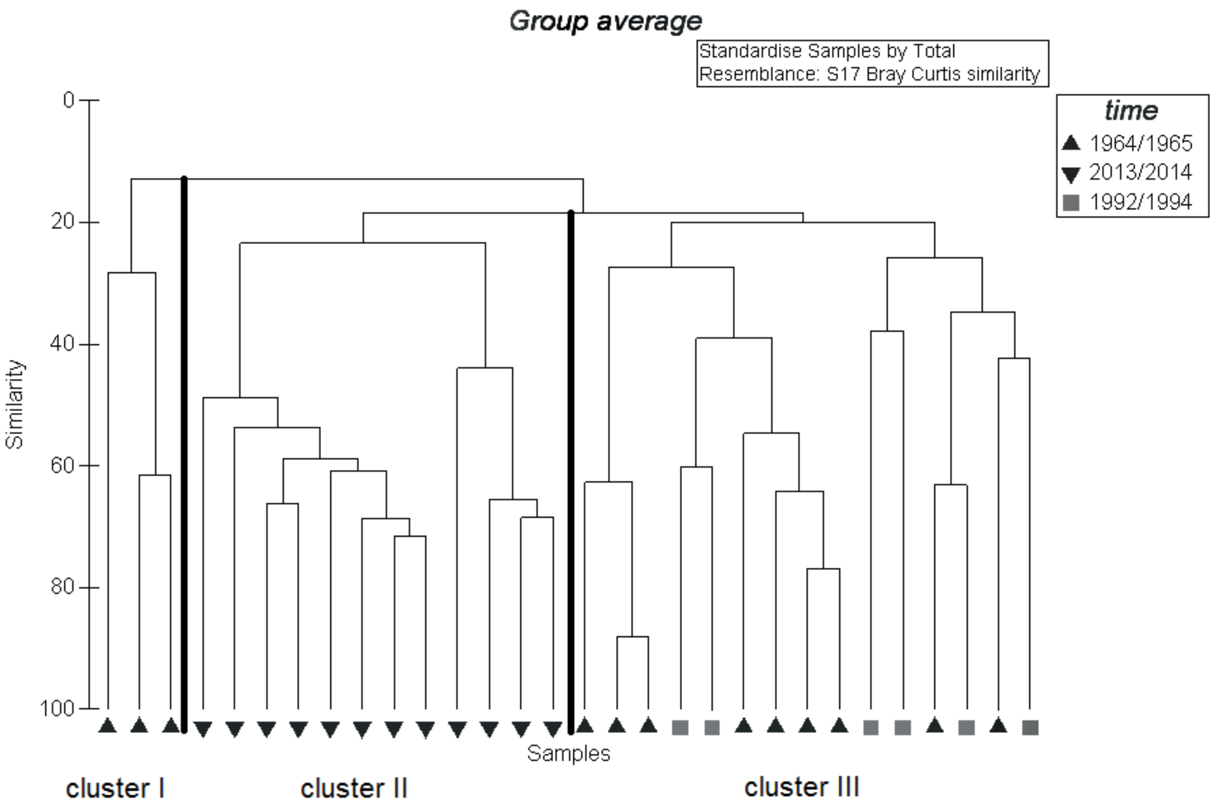

Fig. 12

Cluster analysis based on the Bray-Curtis similarity index for all diatom samples according to the study period

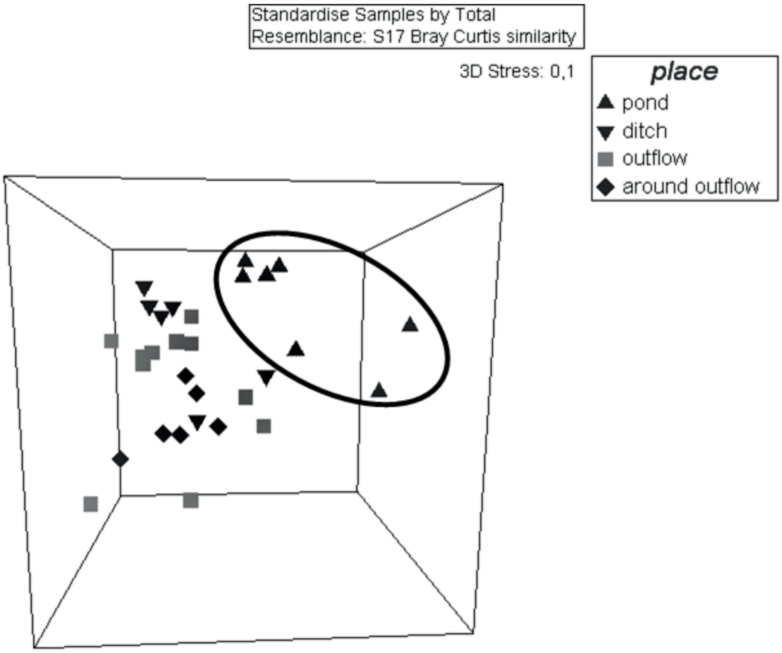

Fig. 13

MDS analysis of diatom samples according to the study sites; marked samples from the pond

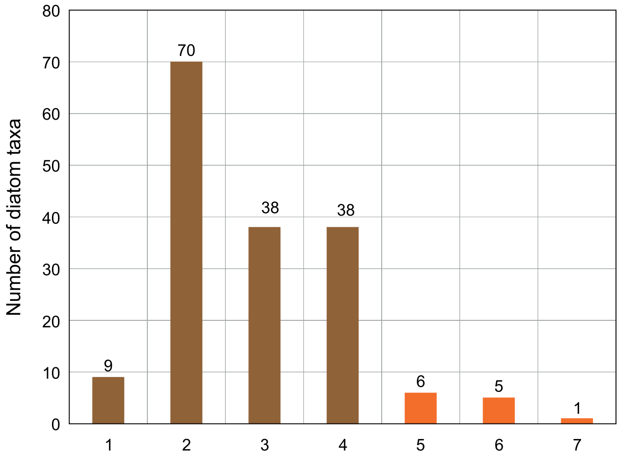

Fig. 14

The number of diatom taxa in each category of the seven-grade halobion system of Van der Werff and Huls (1957-1974) (Denys et al. 1983), with the system of Van Dam et al. 1994 taken into account (dark bars). The classification was based on the OMNIDIA 5.3 software and the references

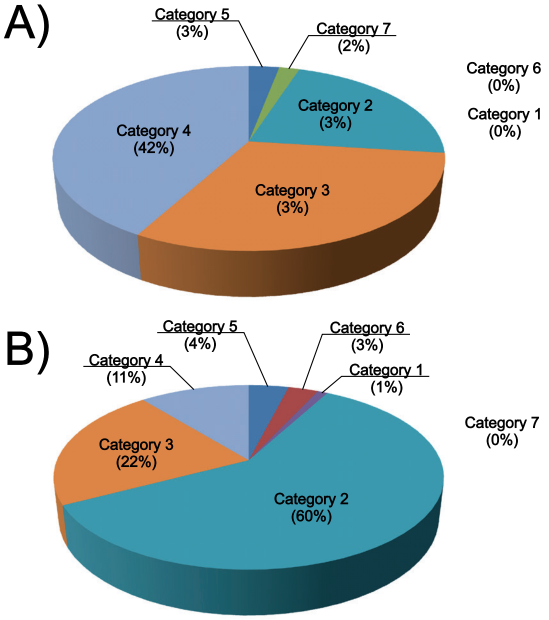

Fig. 15

Relative abundance (%) of diatom taxa in categories of the seven-grade halobion system of Van der Werff and Huls 1957-1974, A. taxa from the outflow; B. taxa from the pond

result from the lower concentration of chlorides in the pond compared with other study sites. In 2013/2014, when the outflow was backfilled with a substrate, the environmental conditions became similar in relation to salinity in the outflow and in the ditch. This caused the change in the structure of diatom assemblages, which in 2013/2014 were characterized by a high degree of similarity.

The identified diatom taxa were classified according to their tolerance to salinity, taking into consideration the isolation of the study sites from marine habitats. The halobion system of Van Dam et al. (1994), which comprises four categories of diatom taxa sensitive to salinity, and occurring in waters with the maximum concentration of chlorides – around 5000 mg l^{-1}, is commonly used for freshwater inland ecosystems. However, it was necessary to apply the halobion system of Van der Werff and Huls 1957-1974, which also includes marine species. This system includes seven categories. The 7th category contains diatom taxa which occur in waters where the concentration of chlorides exceeds 17000 mg l^{-1}. These species are defined as typical marine forms (Table 3) (Denys et al. 1983). Based on the halobion system of Van der Werff and Huls 1957-1974, *Halamphora tenerrima* belongs to the 7th category and may be classified as a marine species (Clavero et al. 2000; Levkov 2009).

Two species were classified as marine-brackish (the 6th category): *Parlibellus crucicula* (W. Smith) Witkowski, Lange-Bertalot & Metzeltin and *Staurophora salina*. Those species are widespread in the North Sea, and they also occur in the Baltic Sea (Witkowski et al. 2000). Both species rarely occurred in the outflow (samples from 1992/1994) and in the area near the outflow (samples from 1964/1965), where the concentration of chlorides ranged between 2500 and 3000 mg l^{-1}. According to the references, three additional taxa may be classified as marine-brackish: *Halamphora dominici*, *H. subsalina* (Cleve) Levkov (Levkov 2009) and *Tabularia tabulata* (Agardh) Snoeijs (Krammer, Lange-Bertalot 1991a).

Three species were classified as brackish-marine (5th category): *Achnanthes brevipes*, *Navicula perminuta* Grunow, and *Opephora mutabilis*. The first species occurred mainly in the ditch and in the pond in the samples from 1964/1965, while in the recent studies, it occurred mainly in the outflow. *N. perminuta* and *O. mutabilis* were present mainly in the samples from 1992/1994. These species occurred in habitats which, according to the concentration of chloride, may be classified as brackish (the 4th category). Moreover, three species according to the references may also be classified as brackish-marine: *Navicula digitoconvergens*, *N. hanseatica* Lange-Bert. & Stachura (Lange-Bertalot 2001) and

N. meulemansii (Mertens et al. 2014).

At the studied sites, 20 species were classified as brackish (the 4th category) and over 30 species as brackish-freshwater (the 3rd category). The changes in the concentration of chloride ions over the 50 years at the study sites could be the cause of the qualitative and the quantitative changes in the diatom assemblages (Table 1). In 1962, the chlorides in the outflow amounted to 2560 mg l^{-1} (Olaczek, unpublished PhD thesis, 1963), while in 1992/1994, it increased to 3000 mg l^{-1} (Żelazna-Wieczorek 2002), and in 2014, it greatly decreased to 1585 mg l^{-1}. In the outflow, an increase in the concentration of orthophosphate ions was also observed. Along with changes in the water chemistry, significant changes in the percentage abundance of some species were observed.

Craticula halophila, considered as a brackish species according to the halobion system of Van der Werff and Huls 1957-1974, was observed in large numbers in the outflow in the period of 1964/1965, while in 2013/2014, it occurred in small numbers. A similar pattern was observed in the case of *Fragilaria famelica* (fresh-brackish) and *Halamphora sydowii*. These species were observed mainly in the area near the outflow (1964/1965) and recently they have been sporadically recorded in the outflow. Due to the apparent disappearance of the above species from the described habitats where the concentration of chloride ions decreased, we propose a change in the classification of the halobion system of Van Dam et al. 1994 concerning these species. For *Fragilaria famelica*, we suggest a transfer from the 2nd to the 3rd category. This species was recorded *inter alia* in alkaline waters with high mineralization in Mexico (Novelo et al. 2007) and fresh to brackish waters of Spanish lakes (Antón-Garrido et al. 2013). For *Halamphora sydowii*, we recommend the 4th category. This species were also recorded in the brackish waters (Levkov 2009) and in the coastal Spanish lakes (Antón-Garrido et al. 2013).

Hippodonta hungarica is a widespread species, however, it prefers waters with high conductivity, which is typical of the study sites. The tolerance range to the chloride concentration of *H. hungarica* is rather broad (Lange-Bertalot 2001; Pavlov et al. 2013). This species was recorded in the outflow and in the area surrounding the outflow in 1964/1965. Recently, it has been recorded mainly in the pond. According to the halobion system of Van der Werff and Huls 1957-1974, this species belongs to the 2nd category, which corresponds to diatoms preferring water with a chloride concentration between 100 and 500 mg l^{-1}. In the samples from 1964/1965, however, the chloride concentration was five times higher than the upper limit of the 2nd category.

Pliński (1969) and Rakowska (1997) published information about the number of taxa identified at each study site in different periods of the study conducted in Pełczyska (Table 2). Part of the samples, collected and published by Pliński (1969), were re-analyzed. Pliński (1969) provides information about 62 taxa identified in all samples, with the highest taxonomic diversity recorded in the outflow. During the re-examination of Piliński's material, 129 taxa were identified, with the highest diversity in the area surrounding the outflow. These differences result from the methods of taxonomic investigation. Presently, the examination is based on the scanning electron microscopy. SEM allows the verification and the description of new taxonomic units among the diatom taxa.

Pliński (1969) and Rakowska (1997) identified the *Amphora coffeaeformis* Agardh (syn. *Halamphora coffeaeformis* (Agardh) Levkov), however, the current taxonomic analysis with the use of SEM did not confirm the presence of this species. Instead, *Halamphora sydowii* was recorded, which might have been previously identified as *Halamphora coffeaeformis* (Archibald, Schoeman 1984). Because of the similarity of *Halamphora tenerrima* to other taxa from the *Halamphora* genus, it could be misidentified (Clavero et al. 2000; Levkov 2009). This species was recorded mainly in marine waters (Clavero et al. 2000; Levkov 2009), inter alia in the Gulf of Mexico (Felder, Camp 2009).

In the pond, three species of the *Halamphora* genus were recorded: *Halamphora veneta*, *Halamphora paraveneta* and *Halamphora dominici*. In the previous studies, they were all identified as *Amphora veneta* Kützing (Lange-Bertalot et al. 2003; Levkov 2009). *Halamphora veneta* is widespread and is noted in many locations around the world (Sala et al. 1998; Żelazna-Wieczorek 2011; Lange-Bertalot et al. 2003; Potapova et al. 2004; Wachnicka, Gaiser 2007; Levkov 2009). Most likely in previous publications by Pliński (1969) and Rakowska (1997), the species recorded as *Amphora veneta* included also *Halamphora veneta, H. paraventa,* and *H. dominici*. *H. veneta* and *H. paraveneta*, which occur in fresh and slightly brackish waters, can be distinguished based on the valve size range (Lange-Bertalot et al. 2003). *Halamphora dominici* prefers inland saline and marine waters, and its valves are characterized by a higher density of striae (Levkov 2009)

In the studied material, *Navicula meulemansi* was recorded in the periods of 1964/1965 and 2013/2014. This species was described in 2014 by Mertens et al. 2014, and it might have been previously identified as the *Navicula* species.

Fragilaria sopotensis has a similar morphology to *Pseudostaurosira cataractarum* (Hustedt) Wetzel, Morales & Ector, and both species can be confused with each other. *P. cataractarum* has shorter striae and a wider central area (Wetzel et al. 2013). *Fragilaria sopotensis* was recorded in the Mediterranean Sea (Witkowski et al. 2000), the Baltic Sea, and in the costal lakes and marshes of the Baltic region (Witkowski, Lange-Bertalot 1993; Witkowski et al. 2000; Lutyńska 2008). During the presented research, the species occurred mainly in the pond in the period of 1964/1965, which may suggest that the species prefers low concentrations of chlorides (to 2500 mg l^{-1}). However, due to lack of data concerning the chloride ion concentration in the pond, we are not able to assess the upper limit of this factor, which would represent the maximum for the species' tolerance and occurrence in the studied habitats.

The long-term research conducted in the athalassic habitats in the village of Pełczyska, varied in terms of hydromorphology (various types of habitats) and hydrochemistry (varying range of salinity), allowed the accurate analysis of spatial and temporal changes in diatom assemblages under different salinity levels. The analysis of changes occurring over time in different types of habitats whose conditions are determined by one predominant factor, is essential for the determination of the species' requirements and tolerance range towards the environmental factor.

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Oświadczenie o współautorstwie

Oświadczam, że mój wkład w przygotowanie oryginalniej pracy polegał na: przygotowaniu koncepcji pracy, zaplanowaniu prac w terenie, identyfikacji taksonomicznej, opracowaniu wyników z wykorzystaniem metod matematycznych. Udział w dyskusji i interpretacji uzyskanych wyników i w przygotowaniu ostatecznej wersji manuskryptu. Swój udział w przygotowanie pracy oceniam na 50%.

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Oświadczam, że mój wkład w przygotowanie oryginalniej pracy polegał na: przygotowaniu koncepcji pracy, zaplanowaniu i realizacji prac w terenie, identyfikacji taksonomicznej, przygotowanie dokumentacji fotograficznej. Udział w analizie i interpretacji uzyskanych wyników. Pracowałem również na ostateczną wersją manuskryptu. Swój udział w przygotowanie pracy oceniam na 50%.

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Taxonomic revision of *Chamaepinnularia krookiformis* LANGE–BERTALOT et KRAMMER with a description of *Chamaepinnularia plinskii* sp. nov.

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Abstract: The benthic diatoms collected during our recent research in athalassic habitats exemplify a high morphological variability within *Chamaepinnularia krookiformis* LANGE–BERTALOT et KRAMMER. The species has already been known from freshwater ecosystems as well as from brackish waters in different regions of Europe. This variability has been also confirmed by the analysis of the literature. However, this is the first time that the morphological variability of the species has been addressed. In order to revise *C. krookiformis* and describe a new species, which has been distinguished on the basis of our material, we compared it with the type material of *Pinnularia krookiformis* KRAMMER 1992 from the periodic saline pool in Nordrhein–Westfalen (Germany). This study deals with a morphological analysis based on a large populations of *C. krookiformis* from Pełczyska (Central Poland). Selected morphological features were analysed, i.e.: valve length and width, number of striae in 10 µm, apices width, constrictions width, apices/constrictions width ratio, central–area length, and valve/central area length ratio. The results of light (LM) and scanning (SEM) microscopy, supported by multidimensional scaling (MDS), allowed for the distinction of three different morphotypes. Two of them match KRAMMER concept of *Pinnularia krookiformis* from 1992, while the third, occurring in our samples in three sampling sites, is clearly different. Thus, we described a new species *Chamaepinnularia plinskii* sp. nov., by the following major features: the valves are linear, symmetric, with rounded, capitate apices, the axial area is narrow, expanding to a wide central area forming a fascia, distal raphe endings are hooked to the same side of the valve and striae slightly radiate in the middle and in the rounded apices, becoming parallel in constrictions, 20–26 in 10 µm. This species occurs in inland waters with a wide range of chloride concentration, from 1000 to 3500 mg.l^{-1}.

Key words: brackish inland waters, *Pinnularia*, *Chamaepinnularia*, type material, new species

INTRODUCTION

The *Chamaepinnularia* LANGE–BERTALOT et KRAMMER genus was described in 1996, based on generitype *Chamaepinnularia vyvermanii* LANGE–BERTALOT et KRAMMER from the periodic saline pool in Nordrhein–Westfalen (Germany). According to the first description, representatives of the genus are characterized by small cells with their dimension not exceeding 25 µm in length and 4 µm in width (LANGE–BERTALOT & METZELTIN 1996); a slightly larger dimension range is characteristic for *Chamaepinnularia gerlachei* VAN DE VIJVER et STERKEN from Antarctica (VAN DE VIJVER et al. 2010).

Diatom frustules of this genus are linear through linear–elliptic to linear–lanceolate shapes with rounded apices. The symmetry and arrangement of the raphe system do not deviate significantly from those observed in representatives of *Navicula* BORY DE SAINT–VINCENT and *Pinnularia* EHRENBERG. Distal raphe endings are hooked towards the same direction. Proximal raphe endings are deflected. Distal raphe arms terminate on a helictoglossa on the valve internal side, while the proximal raphe endings are hooked. Externally, distinguishable single–row striae are formed out of one large areola create foramina, occluded by vela. Internally, striae are open with silica bridges (LANGE–BERTALOT & METZELTIN 1996; WETZEL et al. 2013).

The genus include species, which were earlier classified as *Navicula* or *Pinnularia* (LANGE–BERTALOT & METZELTIN 1996), e.g. those within the *Navicula* genus – *Chamaepinnularia begeri* (KRASSKE) LANGE–BERTALOT, *C. mediocris* (KRASSKE) LANGE–BERTALOT, *C. soehrensis* (KRASSKE) LANGE–BERTALOT et KRAMMER and those within the *Pinnularia* genus – *Chamaepin-*

nularia krookii (Krammer) Lange–Bertalot et Karmmer, *C. krookiformis* (Krammer) Lange–Bertalot et Karmmer. Recently, *Chamaepinnularia parsura* (Hustedt) C.E. Wetzel et Ector, *C. obsoleta* (Hustedt) C.E. Wetzel et Ector, *C. brevissima* (Hustedt) C.E. Wetzel et Ector, *C. perfidissima* (Lange–Bertalot) C.E. Wetzel et Ector, and *C. ventosa* (Hustedt) C.E. Wetzel et Ector (Wetzel et al. 2013) were transferred to *Chamaepinnularia*.

Chamaepinnularia species occur in various fresh–water habitats and in waters with a varying range of salinity. Several species are typical of aerial habitats. Species occurring in antarctic regions are also known (Van de Vijver et al. 2010; Sterken et al. 2015). Some species were observed in the *Sphagnum* peat bogs (Kulikovskiy et al. 2010). In Europe, *Chamaepinnularia schauppiana* Lange–Bertalot et Metzeltin was noted to occur in oligo–dystrophic waters in Finland but also in mire and fen pools (Cantonati & Lange–Bertalot 2009). It was also observed that several other species occurred in Europe both in low pH lakes (Kapetanović et al. 2011) and in mountain fens with a high concentration of minerals (Fránková et al. 2009).
Chamaepinnularia krookiformis was noted to occur in the area of Poland both in seawaters and saline inland waters in the coastal zone (Witkowski 1994; Witkowski et al. 2000; Bąk et al. 2006), in freshwater ecosystem such as upland rivers (Wojtal 2009; Peszek et al. 2015), quaternary springs located near Łódź (Żelazna–Wieczorek 2011), and in athalassic habitats in Central Poland (Żelazna–Wieczorek et al. 2015).

For many years, specimens *of Chamaepinnularia krookiformis* and *C. krookii* have been classified into different genera and species (Fig. 1). *Navicula krockii* was first described in 1882 by Grunow. The description contained information about a similarity of the species with *Pinnularia globiceps* Gregory (1856). The valve length was within 19–28 µm, its width was between 5.5 and 7 µm. The number of striae was between 15 and 17 in 10 µm (Grunow 1882). Several years later, Cleve (1891) transferred the species to the genus *Pinnularia*, at the same time introducing a correction in the species name giving it the name of *Pinnularia krookii* Cleve. The species' name was given to honour Swedish botanist Krook (Cleve 1891). Several years later, Cleve (1895) considered *P. krookii* to be a variety of *P. globiceps*: *P. globiceps* var. *krookii* Cleve. The change resulted from the similarity of *P. krookii* to *P. globiceps*; the most significant difference was that of the cell size (Cleve 1895). *Navicula ignobilis* Krasske was described in 1938. However, specimens shown in the drawing were very similar to the specimen drawing of *Navicula krockii* Grunow published in 1882 (Grunow 1882; Krasske 1938). Krammer and Lange–Bertalot (1986), who referred to some earlier works by Grunow (1882), Cleve (1895) and Krasske (1938) marked *Pinnularia* "*krocki*" and *Pinnularia ignobilis*. Under the name of *Pinnularia ignobilis,* pictures showing in fact *Pinnularia* "*krocki*" (Krammer, Lange–Bertalot 1986, p. 416–417, tafel 187, figs 6–7, 9'; tafel 206, figs 12–19) were published. While photomicrographs of *Pinnularia* "*krocki*" Krammer, Lange–Bertalot (1986, p. 416, tafel 187, fig. 9; tafel 206, figs 8–9) showed the species that had not been previously described. Krammer (1992) described a new species of *Pinnularia krookiformis* making a reference to the specimens illustrated in the publication of Krammer & Lange–Bertalot (1986, p. 416, tafel 187, fig. 9; tafel 206, figs 8–9). Several years later, *P. krookii* and *P. krookiformis* were transferred to the *Chamaepinnularia* genus by Lange–bertalot et Krammer in Lange–Bertalot & Genkal (1999) (see Fig. 1).

In this paper, we present results of the analysis of populations of *Chamaepinnularia krookiformis* from the Pełczyska (Central Poland) and the type material *Pinnularia krookiformis* from Nordrhein–Westfalen (Germany) described by Krammer (1992) with light (LM) and scanning electron microscope (SEM) techniques using multivariate analysis of multidimensional scaling (MDS) to explore the morphological variability of the different populations up to now identified as *C. krookiformis*. The analysis enabled us to describe one new species *Chamaepinnularia plinskii* sp. nov., which is detailed below.

Material and Methods

The study area is located in Pełczyska (51°58'34.5"N, 19°14'20.4"E) (Central Poland), a village next to the city of Łęczyca in the Łódź Province. The study area included three sampling sites, i.e. outflow, ditch and pond, with a varying range of salinity. Chemical and physical parameters, also the structure of diatom assemblages, clearly differentiate the ditch environment from the two other ecosystems of an outflow and pond (Żelazna–Wieczorek et al. 2015). All the data is compiled in Table 1.

The following samples have been examined. 30 samples altogether were collected from all three habitats in 1964 (X, XI and XII); 1992 (IV); 1994 (VI, IX); 2013 (VII, XII); 2014 (II, III). For the diatomological analysis, 15 samples collected by Pliński in 1964/1965 (1969), 3 samples collected by Żelazna–Wieczorek (2002) in 1992/1994, and 12 samples collected recently, in 2013/2014, were used. The morphological analysis was based on samples in which *Chamaepinnularia krookiformis* occurred with relative abundance greater than 5%. The recent benthic samples were collected and purified according to Żelazna–Wieczorek (2011).

The analysis of selected morphological features of *Chamaepinnularia krookiformis* was made by examining 4 permanent slides with a light microscope (Nikon YS 100 and Nikon Eclipse E400) with 1000× magnification (plan oil–immersion objective 100×/1.25). A total of 170 specimens were analysed in 4 permanent slides. The following features were taken into account: 1) valve width and 2) length, 3) number of striae in 10 µm, 4) width of capitate apices, 5) width of constrictions, 6) ratio of apices width and constrictions width, 7) length of central area and 8) ratio of valve length and central area length (Fig. 2A) in the MDS analysis (Multi–Dimensional–Scaling – MDS) based on the Bray–Curtis similarity (Żelazna–Wieczorek 2011) was used. The PRIMER 6.1.10 software was used for calculations.

The new permanent slides from unmounted type material of *Pinnularia krookiformis* Krammer 1992 from saline puddles in Nordrhein–Westfalen were prepared (*Salzgebiet südlich von Salzkotten, Nordrhein–Westfalen* Germany, leg. Wygasch, 17.04.1982 – KR604B Friedrich–Hustedt–Zentrum für Diatomeenforschung Institute for Polar and Marine Research, Bremerhaven). The unmounted sample was washed, first in 95% ethyl alcohol and then several times in deionized water. The new permanent slides were mounted with Naphrax® according to Żelazna–Wieczorek (2011). 45 specimens from the type material morphological features were analysed.

All materials were analysed using the Phenom Pro X (with 8 nm gold layer, at 10 kV) scanning microscope at the Laboratory of Microscopy Imaging & Specialist Biological Techniques at Faculty of Biology & Environmental Protection. SEM photomicrographs were taken at a resolution of 1024×1080 pixels.

RESULTS AND DISCUSSION

The Bray–Curtis similarity index was used to determine the similarity between *Chamaepinnularia krookiformis* specimens. The MDS procedure distinguished three groups of specimens determined as: Morphotype 1 (N=66 specimens), Morphotype 2 (N=66 specimens) and Morphotype 3 (N=38 specimens) (Fig. 2B.). The variation range of morphological features of specimens and species variability by Lange–Bertalot & Genkal (1999) are listed in Table 2.

Chamaepinnularia plinskii Żelazna–Wieczorek et Olszyński sp. nov. (Figs. 3–19)

Morphotype 1 (Fig. 2B)

Synonyms: *Pinnularia* spec. in: Krammer, K. & Lange–Bertalot, H. 1986 *pro parte*: fig. 206: 10;
Pinnularia krooki in: Krammer, K. 1992 *pro parte*: tafel: 18, fig. 2;
Pinnularia krookiformis Krammer 1992, in: Witkowski, A. 1994 *pro parte*: plate: XXXVIII, figs 16–17.

Description: LM: Linear, symmetric valves with rounded capitate apices. Valve dimension: length 18–24 µm, width 4–5 µm, width of apices: 3–4 µm. The narrowest points of the valves are close to the capitate apices, width of constrictions 2.5–3.5 µm. The ratio of the width of apices to the width of constrictions is 0.9–1.4. The axial area is narrow, length 6–11 µm, width 3–5 µm, and expands to a wide central area, forming a fascia. The ratio of the valve length to the length of the central area is 1.9–3.4. Raphe is straight and filiform. Distal raphe endings are hooked towards the same direction. Proximal raphe endings are straight, expanded and pore–like. Striae are slightly–radiate in the middle and in the rounded apices to become parallel in the constrictions: 20–26 in 10 µm.

SEM: Externally, striae are composed of a chamber–like areola and covered by vela. Striae on the valve face are separated from the mantle striae by a hyaline area (Fig. 50). Distal raphe endings are hooked, curving down to the mantle (Fig. 51). Internally, striae on the valve face are interrupted and divided by a silica bridge to form two parts, the first one is small and rounded, and located on the valve face–mantle junction, while the second one is elongated, and located on the valve face (Fig. 52). Proximal raphe endings are hooked and deflected to the same direction, distal raphe endings terminate in a helictoglossa. The thick sternum expands proximally to a wide, rhomboidal fascia, distally to polar bars (Fig. 52).

Holotype: slide: D.PEDB040713 No. 00065 Algae Collection Department of Algology and Mycology, University of Łódź. Holotype illustrated in Fig. 7 (designated here).

Type locality: POLAND. Łódź Province, Pełczyska village N 51°58'34.5", E 19°14'20.4", brackish inland waters.

Etymology: This species is named in honour of Professor Marcin Pliński, a Polish phycologist, who began exploring diatoms in Pełczyska village in 1964.

Differential diagnosis: *Chamaepinnularia plinskii* possesses the characteristic feature of the genus, i.e. a chamber–like areola covered by vela divided into two parts by a silica bridge on the internal valve side (Lange–Bertalot & Metzeltin 1996). Unlike *C. krookiformis, C. plinskii* has wide fascia reaching the valve edge, a greater number of striae in 10 µm, wider constrictions and a lower ratio of the valve length to the width of the central area as shown in Table 2. *C. plinskii* may be also confused with *Pinnularia bertrandii* Krammer. However, *P. bertrandii* has a smaller number of striae in 10 µm, these are arranged more radially and they are shorter at the axial area apices. Frustules in *P. bertrandii* are larger and proximal endings of the raphe are slightly curved towards the same direction (Krammer 2000). Specimens similar to *C. pliński* were not found in the type material of *Pinnularia krookiformis*.

Ecology and Distribution: A description of *Chamaepinnularia plinskii* was made based on specimens mainly from a small ditch through which water flows from the backfilled outflow. The water is characterized by slightly alkaline to alkaline conditions and high conductivity values up to 5170 µS.cm^{-1}. The concentration of chlorides varied from 1000 to 3500 mg.l^{-1}. As far as the type locality is concerned, this species was primarily found in benthic samples taken from the bottom of the ditch. *C. plinskii* was already reported in the freshwater springs of the Łódź Hills (Żelazna–Wieczorek 2011, p. 103, plate 103, fig. 34) wherein the concentration of chlorides was between 12.09

Table 1. Physical and chemical parameters of water at the studied sites analysed according to Żelazna–Wieczorek et al. 2015 (modified).

	1962	**1965**		**1994**		**2013**			**2014**		
	X–OUTFLOW	**I–DITCH**	**I–POND**	**IV–OUT-FLOW**	**X–OUT-FLOW**	**VII–OUT-FLOW**	**VII–DITCH**	**VII–PO-UND**	**III – OUT-FLOW**	**III – DITCH**	**III – POND**
T (°C)	7.00			7.00	8.00	20,70	19,5	20.4	6.70	7.80	8.20
pH	7.40	7.2	7.3	7.20	7.22	n/d	n/d	n/d	7.99	6.40	7.82
Cond (µS)	n/d			7764.00	7681.00	9190.00	3740.00	2209.00	4450.00	5170.00	2930.00
T.Hard (mvaL.l^{-1})	37.60			10.84	11.85	n/d	n/d	n/d	n/d	n/d	n/d
CL^{-} (mg.l^{-1})	2560.00	2668.40	738.40	2910.86	3116.19	1672.03	3468.23	925.21	1585.00	1006.00	685.00
SO_4^{2-} (mg.l^{-1})	225.00			154.44	214.44	141.24	279.6	254.97	187.60	165.00	166.20
NH_4^{+} (mg.l^{-1})	2.00			2.38	2.20	0.99	n/d	n/d	2.09	0.19	0.46
NO_2^{-} (mg.l^{-1})	n/d			0.02	0.01	n/d	n/d	n/d	n/d	n/d	n/d
NO_3^{-} (mg.l^{-1})	n/d			0.51	1.50	0.02	0.03	0.16	n/d	n/d	n/d
PO_4^{3-} (mg.l^{-1})	n/d			0.18	0.05	20.04	2.31	11.41	12.46	8.57	9.10
Na^{+} (mg.l^{-1})	n/d			1528.00	1413.00	471.11	925.17	252.32	500.70	453.30	277.30
K^{+} (mg.l^{-1})	n/d			19.65	22.60	73.79	110.81	54.57	124.80	109.80	59.00
Ca^{2+} (mg.l^{-1})	480.00			132.10	144.80	160.22	179.53	121.46	171.30	165.00	75.80
Mg^{2+} (mg.l^{-1})	195.00			51.68	56.16	28.99	9.33	17.56	43.8	35.90	25.6

Table 2. Range of selected morphological features of *Chamaepinnularia krookiformis* sensu lato in original and type material of *Pinnularia krookiformis* Krammer 1992.

	Length (µm)	**Width (µm)**	**Striae in 10 µm**	**Width of apices (µm)**	**Width of con-strictions (µm)**	**Ratio of width of apices/width of constriction**	**Length of axial area (µm)**	**Width of axial area (µm)**	**Ratio of length of valve length of central area**
Material from Pełczyska (Poland)									
Chamaepinnularia plinskii sp. nov. = Morphotype 1	18–24	4–5	20–26	3.0–4.0	2.5–3.5	0.9–1.4	6–11	3.0–5.0	1.9–3.4
Chamaepinnularia krookiformis Morphotype 2	17–22	4–5	16–20	3.0–3.5	2.0–2.5	1.2–1.8	6–10	2.0–3.5	1.9–3.5
Chamaepinnularia krookiformis Morphotype 3	19–21	4–5	16–20	2.5–3.0	1.5–2.0	1.3–2.0	11–14	2.5–5.0	1.4–1.9
Type material of *Pinnularia krookiformis* Krammer 1992									
Chamaepinnularia krookiformis Morphotype 2	14–17	4–5	19–21	3–4	2.0–2.5	1.0–1.3	5–7	2.0–2.6	2.3–2.8
Chamaepinnularia krookiformis Morphotype 3	16–18	4–5	19–20	2–3	1.3–2.0	1.3–2.0	7–10	2.0–2.6	1.8–2.4

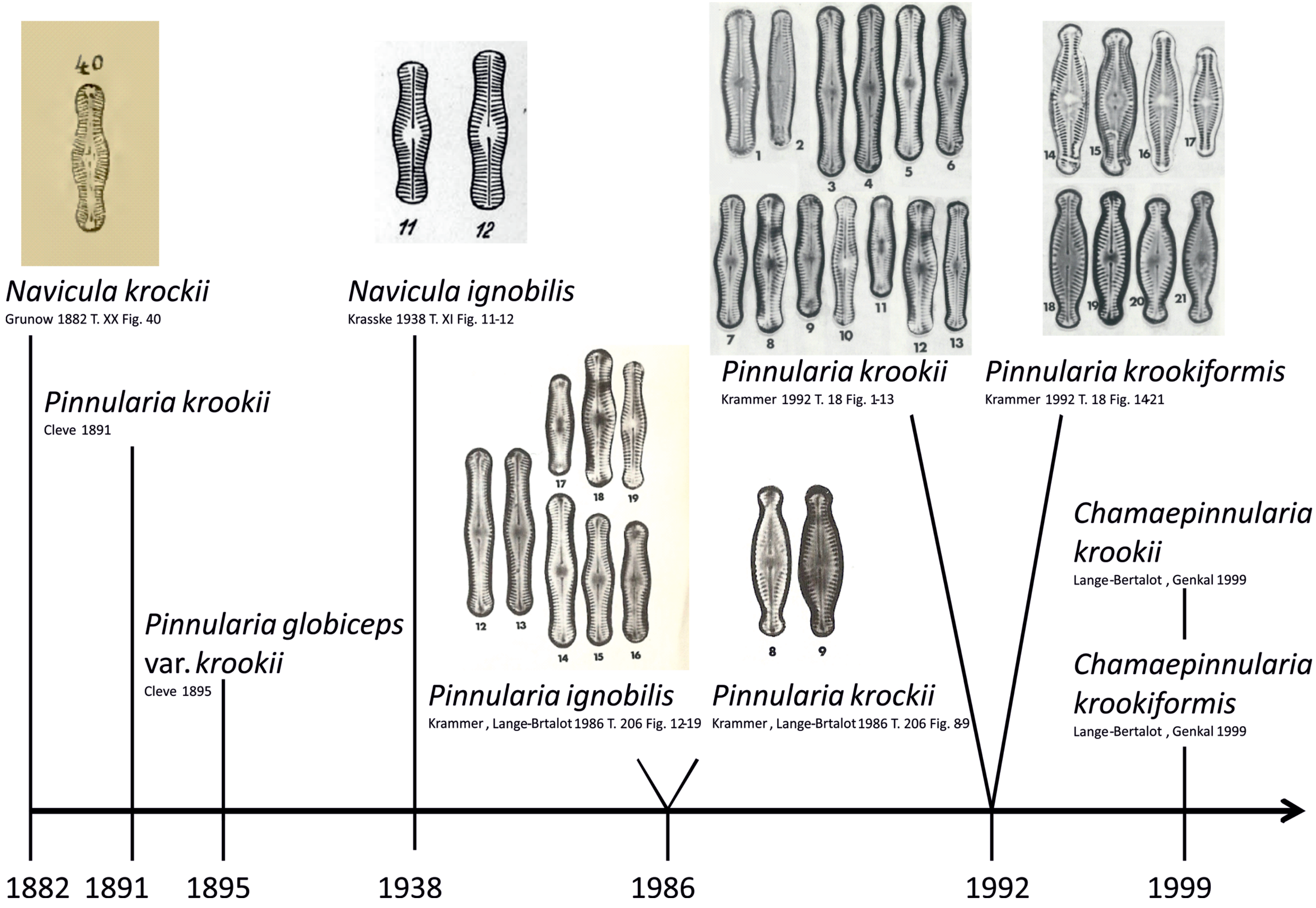


Fig. 1. Timeline of interpretations of the *Chamaepinnularia krookiformis* species.

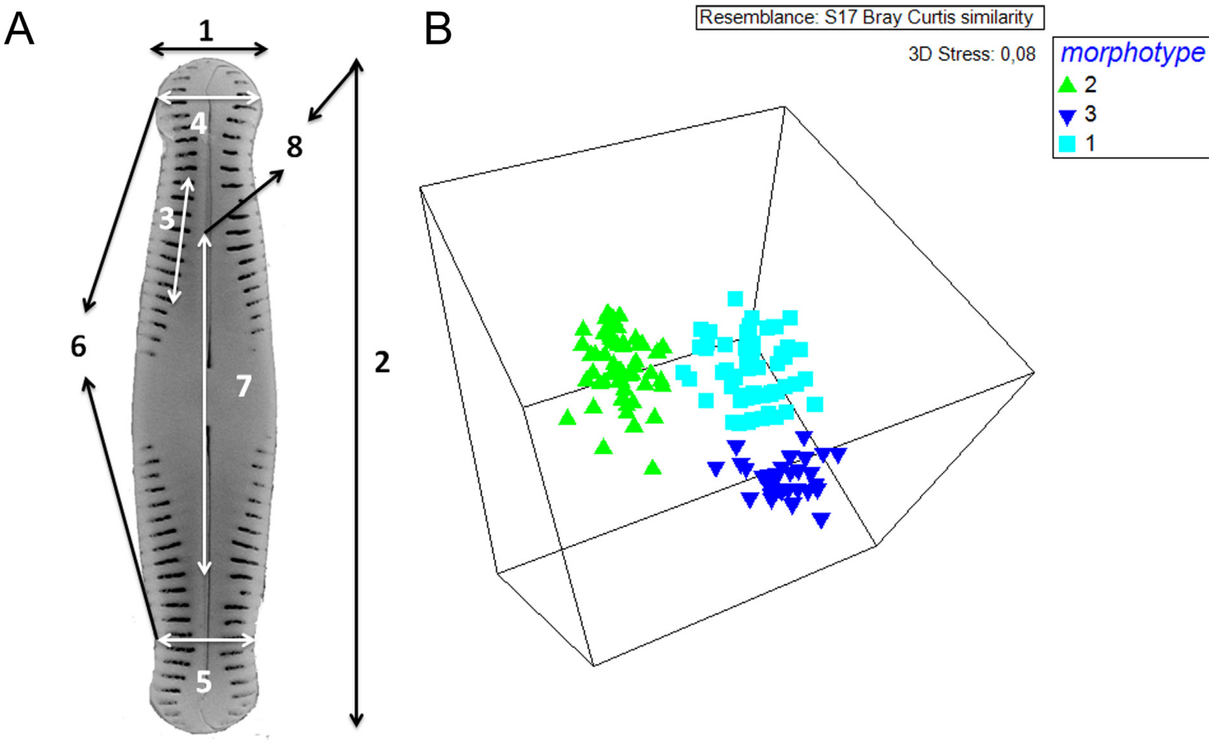


Fig. 2. The MDS procedure distinguished three groups of specimens based on eight features: (A) morphological features: (1) valve width and (2) length, (3) number of striae in 10 µm, (4) width of capitate apices, (5) width of constrictions, (6) ratio of apices width to constrictions width, (7) length of central area, and (8) ratio of the valve length to the central area length; (B) MDS analysis revealed three morphotypes of *Chamaepinnularia krookiformis*.

to 19.49 $mg.l^{-1}$. This species was noted in the costal shallows of Puck Bay (Witkowski 1994, p. 176–177, plate XXXVIII, fig. 16–17) where the concentration of chloride ions was higher. Żelazna–Wieczorek (2011) identified several specimens of this species in samples from springs; however, in samples from the Puck Bay the presence of this species was noted from rare to frequent (Witkowski 1994). In the present research, the species percentage exceeded 10% in samples from the ditch. Since *Chamaepinnularia plinskii* occurs largely in waters with an increased concentration of chloride ions (over 1000 $mg.l^{-1}$ Cl^{-}), according to the halobion system of Van der Werff and Huls 1957–1974 (Denys et al. 1983; Żelazna–Wieczorek et al. 2015) *Chamaepinnularia plinskii* can be classified as a brackish species (the 4th category of salinity). *Chamaepinnularia plinskii* occurred in a habitat which dries up periodically. According to Van Dam et al. (1994), the classification of this species can be described as typical for wet and moist or temporarily dry habitats (the 4th category of moisture).

Chamaepinnularia krookiformis **(Krammer) Lange–Bertalot et Krammer 1999 (tafel 45, fig. 6–10)**

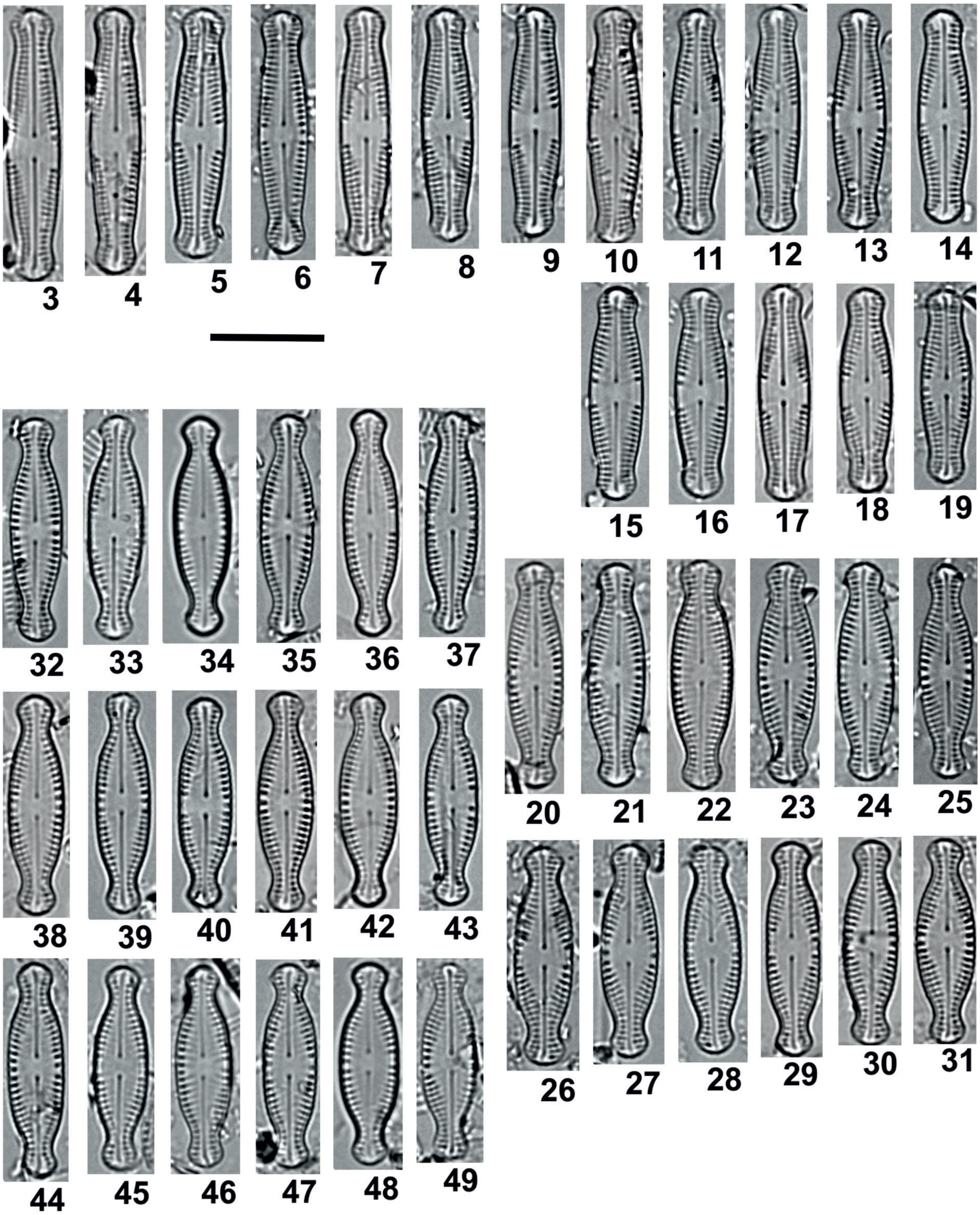
Description species according to Krammer 1992

Valve length 14–40 µm; valve width 5–11 µm; number of striae 17–21/10 µm; ratio of the length to the width 2.8–4. The valve is elliptic in shape, convex, ends of the valve are rounded with constrictions. The axial area is narrow; the central area is wide and of rhomboid–elliptic shape. The raphe is straight and filiform.

Based on the MDS analysis two morphological forms defined as: Morphotype 2 and Morphotype 3, *Chamaepinnularia krookiformis* have been distinguished (Fig. 2B). Morphotype 2 (Fig. 20–31) and Morphotype 3 (Fig. 32–49).

Description: LM: the main differentiating feature of Morphotype 2 from Morphotype 3 is the central area length and width. In Morphotype 2, the central area dimensions are 6–10 µm in length and 2–3.5 µm in width. In specimens belonging to Morphotype 3, the central area dimensions are 11–14 µm in length and 2.5–5.0 µm in width; the ratio of the valve length to the central area length is 1.4–1.9. Morphotype 3 has more capitate apices than Morphotype 2 (Table 2).

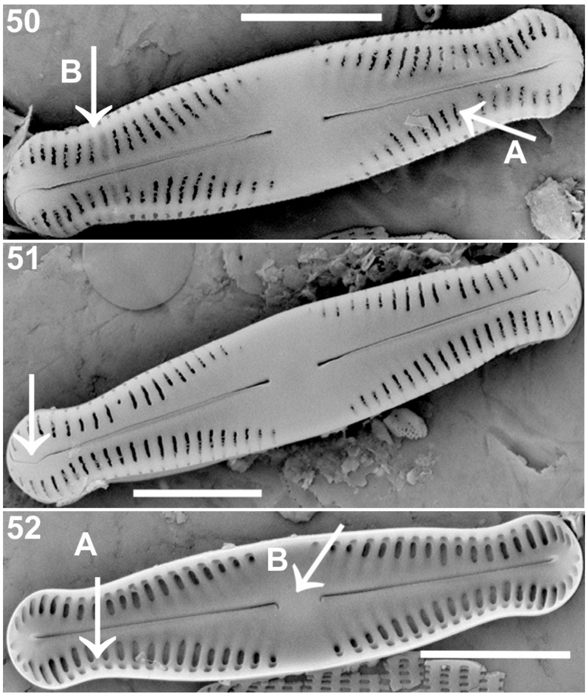
SEM: Externally, striae are composed of simple, chamber–like areole, with external openings covered by vela. The valve striae are separated from the mantle striae by a hyaline area along the entire valve circumference. The raphe is straight, its proximal endings have a tear drop shape. Distal raphe endings are hooked in the case of Morphotype 2 (Figs 53–55), while they are strongly hooked in the case of Morphotype 3 (Figs. 56–58). Internal view: striae make foramina, they are arranged in the same way as those on the ex-



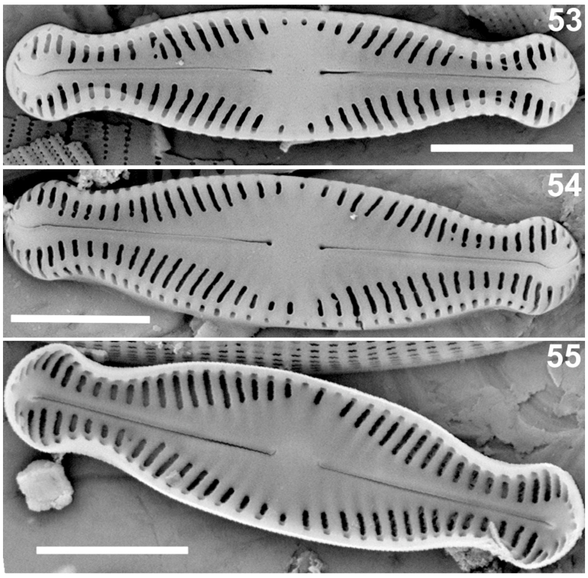
Figs 3–49. LM photomicrographs of *Chamaepinnularia* species: (3–19) *Chamaepinnularia plinskii* sp. nov. (Morphotype 1); (20–31) *Chamaepinnularia krookiformis* – Morphotype 2; (32–49) *Chamaepinnularia krookiformis* – Morphotype 3. Scale bar 10 µm.

ternal side. Striae on the valve face are interrupted and separated by a silica bridge to form two parts, the first one is small and rounded and is located on the valve face–mantle junction, the second one is elongated, and located on the valve face. Raphe is straight, distal endings form a helictoglossa, the proximal ones are hooked in the same direction.

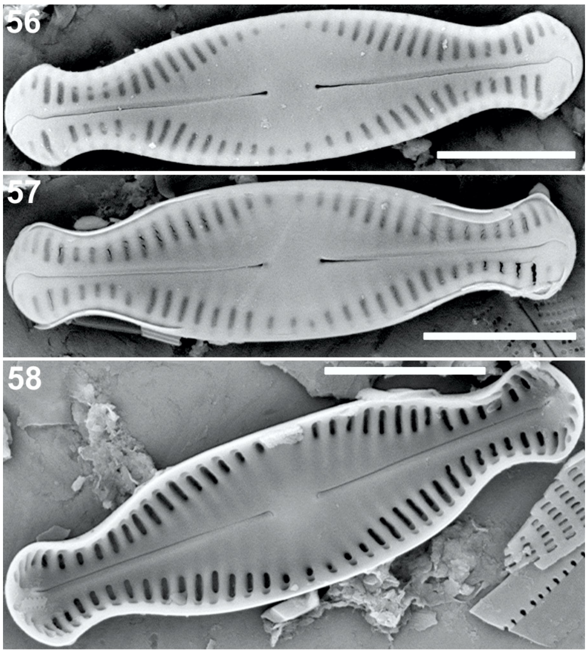
Specimens from the type material of *Pinnularia krookiformis* correspond to both morphotypes in the recent material: Morphotype 2 (Figs 62–69) and Morphotype 3 (Figs 70–77). The analysis of morphological features of Morphotypes 2 and 3 in SEM (Figs 59–61) allows a high similarity between the type and recent material to be confirmed. However, the valve dimensions in the



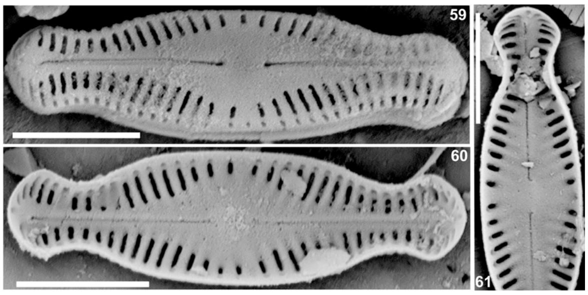
Figs 50–52. SEM images of *Chamaepinnularia plinskii* sp. nov. from Pełczyska (Central Poland): (50) External view, (arrow A) striae composed of chamber–like areola and covered by vela, (arrow B) striae on the valve face separate from the mantle striae by a hyaline area; (51) external view, (arrow) distal raphe endings hooked, curving down to the mantle; (52) internal view, (arrow A) silica bridge, divided areola, (arrow B) thick sternum forming a fascia. Scale bars 5 µm.



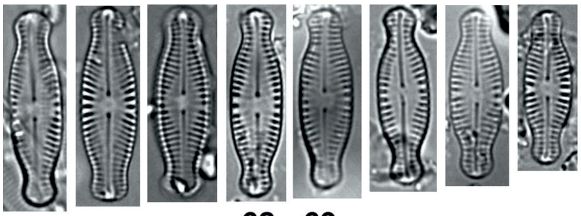
Figs 53–55. SEM images of *Chamaepinnularia krookiformis* – Morphotype 2 from Pełczyska (Central Poland): (53–54) internal view, chamber–like areola, tear–drop–shaped proximal raphe endings, distal raphe endings hooked; (55) internal view, distal raphe endings form helictoglossa, the proximal ones are hooked and turned towards the same valve edge. Scale bars 5 µm.



Figs 56–58. SEM images of *Chamaepinnularia krookiformis* – Morphotype 3 from Pełczyska (Central Poland): (56–57) internal view, chamber–like areola, tear–drop–shaped proximal raphe endings, distal raphe endings strongly hooked; (58) internal view, distal raphe endings form helictoglossa, the proximal ones are hooked and turned towards the same side of the valve. Scale bars 5 µm.



Figs 59–61. SEM photomicrographs of the type material of *Pinnularia krookiformis* Krammer from Germany [=*Chamaepinnularia krookiformis* (Krammer) Lange–Bertalot et Krammer 1999]: (59) valve external view, (60) valve internal view; (61) valve internal view with hooked distal raphe endings. Scale bars 5 µm.



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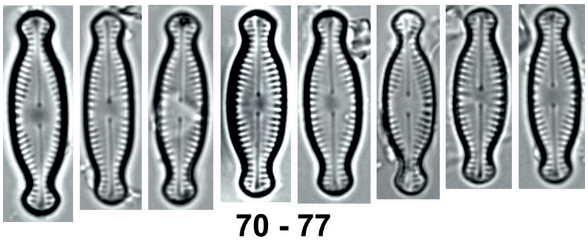

70 - 77

recent and type material are narrower (length 14–22 µm and width 4–5 µm) than those given in the description by Krammer (1992) for *Pinnularia krookiformis* (lenght 14–40 µm, width 5–11 µm).

Summary

In the athalassic habitats studied, a wide morphological variability of forms earlier classified as *Chamaepinnularia krookiformis* was observed. The analysis allowed three morphotypes to be distinguished. Morphotype 1 was distinguished as a new species, *Chamaepinnularia plinskii,* based on the following most important features: the presence of a wide fascia reaching the valve margin, number of striae in 10 µm, wider apical constrictions, and a lower ratio of valve length to central area width. However, features differentiating the two Morphotypes (2 and 3) have not provided a sufficient basis for distinguishing them as separate species.

Chamaepinnularia plinskii and *C. krookiformis*, occurred in the same habitats in Pełczyska. In Poland, the species were noted in various types of ecosystems as *Chamaepinnularia krookiformis*; however, the analysis of micrographs in publications enables their verification and classification as *Chamaepinnularia krookiformis* and *C. plinskii.* Żelazna–Wieczorek (2011) observed both species in the springs of the Łódź Hills, i.e.: *Chamaepinnularia plinskii* as *C.* cf. *krookiformis* (plate 103, fig. 34) and *Chamaepinnularia krookiformis* – Morphotype 3 (plate 103, figs 35–36). Wojtal (2009) noted *Chamaepinnularia krookiformis* in the Kobylanka River; however, the photomicrographs documentation does not provide sufficient information for the correct classification of Morphotypes 2 and 3 (p. 164, plate 26, fig. 5; plate 82, fig. 3). In Puck Bay, Witkowski (1994) could also observe the occurrence of *Chamaepinnularia krookiformis* Morphotype 2 (plate XXXVIII, figs 14–15) and *Chamaepinnularia plinskii* (Plate XXXVIII, Figs 16–17).

Chamaepinnularia plinskii is a species occurring in various types of aquatic ecosystems which dry up periodically; however, conditions favorable for its development are alkaline water with high chlorides concentrations.

← Figs 62–77. LM photomicrographs of type material of *Pinnularia krookiformis* Krammer from Germany [=*Chamaepinnularia krookiformis* (Krammer) Lange–Bertalot et Krammer 1999]: (62–69) LM photomicrographs of specimens corresponding to *Chamaepinnularia krookiformis* Morphotype 2 from recent material; (70–77) LM photomicrographs of specimens corresponding to *Chamaepinnularia krookiformis* Morphotype 3 from recent material. Scale bar 10 µm.

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Oświadczenie o współautorstwie

Oświadczam, że mój wkład w przygotowanie oryginalniej pracy polegał na: przygotowaniu koncepcji pracy, zaplanowaniu prac w terenie, identyfikacji taksonomicznej, opracowaniu wyników z wykorzystaniem metod matematycznych. Udział w dyskusji i interpretacji uzyskanych wyników i w przygotowaniu ostatecznej wersji manuskryptu. Swój udział w przygotowanie pracy oceniam na 50%.

dr hab. Joanna Żelazna-Wieczorek prof. UŁ
Katedra Algologii i Mykologii

Oświadczam, że mój wkład w przygotowanie oryginalniej pracy polegał na: przygotowaniu koncepcji pracy, zaplanowaniu i realizacji prac w terenie, identyfikacji taksonomicznej, przygotowanie dokumentacji fotograficznej. Udział w analizie i interpretacji uzyskanych wyników. Pracowałem również na ostateczną wersją manuskryptu. Swój udział w przygotowanie pracy oceniam na 50%.

mgr Rafał M. Olszyński

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Aulacoseira pseudomuzzanensis sp. nov. and other centric diatoms from post iron ore mining reservoirs in Poland

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Post iron ore mining reservoirs in Łęczyca (Poland) can be considered extreme habitats because they are characterized by high pH, conductivity, and sulfate, sodium and potassium concentrations. We observed high abundances in these water bodies of several centric diatom species that are otherwise known from less extreme environments. These included *Thalassiosira duostra*, *Cyclostephanos dubius*, *Stephanodiscus binatus*, *Stephanodiscus invisitatus, Stephanodiscus parvus*, *Stephanodiscus hantzschii*, *Lindavia* aff. *balatonis*, *Discostella woltereckii*, *Discostella pseudostelligera*, and *Cyclotella meneghiniana*, revealing the eurytopic nature of these species. Morphological differences between populations from extreme habitats and their counterparts from less extreme environments may indicate phenotypic responses of eurytopic taxa to the differing environmental regimes, or the presence of distinct, more stenotopic taxa. In both cases, this could be relevant for biomonitoring. For this reason, we carefully observed the morphological variability of our target taxa in the Łęczyca reservoir samples compared with literature accounts from less extreme habitats, and in some cases, with type material of the species concerned. Specimens similar to *Aulacoseira muzzanensis* α-chains were observed. Following comparison with the type material of *Melosira muzzanensis* Meister ($\equiv$ *A. muzzanensis*), we concluded that the Łęczyca populations are sufficiently different to be recognized as a separate species, here described as *Aulacoseira pseudomuzzanensis* sp. nov. The importance of identifying centric diatoms to species level for water management surveys is emphasized, even for those taxa that are difficult to identify using only LM.

Keywords: Aulacoseira*, centric diatoms, morphology, autecology, post-mining reservoirs, new species*

Introduction

Diatoms are the most abundant group of autotrophic microorganisms commonly inhabiting different types of freshwater ecosystems, both natural, such as springs, rivers, lakes, and ponds, and those of anthropogenic origin, such as post-mining reservoirs, dam reservoirs, and small artificial ponds. To increase our knowledge of the morphological and ecological features of diatoms, the most interesting artificial water ecosystems are post-mining reservoirs, created by flooding the depleted mineral deposits of open-pit mines. This results in unique aquatic ecosystems with specific hydro-chemical compositions related to the excavated deposits (Jankowski et al. 2005).

Due to their origin, post-mining reservoirs are characterized by unique physico-chemical conditions, impacted by minerals within the basin of any given reservoir. The geological bed of post-mining reservoirs affects the concentration of individual ions, including cations and heavy metal salts in the reservoirs, but the ion concentration is also influenced by the water chemistry. When pH is high, heavy metal cations are bonded to salts, and thus their presence in water may become undetectable. On the other hand, low pH allows heavy metal salts to be dissolved, releasing cations into the water and resulting in higher heavy metal ion concentrations (Wright et al. 2007). When diatoms in such environments are exposed to heavy metals abnormal valve development and teratological forms can ensue (Medley & Clements 1998, Falasco et al. 2009a, 2009b, Anantharaj et al. 2011, Pandey et al. 2014).

Diatoms have often been used as indicators of changes in the environmental conditions of running waters polluted by mine drainage (DeNicola & Stapleton 2002, John 2004, Smucker & Vis 2009, Herlory et al. 2013, Valente et al. 2016). Some studies of post-mining reservoirs have been related to diatom paleoecology and their role as indicators of past climatic or environmental change (de Haan et al. 1993, Rakowska 1996, Thomas & John 2006, Sienkiewicz & Gąsiorowski 2016). Until now, the autecology of diatoms in post-mining reservoirs, characterized by specific chemical compositions, has rarely been studied (Van Landingham 1968, de Haan et al. 1993, Rakowska 1996, Sienkiewicz & Gąsiorowski 2016).

In standing freshwater ecosystems, centric diatoms commonly occur in large numbers. Most of them are euplanktonic (e.g. *Aulacoseira muzzanensis* (Meister) Krammer, *Cyclostephanos dubius* (Fricke) Hustedt,

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Lindavia radiosa (Grunow) De Toni & Forti) or tychoplanktonic (e.g. *Cyclotella meneghiniana* Kützing, *Discostella woltereckii* (Hustedt) Houk et Klee) forms of benthic origin (Denys 1991). In shallow, standing water ecosystems, planktonic forms and forms related to the substratum occur together in benthic samples (DeNicola & Stepleton 2002, Szabó et al. 2004, Tanaka 2007, Reavie & Kireta 2015). However, centric diatoms are frequently only identified as far as genus, particularly when such diatoms are scarce in any given sample. This applies particularly to those forms that are especially difficult to identify by light microscopy, such as *Stephanodiscus binatus* Håkansson et Kling, *Stephanodiscus minutulus* (Kützing) Cleve & Möller and *Stephanodiscus parvus* Stoermer et Håkansson (Håkansson 2002, Houk et al. 2014). The morphological features used to differentiate species are often only visible with scanning electron microscopy (SEM), i.e. the morphology of areolae, fultoportulae and rimoportulae (Håkansson 2002, Houk et al. 2014).

In order to identify diatoms accurately, which is the basis of ecological research, including biomonitoring, it is necessary to determine the intraspecific variation, the variability of specimens within a population and variability between populations from different locations (Houk 2003). In our opinion habitats characterized by extreme physicochemical conditions, such as post-mining reservoirs, create an opportunity to identify possible morphological change with respect to a given habitat.

The aims of this study were to: (1) investigate the species diversity and morphological variability of centric diatoms in post-mining reservoirs; (2) reveal the morphological distinction of *Aulacoseira pseudomuzzanensis* sp. nov. from the type material of *Melosira muzzanensis Meister*; (3) verify the environmental requirements of some centric diatoms.

Material and methods

Study area and hydro-chemical background

The study was conducted in three shallow reservoirs created by flooding an open-pit iron ore mine in Łęczyca (Łódź Province, Central Poland) (Fig. 1). The reservoirs are connected to each other by small canals. The mine operated until the early 1990s (Solarski 2015). Sphaerosiderite, siderite clay and other siderite ores had been excavated from a large geological structure of the Kłodawsko-Łeczycka anticline, which also contained salt from Jurassic and Zechstein formations. Siderite iron ores are accompanied by calcite and highly pyritized carbon (Ziomek 2008).

The water temperature, pH, and conductivity were measured *in situ* (Elmetron CP-401 and CC-401 devices) and water samples for chemical and physical analyses were collected and taken to the Laboratory of Geology, at the Faculty of Geographical Sciences, University of Łódź, where the analyses were performed. The chemical

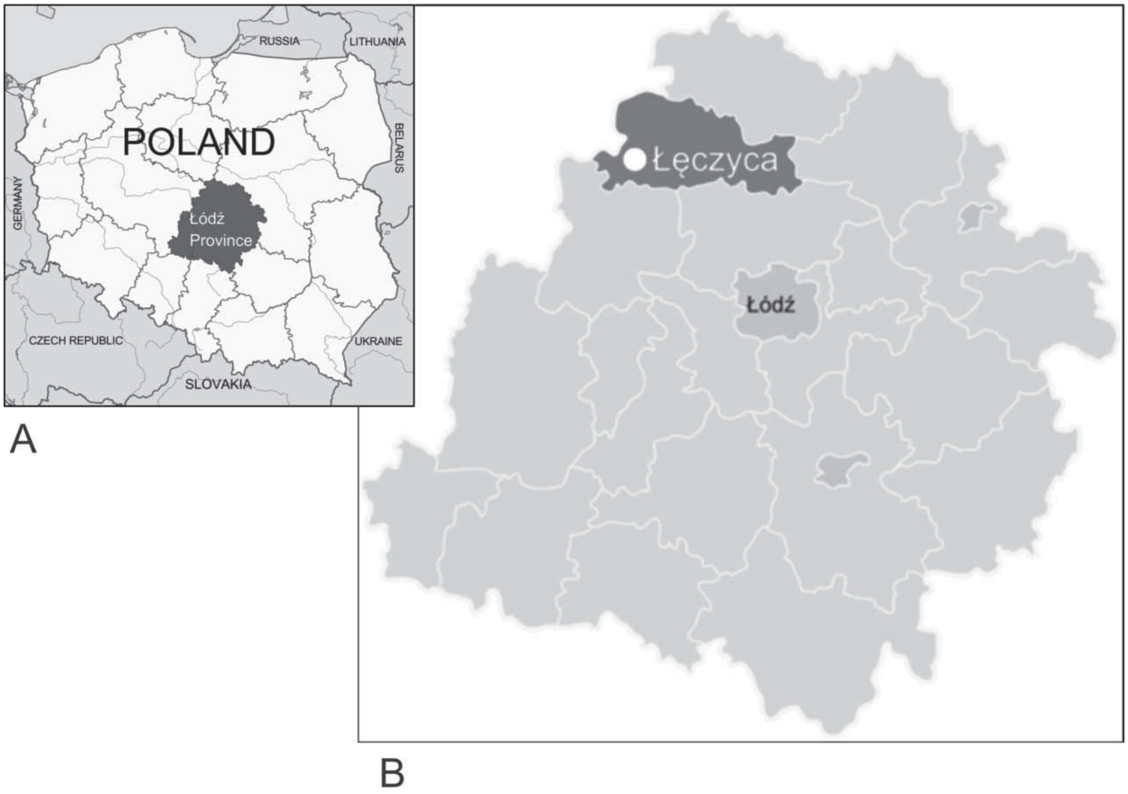


Fig. 1. Location of sampling sites, **A**: location of Łódź Province, **B**: Łódź Province; white point: Łęczyca.

Table 1. Range of physical and chemical parameters of water from Łęczyca reservoirs (two seasons); UND – undetectable.

	Łęczyca 1	Łęczyca 2	Łęczyca 3
pH	7.9–8.6	7.5–8.6	7.5–8.8
SEC (µS cm^{-1})	649.0–865.0	558.0–836.0	472.0–778.0
T (°C)	1.6–20.6	0.6–21.1	2.2–22.4
HCO^{3-} (mg L^{-1})	164.7–338.6	167.8–274.5	183.0–289.8
$CO_2[HCO^{3-}]$ (mg L^{-1})	59.4–122.1	60.5–99.0	66.0–104.5
Cl^- (mg L^{-1})	57.4–92.8	59.9–92.5	67.1–105.5
N-NH_4 (mg L^{-1})	UND–0.7	UND–0.4	UND–1.2
NH^{4+} (mg L^{-1})	UND–0.5	UND–0.4	UND–0.9
$PO_4{}^{3-}$ (mg L^{-1})	0.2–0.5	0.1–0.5	0.1–0.6
P-PO_4 (mg L^{-1})	0.1–0.2	0.1–0.2	UND–0.2
$SO_4{}^{2-}$ (mg L^{-1})	70.5–146.9	84.1–122.4	62.9–136.2
S-SO_4 (mg L^{-1})	23.5-49.0	28.1–40.8	21.0–45.4
Mn_{tot} (mg L^{-1})	UND–0.1	UND	UND–0.1
$Fe^{2+/3+}$ (mg L^{-1})	UND–0.3	UND–0.1	UND–0.3
Mg^{2+} (mg L^{-1})	11.6–19.1	14.5–18.3	9.5–15.3
Ca^{2+} (mg L^{-1})	42.9–104.5	50.5–117.6	48.0–86.4
Na^+ (mg L^{-1})	15.6–42.0	20.3–44.2	22.0–42.4
K^+ (mg L^{-1})	4.5–9.5	5.1–8.5	5.6–10.7

and physical parameters are shown in Table 1. The studied reservoirs are characterized by alkaline water, high conductivity, increased sulfate, sodium, and potassium concentrations.

Diatom preparation and identification

The phytobenthos samples were collected every three months from March 2014 to December 2015. The benthic samples were collected with a glass pipette in a plastic 100 mL container and transported to the laboratory. Qualitative and quantitative analyses of diatoms were conducted on permanent slides.

The following species were also examined from material in the Hustedt collection (BRM): *Melosira muzzanensis* Meister (≡*A. muzzanensis* (Meister) Krammer) from Lago di Muzzano, Italy E1289, *Cyclotella pseudostelligera* Hustedt (≡*Discostella pseudostelligera* (Hustedt) Houk et Klee) from Ems, Station 197, Profil L, nahe Neue Schleuse bei Papenburg E524 and *Cyclotella woltereckii* Hustedt (≡*D. woltereckii* (Hustedt) Houk et Klee) from Java, Butenzorg C. 98 Teich in Motanischen Garten, Wallacea-Expedition AS1329. These were held as wet unmounted material in formalin and glycerin. Before preparing permanent slides, the samples were first washed in 95% ethyl alcohol and then several times in deionized water. Permanent slides were mounted with Naphrax® according to Żelazna-Wieczorek (2011).

Light microscopy (LM)

The morphological analysis of diatom valves was conducted by examining 24 permanent slides using a Nikon Eclipse 50i light microscope under 1000 × magnification (plan oil-immersion objective 100 × /1.25). Light photomicrographs were taken with the OPTA-TECH digital camera.

Linking and separation valves from the type material of *M. muzzanensis* and specimens from the Łęczyca reservoirs were measured individually. The following features were measured: valve face diameter or (and) mantle width, mantle height (MH), number of striae in 10 µm, number of areolae in 10 µm and number of rimoportulae. To determine the ratio of linking to separation valves, 260 valves were counted: 100 of the type material and 160 of Łęczyca reservoirs, from two seasons (early spring and summer).

SEM observation

The morphological features in the SEM observations were determined from cleaned material that had been air-dried on aluminium stubs and examined in a Phenom Pro X (gold layer of 8 nm, at 10 kV) scanning microscope at the Laboratory of Microscopy Imaging & Specialist Biological Techniques, Faculty of Biology & Environmental Protection, University of Łódź. SEM photomicrographs were taken with a resolution of 1024 × 1080 pixels.

Results and discussion

Aulacoseira pseudomuzzanensis Olszyński & Żelazna-Wieczorek sp. nov. (Figs 2–52)

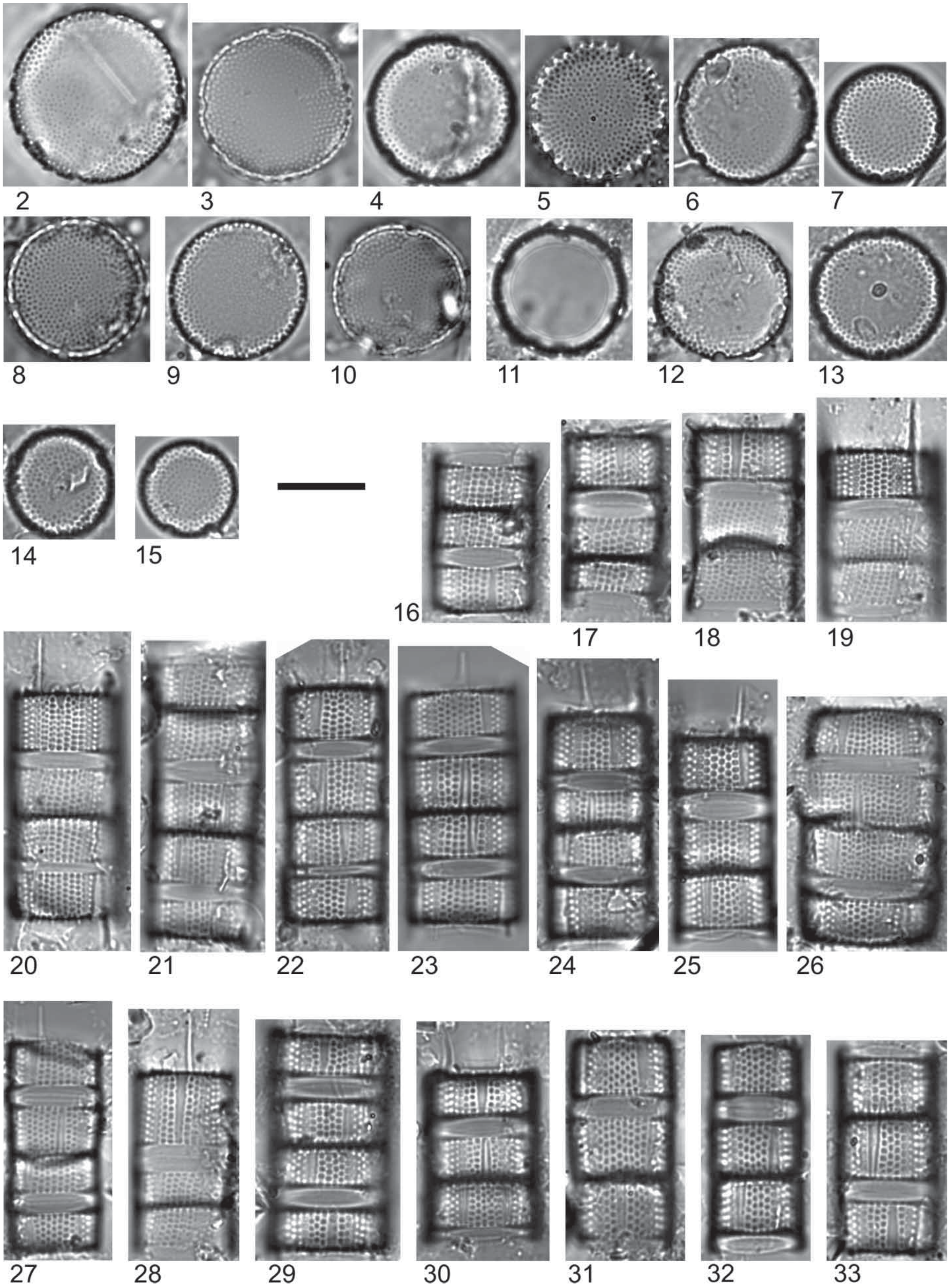
Synonyms: *Aulacoseira muzzanensis* (Meister) Krammer in: Krammer 1991, figs 10, 10a, 12, 12a, 13, 14, 17, 17a.

Aulacoseira muzzanensis (Meister) Krammer in Krammer & Lange-Bertalot 1991, tafel 20, figs 1, 7, 8.

Aulacoseira cff. *muzzanensis* in Houk 2003, tab XXVII, figs 1–7.

Description

LM observations: Valve diameter: 10–20 µm (Figs 2–15), MH: 5–9 µm (Figs 16–33), MH/VD: 0.37–0.86, striae: 12–16 in 10 µm, areolae arranged in spiral rows, running dextrorse on the mantle: 12–15 in 10 µm on linking valves



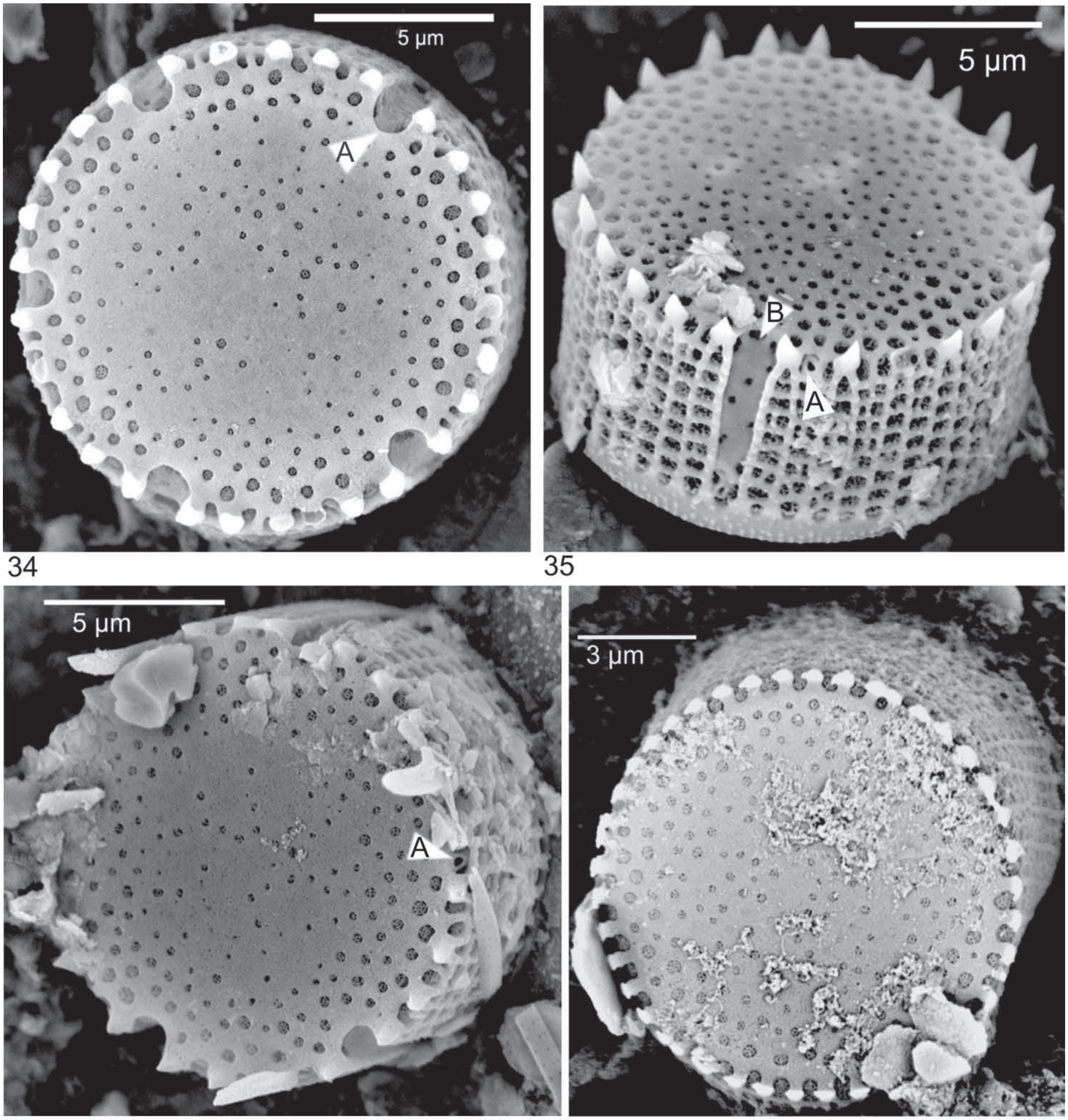
Figs 2–33. LM photomicrographs of *Aulacoseira pseudomuzzanensis* sp. nov. Olszyński & Żelazna-Wieczorek. **Figs 2–15.** Valve view. **Figs 16–33.** Girdle view. Scale bar = 10 µm.

or running straight: 12–14 in 10 µm on separation valves. The collum is usually narrow (Figs 16–33).

Separation valves: Diameter (VD): 11.3–20.0 µm, MH: 5.9–8.0 µm (Figs 16–33), ratio of MH to valve diameter (MH/VD): 0.37–0.64, pervalvar rows of areolae: 14–16 in 10 µm (Figs 16–33), areolae: 12–14 in 10 µm (Figs 16–33). Striae are parallel to the edge of the mantle (Figs 16–33).

Linking valves: Diameter: 10.0–15.0 µm (Figs 16–21, 28), MH: 5.0–9.0 µm (Figs 16–21, 28), MH/VD: 0.47–0.86, striae: 12–15 in 10 µm (Figs 16–21, 28), areolae arranged in spiral rows, running dextrorse on the mantle: 12–15 in 10 µm (Figs 16–21, 28).

Over 80% of the *A. pseudomuzzanensis* valves were separation valves.



Figs 34–37. SEM, external views of *Aulacoseira pseudomuzzanensis* sp. nov. Olszyński & Żelazna-Wieczorek. **Fig. 34.** The valve face of separation valve. Arrow A: groove from long separation spine. **Fig. 35.** The valve face/mantle junction of separation valve. Arrow A: rimoportula opening. Arrow B: groove from separation spine. **Fig. 36.** The valve face of separation valve. Arrow A: rimoportula opening. **Fig. 37.** The valve face of linking valve.

SEM observations

External views: The valve face of separation valves is circular and flat. The areolae on the valve face are scattered, larger at the edge of the valve face, becoming smaller and less dense toward the valve centre (Figs 34–36). The areolae on the mantle are rectangular or more or less circular, arranged parallel to the pervalvar axis and composed of one row of areolae (Figs 35, 43, 44, 46). The areolae on the valve face and mantle are occluded. At the valve face/mantle junction, at the end of every second areola, short triangular separation spines are located, rising from two pervalvar ribs (Figs 35, 43, 44, 46). Long separation spines/grooves (four or more) are triangular; they may be as long as the mantle is high and are located at the end of one row of areolae (Figs 34–36, 43, 44, 46). External rimoportula openings (from one to three) are observed between separation spines at the valve face/mantle junction near the ringleiste (Figs 35–36, 43).

The valve face of linking valves is circular and flat. The valve face areolae are scattered, larger at the edge of the valve face, becoming smaller and less dense toward the valve centre (Fig. 37). The mantle areolae are rectangular or more or less circular, arranged dextrorse to the pervalvar axis and composed of one row of areolae (Fig. 45). The areolae on the valve face and mantle are occluded (Figs 37, 45). At the valve face/mantle junction, bifurcated linking spines are located, rising from one pervalvar rib (Fig. 45). However, the shape of the linking spines can vary markedly from bifurcated (Fig. 45), Y-shaped (Fig. 47), spathulate (Fig. 48), tridentate (Fig. 49), forked (Fig. 50), to strongly forked (Figs 51, 52). The external rimoportula openings are small, located near the ringleiste (Fig. 45).

Internal views: The areolae on the valve face and mantle are occluded (Fig. 40). The ringleiste is narrow (Figs 38, 39, 41). One to three stalked rimoportulae with coiled openings are present near the valve face/mantle junction and the ringleiste (Figs 38–42).

Holotype: Slide: D.ŁEP1.031214 No. 00214. Algae Collection Laboratory of Algology and Mycology, University of Łódź. Holotype illustrated in Fig. 20 (designated here).

Isotype: Slide No. Zu10/98 BRM.

Type locality: POLAND. Łódź Province, Łęczyca. Post-iron ore mining reservoirs.

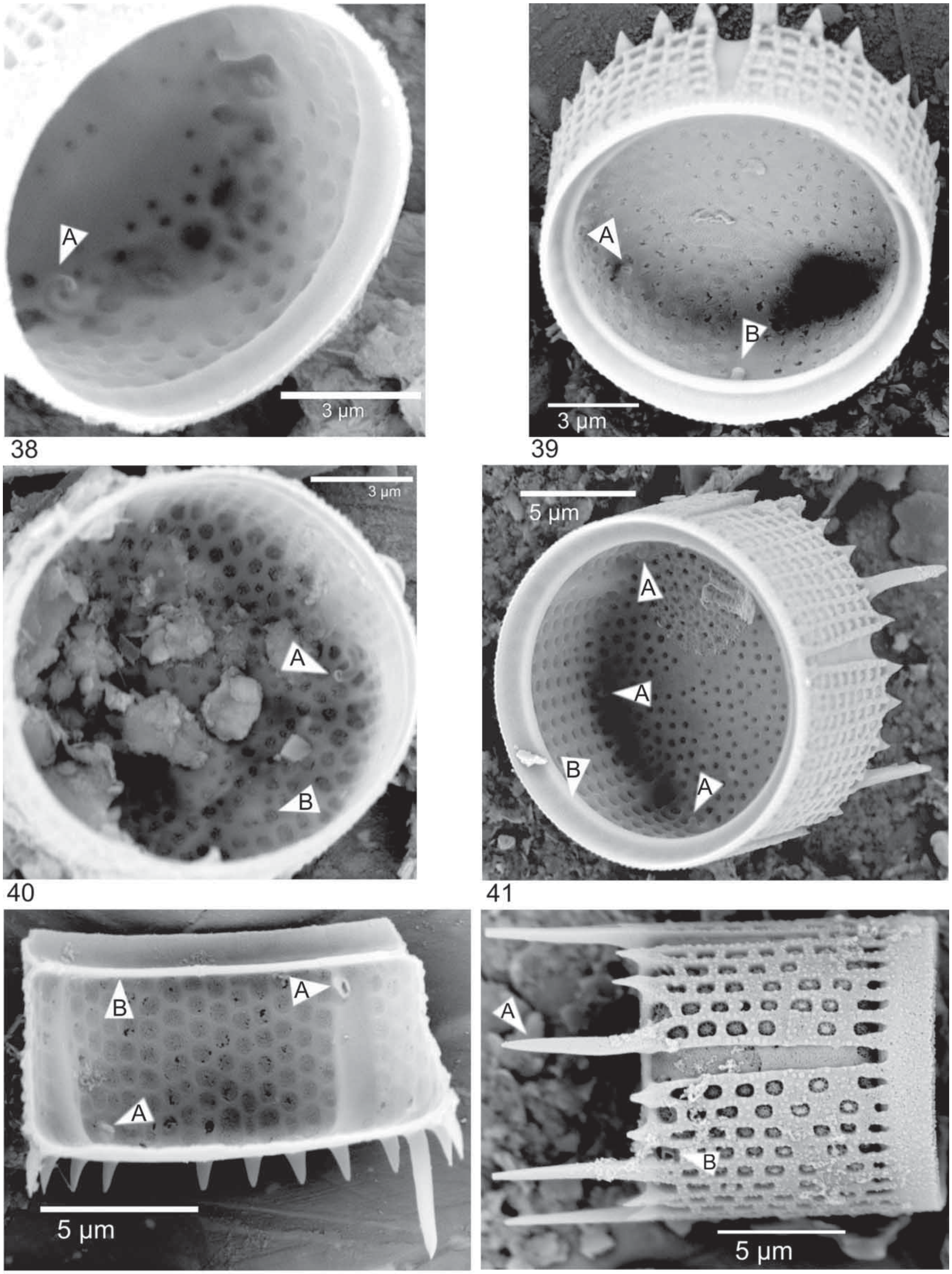
Etymology: The specific epithet *pseudomuzzanensis* refers to the similarity in the valve morphology with *A. muzzanensis*.

Differential diagnosis and discussion

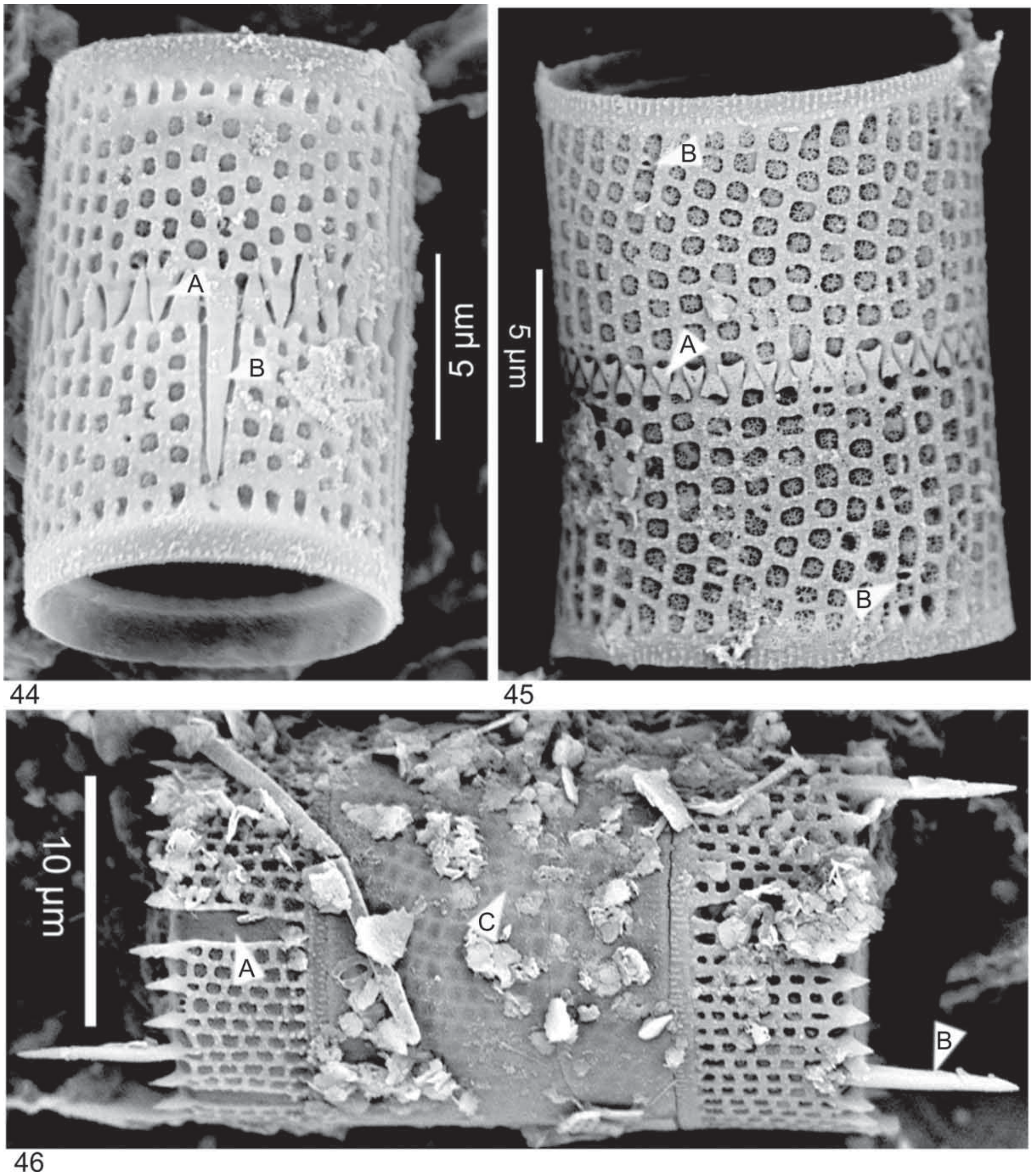
Aulacoseira was described by Thwaites (1848); however, the description of the new genus was insufficient. Crawford (1981) provided a detailed description of *Aulacoseira* based the type material of Thwaites and other samples. He (Crawford 1981) also noticed that, in some cases specimens had two different types of valves, one with linking spines, the other with separation spines. The ratio of linking to separation valves determines the length of the chain. Davey (1986, 1987) showed the interrelationship between the numbers of separation valves and seasons of the year. During autumn and summer diatom blooms, the number of separation valves was lower than in spring loss periods (Davey 1986, 1987). The morphology of linking valves and separation valves can be different in particular species (Round et al. 1990, Siver & Kling 1997, Houk 2003).

The first description of *M. muzzanensis* contained drawings of the valve face without any ornamentation at the valve centre, which was slightly oblique to the pervalvar axis (Meister 1912). Krammer (1991) transferred *M. muzzanensis* to *Aulacoseira* and observed two morphs in the type material: 'status τ' corresponding to the Meister drawings, and 'status α' with different morphology. The latter corresponds to specimens from post-iron ore mining reservoirs in Łęczyca. Tremarin et al. (2012) analysed the type material of *M. muzzanensis* and revealed ornamentation, not at the centre of the valve face, but only at its edge. The authors also noticed that the rows of areolae on the mantle have a spiral dextrorse pattern; therefore, *A. muzzanensis* 'status α' (Krammer 1991) represents *A. pseudomuzzanensis*.

The morphological features of *A. pseudomuzzanensis* and *A. muzzanensis* are compared in Table 2. The most significant differentiating features (Figs 53–67) visible in LM are the lower density of areolae in *A. pseudomuzzanensis* and invisible rimoportulae. *Aulacoseira muzzanensis* has a flat valve face with areolae only at edge (Fig. 70), unlike *A. pseudomuzzanensis* which has areolae over the entire surface of the valve face; the striae on separation valves are less dense in *A. pseudomuzzanensis*. The differential morphology of the striae was observed using both SEM and LM. The striae of *A. muzzanensis* are denser (14–19 in 10 µm) and finely structured, composed of one to two rows of areolae (Figs 68, 71), while in *A. pseudomuzzanensis,* they are coarsely structured, composed of one row of areolae (Figs 43–46). The Y-shaped linking spines of *A. pseudomuzzanensis* differ from those of *A. muzzanensis* (Figs 68, 71–73). Separation spines/grooves of both species are as long as the MH (Fig. 70). The internal rimoportula opening of *A. pseudomuzzanensis* is coiled while that of *A. muzzanensis* is larger and strongly coiled (Fig. 69); the external openings are small in both (Fig. 68). *A. pseudomuzzanensis*-like morphology (number of areolae on the mantle, ornamentation on the valve face and parallel rows of areolae on the mantle) had also been noted by Krammer & Lange-Bertalot (1991, tafel 20, fig. 7, 8), Houk (2003, tab. XXVII. figs 1–7), Potapova & English (2011) and Kiss et al. (2012).



Figs 38–43. SEM, internal (**Figs 38–42**) and external (**Fig. 43**) view of images of *Aulacoseira pseudomuzzanensis* sp. nov. Olszyński & Żelazna-Wieczorek. **Fig. 38.** The valve face/mantle junction. Arrow A: coiled rimoportula. **Fig. 39.** The valve. Arrow A: rimoportula opening at the valve valve/mantle junction. Arrow B: rimoportula opening near the ringleiste. **Fig. 40.** The valve. Arrow A: coiled rimoportula opening. Arrow B: areolae covered by cribra. **Fig. 41.** The valve. Arrows A: rimoportula openings. Arrow B: the ringleiste. **Fig. 42.** The valve. Arrows A: rimoportula openings. Arrow B: the ringleiste. **Fig. 43.** The mantle. Arrow A: long separation spine. Arrow B: rimoportula opening.



Figs 44–46. SEM, external views of *Aulacoseira pseudomuzzanensis* sp. nov. Olszyński & Żelazna-Wieczorek. **Fig. 44.** Two separation valves. Arrow A: short, triangular and sharply pointed separation spine. Arrow B: long, triangular and sharply pointed separation spine. **Fig. 45.** Two linking valves with a spiral dextrorse pattern. Arrow A: bifurcated linking spines. Arrows B: rimoportula openings. **Fig. 46.** Two cells. Arrow A: groove from long separation spine Arrow B: long separation spine. Arrow C: connection between two linking valves.

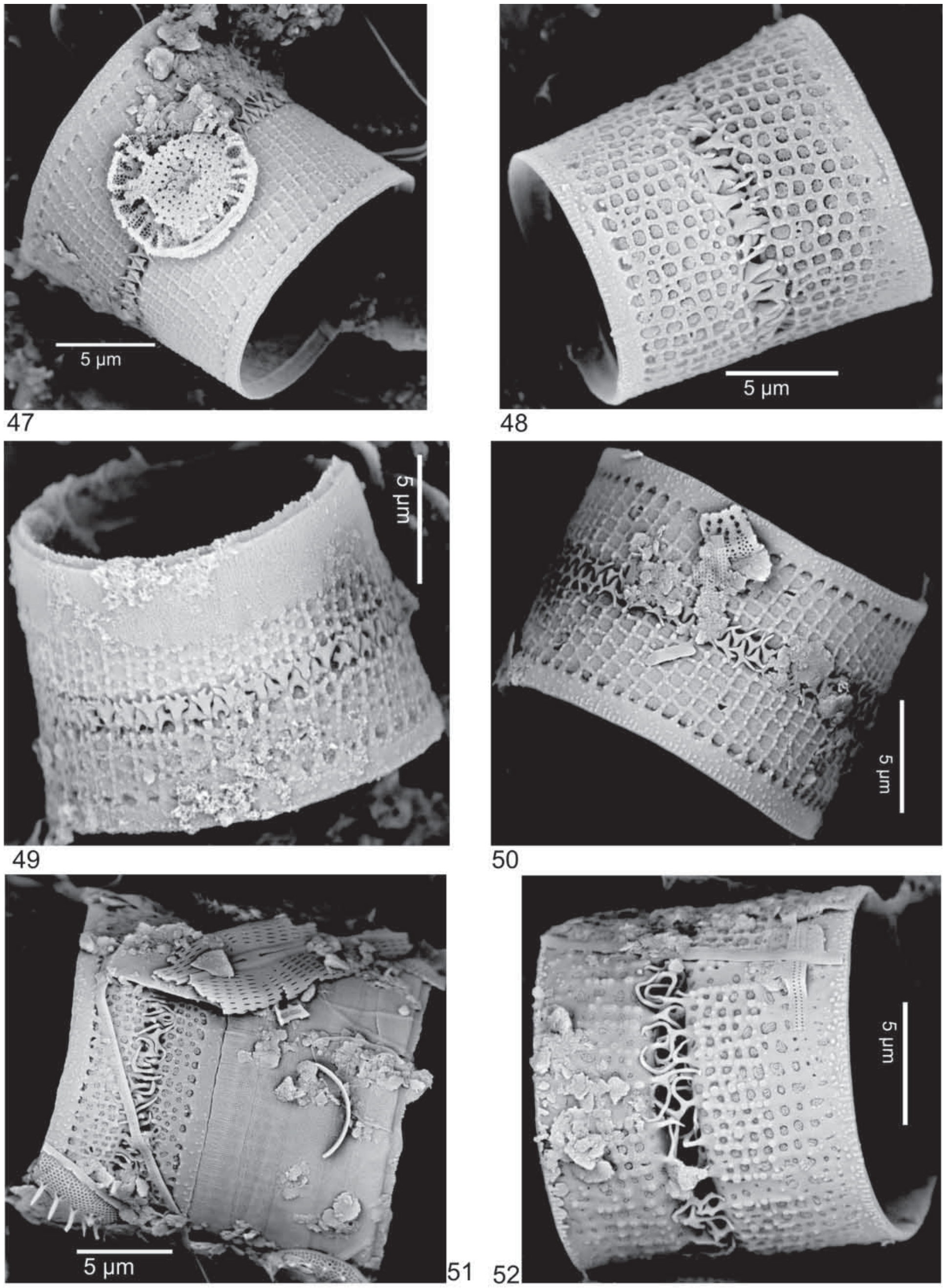
Aulacoseira pseudomuzzanensis can be confused with *A. veraluciae* Tremarin, Torgan & T. Ludwig, however *A. pseudomuzzanensis* has a bigger valve diameter and a smaller MH/VD ratio. The SEM analysis revealed more differences from *A. veraluciae*, i.e. smaller, elongated rimoportulae, and broadly spathulate linking spines (Tremarin et al. 2014). *Aulacoseira gesneri* (Hustedt) Simonsen can also be confused with *A. pseudomuzzanensis* but it has a higher MH/VD ratio. Areolae are more densely arranged, rimoportulae are sessile and linking spines are spathulate, with an absence of long separation spines. In the SEM figures from Tremarin et al. (2011), parallel areolae can be observed on the linking valves.

Cyclostephanos dubius (Fricke) Round (Figs 74–92)

Synonyms: *Cyclotella dubia* Fricke; *Stephanodiscus dubius* (Fricke) Hustedt

Description

LM observations (Figs 74–86): Valve diameter 6.6–16.0 µm; interfascicles 13–16 in 10 µm, disorganized areolae at valve centre.



Figs 47–52. SEM, external views of *Aulacoseira pseudomuzzanensis* sp. nov. Olszyński & Żelazna-Wieczorek. Shape diversity of linking spines. **Fig. 47.** Y-shape linking spines. **Fig. 48.** Spathulate linking spines. **Fig. 49.** Tridentate linking spines. **Fig. 50.** Forked linking spines. **Figs 51–52.** Strongly forked linking spines.

SEM observations (Figs 87–92): Scanning microscopy confirms the morphological features recorded in the literature (Pienaar & Pieterse 1990, Håkansson 2002, Kiss et al. 2012, Houk et al. 2014).

Distribution: *Cyclostephanos dubius* is a widespread species (Pienaar & Pieterse 1990a, Schefler & Morabito 2003, Wojtal & Kwandrans 2006, Żelazna-Wieczorek 2011, Houk et al. 2014, Reavie & Kireta 2015) found in various types of aquatic ecosystems. *Cyclostephanos dubius* occurs mostly in freshwater ecosystems with a chloride concentration between 300 and 1000 mg L^{-1} (Clarke 1989). It was the most abundant taxon in our studies, occurring in all samples as the dominant species.

Studies of post-mining reservoirs in Łęczyca confirm that this species occur frequently in ecosystems under a high human impact.

Table 2. Comparison of morphological features of *Aulacoseira pseudomuzzanensis* and related species; N/D – no data available.

	A. pseudomuzzanensis	*A. muzzanensis* type material	*A. muzzanensis* τ Krammer 1991	*A. muzzanensis* α Krammer 1991	*A. veraluciae* Tremarin et al. 2014	*A. gessneri* Tremarin et al. 2011
Valve diameter	10.0–20.0	10.0–22.8	N/D	N/D	3–15	8–21
Mantle height	5.0–9.0	5.6–10.0	N/D	N/D	4–12	4–11
MH/VD	0.37–0.86	0.28–0.79	N/D	N/D	0.35–2.01	0.25–1.12
Striae in 10 μm	12–16,1 row of areolae	12–19, 1–2 rows of areolae	13–15	13–15	10–16, 1 row of areolae	10–14, 1–2 rows of areolae
Areolae in 10 μm	12–15, rectangular	14–20, rectangular	17–21, finely structured	12–13, coarsely structured	10–15, rounded	14–20 rounded, ellipticalor irregular
Rimoportulae	1–3 coiled at valve face/mantle junction and near the ringleist	1–2 strongly coiled, visible in LM, near the ringleist	N/D	N/D	1–3, elongate	At least 4, sessile
Linking spines	usually bifurcated, raised from one pervalvar rib	bifurcated, raised from one pervalvar rib	triangular to bifid[a]		broadly spathulate	spathulate
Separation spines	short and long as mantle height	triangular, wide and sharply pointed	shorter and longer[a]		triangular, shorter than mantle height	absent

[a] Without distinguishing between the two morphs.

Cyclostephanos invisitatus (Hohn & Hellerman) Theriot, Stoermer & Håkansson (Figs 93–112)

Synonym: *Stephanodiscus invisitatus* Hohn & Hellerman

Description

LM observations (Figs 93–108): Valve diameter: 7.9–13.2 μm; interfascicles 13–21 in 10 μm, bright points on spines on the valve margin can be observed.

SEM observations (Figs 109–112): Scanning microscopy confirms the morphological features reported in the literature (Genkal & Kiss 1991, Håkansson 2002, Kiss et al. 2012, Houk et al. 2014, Reavie & Kireta 2015). However, we noticed that the interfascicles are divided at the valve face/mantle junction although they do not create alveoli (Figs 110–112).

Distribution: *Cyclostephanos invisitatus* is a ubiquitous freshwater species. It occurs in different environments, including unpolluted rivers and habitats with a high anthropogenic influence (Reavie & Smol 1998, Wojtal & Kwandrans 2006, Kiss et al. 2012, Houk et al. 2014, Reavie & Kireta 2015). Our studies confirm that this species occurs abundantly in artificial aquatic ecosystems under high human impact.

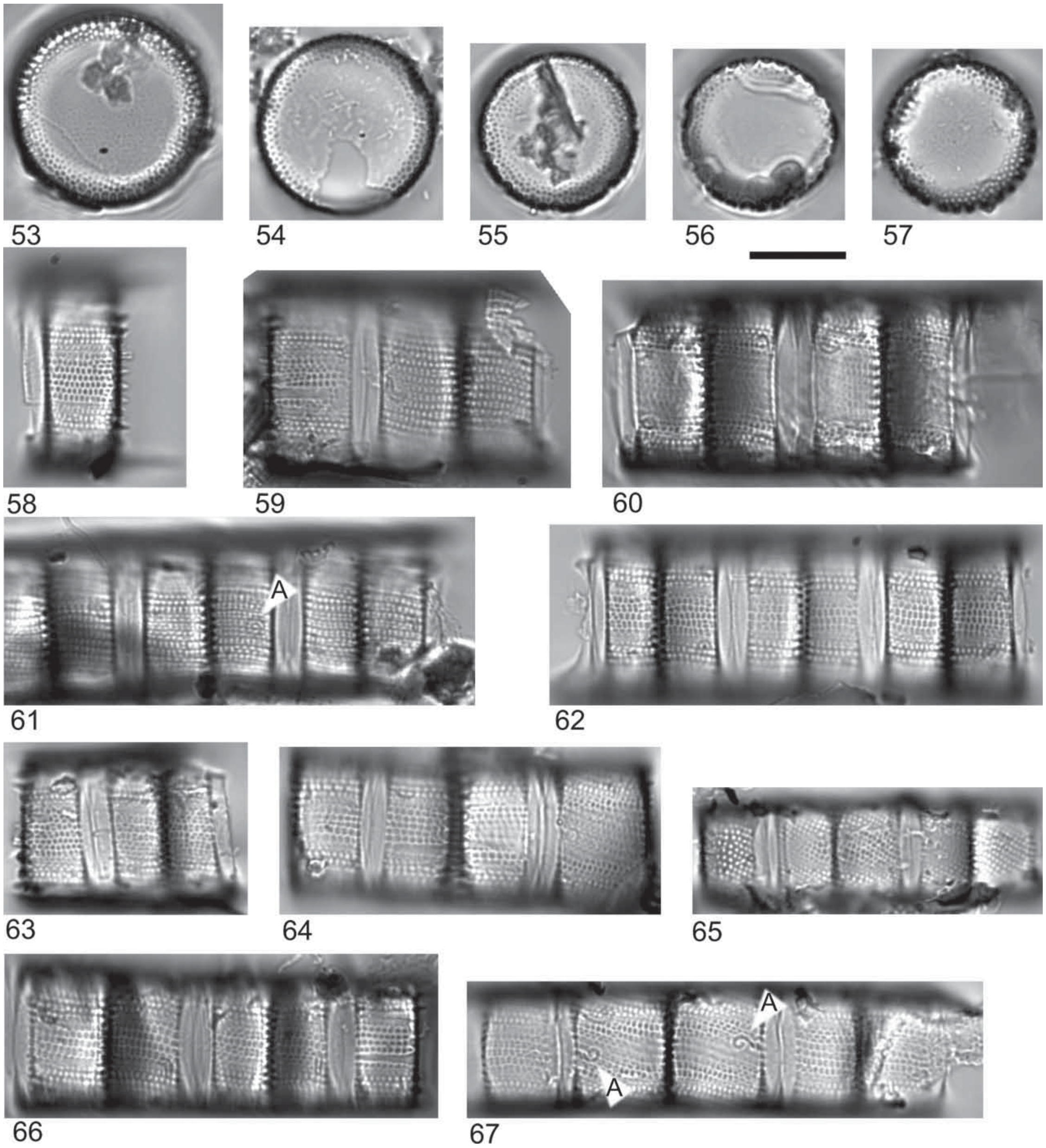
Remarks: Houk et al. (2014) described *C. invisitatus* but did not mention the alveolate structure. However, they noted that the positions of the rimoportula (below spine ring) and the external openings of the rimoportulae without tubular projections are features that differ from *Stephanodiscus*.

Cyclotella meneghiniana Kützing (Figs 113–138)

Description

LM observations (Figs 113–120, 127–133): Valve diameter: 7.1–17.4 μm; striae: 9–10 in 10 μm.

*SEM observations***:** The analysis revealed the presence of two morphs. The first (A) (Figs 113–126) has an externally flat or slightly concave, or convex central area, while the second (B) (Figs 127–138) has an undulate central area. Within the first morph (A), three indistinct sub-types can be recognized: (A1) without any ornamentation (Fig. 121); (A2) with granules (Fig. 122); and (A3) with a star-like pattern created by delicate wrinkles (Fig. 123). Within the second morph (B), two sub-types can be distinguished: (B1) with star-like ornamentation (Fig. 134) and (B2) without ornamentation (Fig. 135). There are one to three fultoportulae in the central area (Figs 121, 122, and LM Fig. 129). Striae are raised and composed of five to eight rows of circular or elongate areolae. Close to the valve face/mantle junction, the striae become flat and extend onto the mantle (Fig. 122). Interstriae lie in the same plane as the central part of the valve. The interstriae are usually pore free (Fig. 124), however, some specimens show external silica bridges over the interstriae, linking the striae



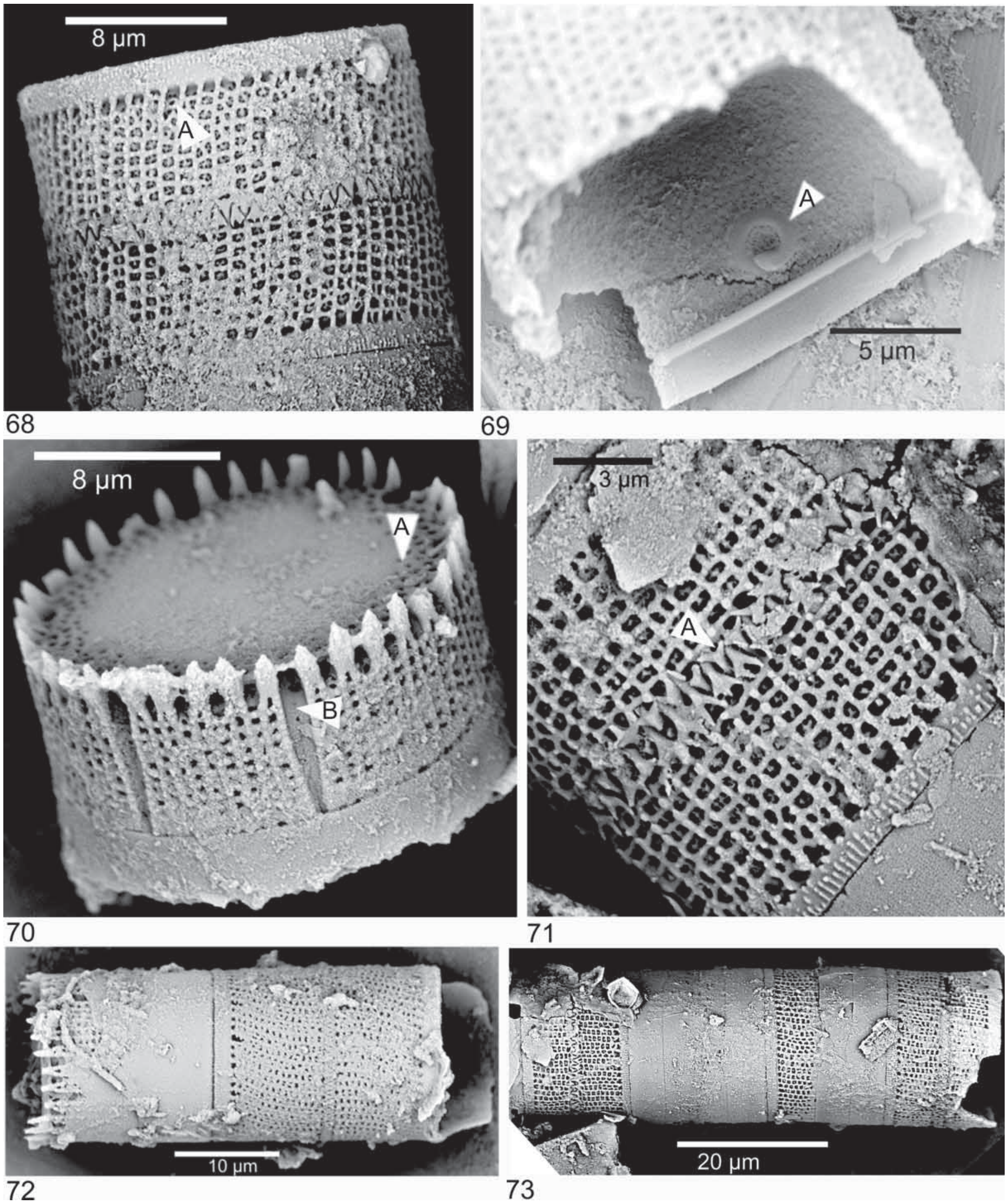
Figs 53–67. LM photomicrographs of the type material of *Melosira muzzanensis* (≡*A. muzzanensis*) Meister. **Figs 53–57.** Valve view. **Figs 58–67.** Girdle view. **Fig. 61.** Arrow A: strongly coiled rimoportula. **Fig. 67.** Arrows A: strongly coiled rimoportulae. Scale bar = 10 µm.

(Fig. 125). Spines are located at the end of each interstria, surrounded by four circular pores. The external openings of the fultoportulae, surrounded by 2–3 small spinulae are located beneath the spines (Fig. 126).

Internally, the valve face fultoportulae are short tubes with three satellites pores. Alveoli are present between each pair of interstriae (Fig. 136). A fultoportula tube with three satellites pores is located on almost every interstria, with one exception, where the stalked rimoportula is located (Fig. 137). In some cases, an additional single costa or mantle fultoportula is present in an alveolus (Fig. 138).

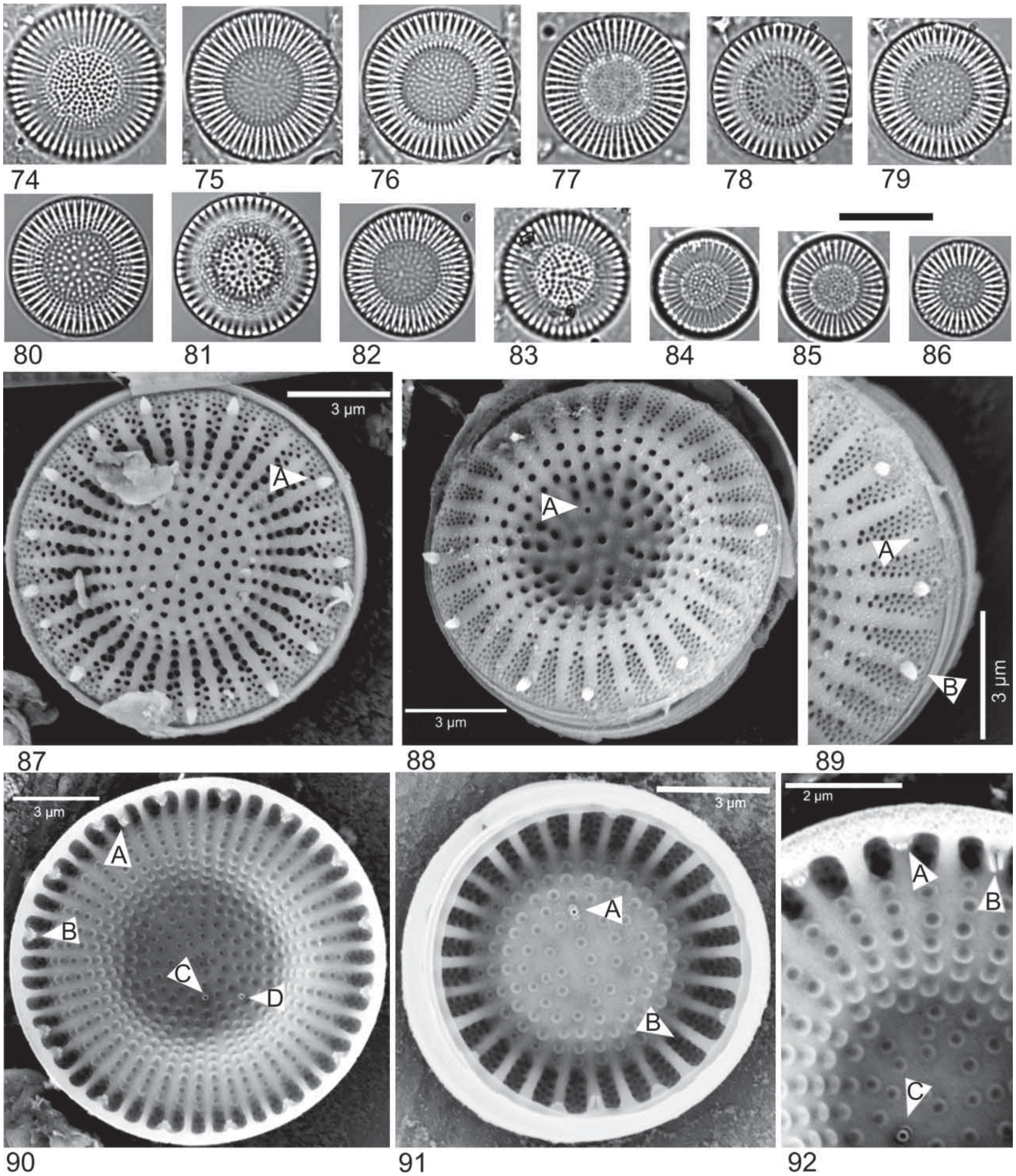
Distribution: *Cyclotella meneghiniana* is a common species, most frequently identified from different types of ecosystems. It has been recorded from eutrophic lakes, reservoirs (Houk et al. 2010) and slow-running rivers (Wojtal & Kwandrans 2006), brackish waters with various degrees of salinity (Håkansson & Chepurnov 1999), oligotrophic lakes, strongly polluted environments (Håkansson 2002), freshwater springs (Żelazna-Wieczorek 2011), and coastal zones (Tanaka 2007).

Remarks: Cyclotella meneghiniana is a well-known species (Håkansson & Chepurnov 1999, Håkansson 2002,



Figs 68–73. SEM, external (**Figs 68, 70–73**) and internal (**Fig. 69**) views of the type material of *Melosira muzzanensis* (≡ *Aulacoseira muzzanenis*) Meister. **Fig. 68.** Two linking valves. Arrow A: rimoportula opening. **Fig. 69.** The valve. Arrow A: strongly coiled rimoportula. **Fig. 70.** Separation valve. Arrow A: areolae only at the edge of the valve face. Arrow B: groove from long separation spine. **Fig. 71.** Two linking valves. Arrow A: Y-shape linking spines. **Figs 72–73.** Chains linking and separation valves.

Beszteri et al. 2007) in which, according to Håkansson & Chepurnov (1999), there is a link between the diversity of the morphology and the environmental conditions. The authors studied variation in cell morphology with salinity, revealing that some morphological features are linked to salinity. However, we noted significant differences in valve morphology regardless of any changes in salinity. It seems that other environmental conditions, not only salinity, can influence the valve morphology of *C. meneghiniana* in relation to stria length, valve face morphology and fultoportula shape. It can be difficult to identify ecotypes of *C. meneghiniana* correctly and invalid descriptions of new species may result (Håkansson 2002). The high morphological differentiation of *C. meneghiniana* in the samples from post-mining reservoirs in the city of Łęczyca may support the suggestion of Beszteri et al. (2007) that this could be a ‘cryptic’ species. Further morphological investigations supported by molecular analysis



Figs 74–92. *Cyclostephanos dubius* (Fricke) Round. **Figs 74–86**. LM photomicrographs. Scale bar = 10 µm. **Figs 87–92.** SEM, external (**Figs 87–89**) and internal (**Figs 90–92**). **Fig. 87.** Convex valve face. Arrow A: a spine. **Fig. 88.** Concave valve face. Arrow A: valve face fultoportula opening. **Fig. 89.** Valve face/mantle junction. Arrow A: rimoportula opening. Arrow B: mantle fultoportula opening. **Fig. 90.** Convex valve face. Arrow A: rimoportula opening. Arrow B: mantle fultoportula opening with two satellites pores. Arrows C and D: valve face fultoportula openings with two satellite pores. **Fig. 91.** Concave valve face. Arrow A: valve face fultoportula opening with two satellite pores. Arrow B: alveolus. **Fig. 92.** Valve face/mantle junction. Arrow A: mantle fultoportula opening with two satellite pores. Arrow B: sessile rimoportula opening. Arrow C: valve face fultoportula opening with two satellite pores.

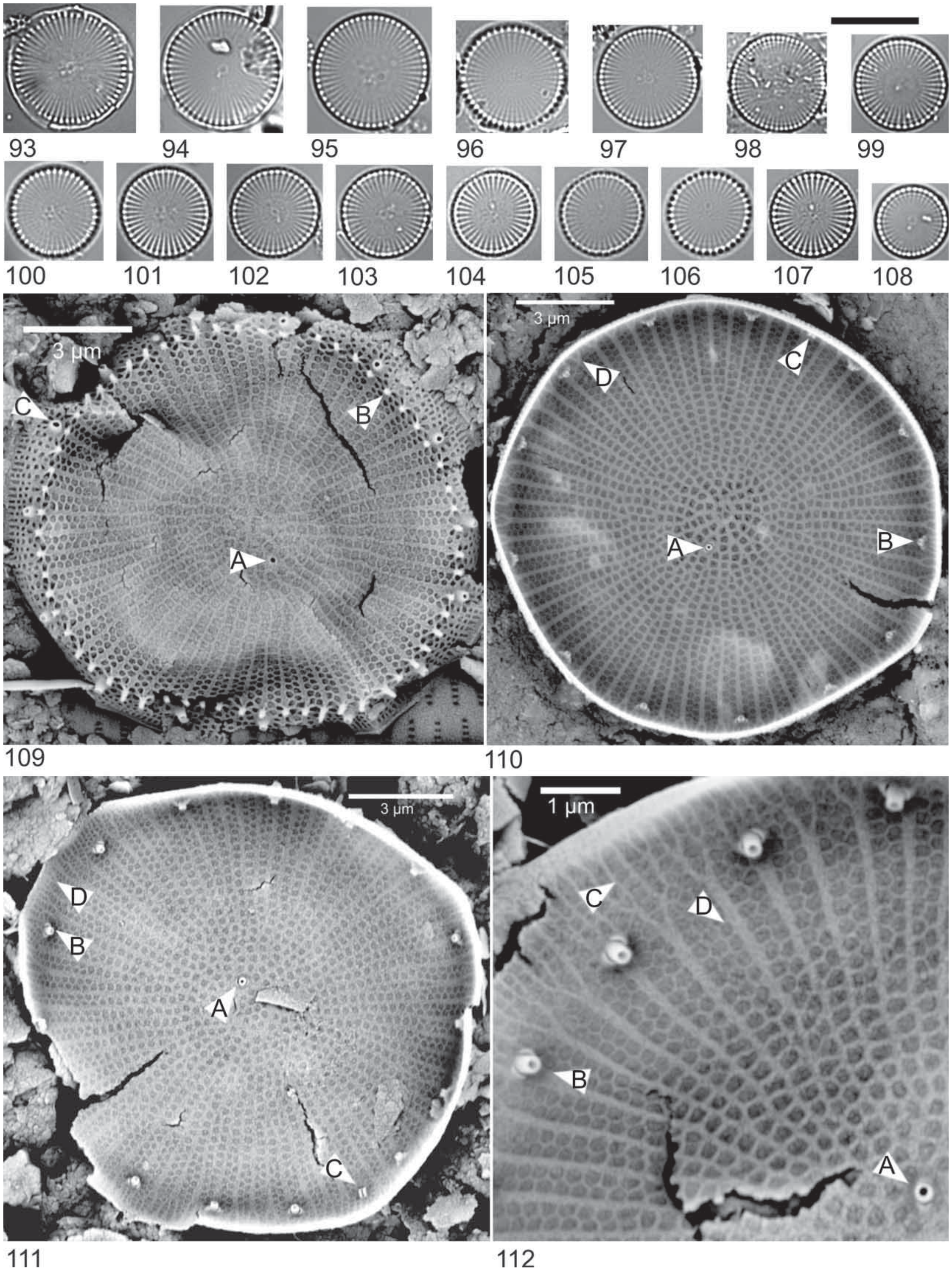
may clarify the taxonomic position of *C. meneghiniana* morphs.

Discostella pseudostelligera (Hustedt) Houk et Klee (Figs 139–176)

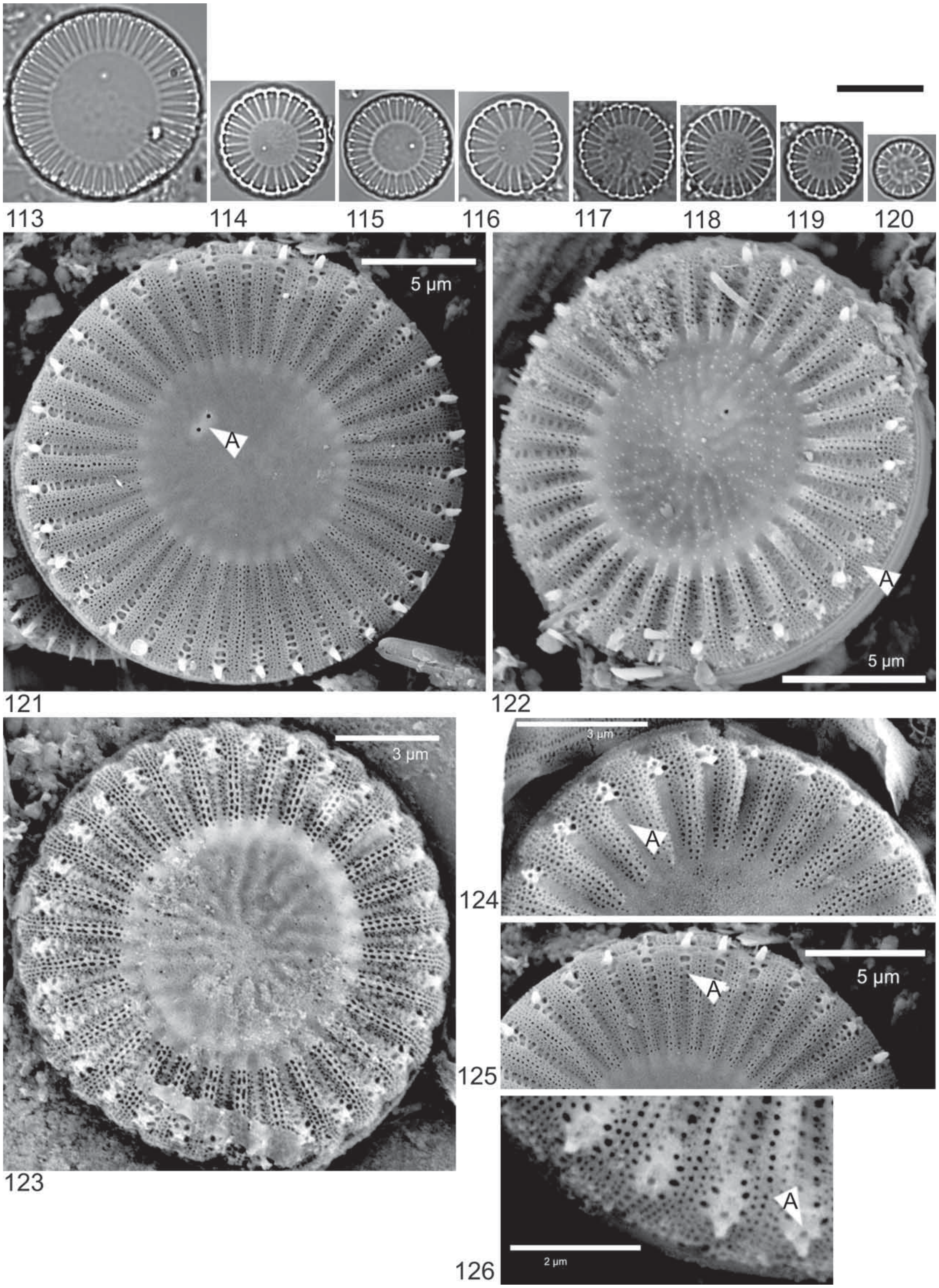
Synonym: *Cyclotella pseudostelligera* Hustedt

Description

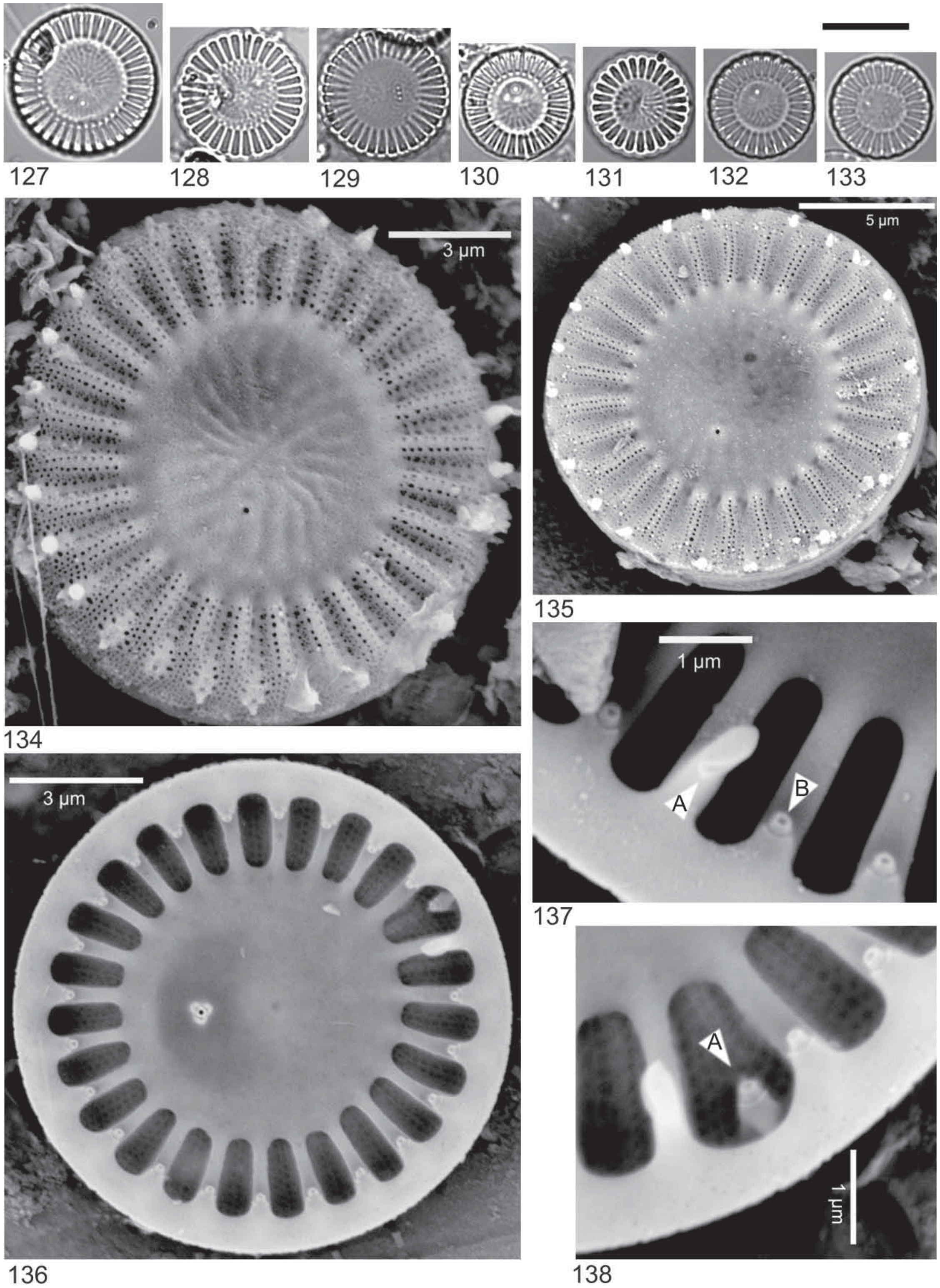
LM observations (Figs 139–172): Valve diameter: 5.5–10.0 µm; striae 17–20 in 10 µm, a star-like pattern close to the centre of the valve is visible. Depending on the focal plane, a hyaline ring may be visible between the star-like pattern and striae.



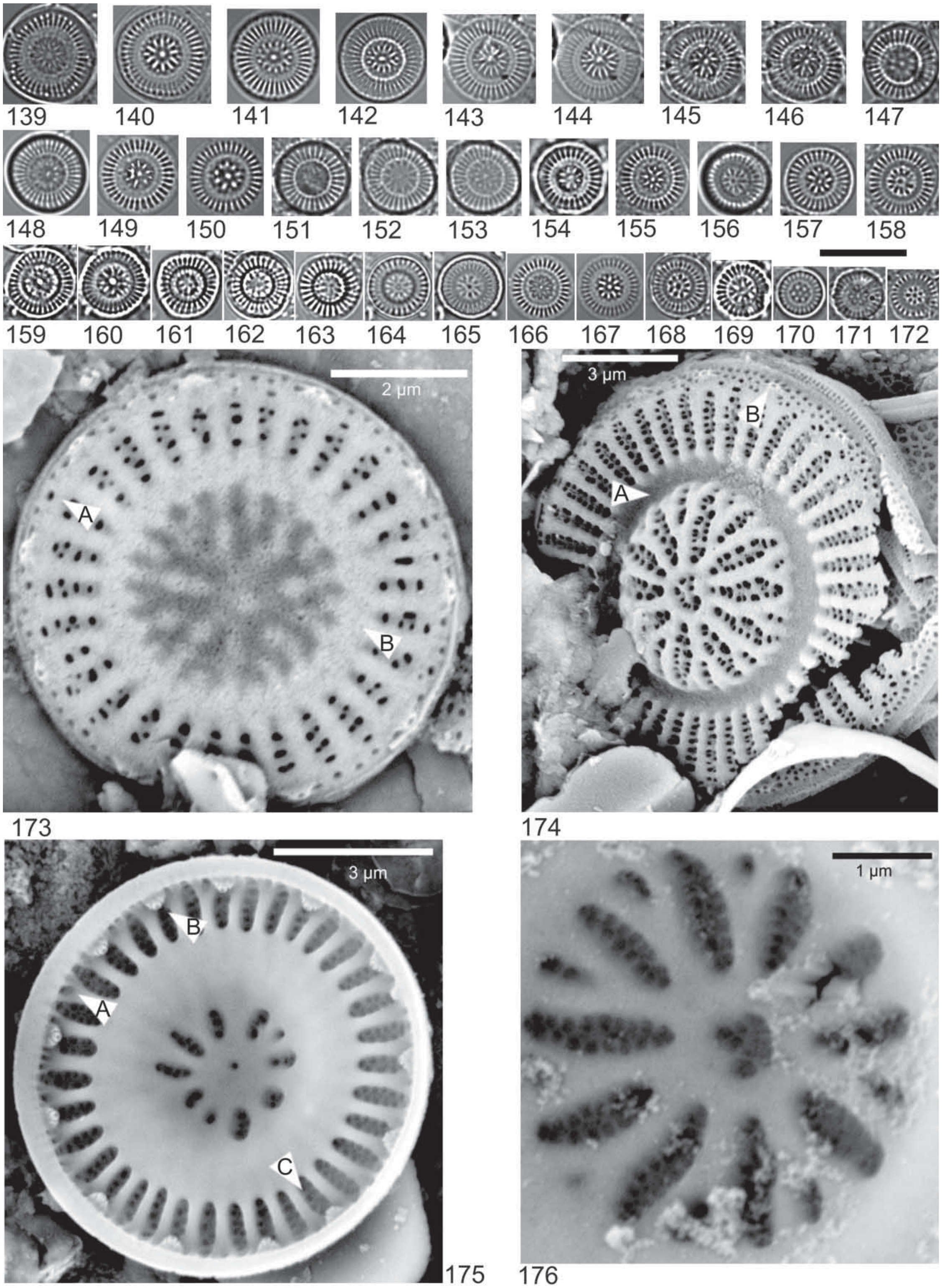
Figs 93–112. *Cyclostephanos invisitatus* (Hohn & Hellerman) Theriot, Stoermer & Håkansson. **Figs 93–108**. LM photomicrographs. Scale bar = 10 µm. **Figs 109–112**. SEM, external (**Fig. 109**) and internal (**Figs 110–112**). **Fig. 109.** The valve face. Arrow A: valve face fultoportula opening. Arrow B: a spine. Arrow C: mantle fultoportula opening. **Figs 110–111.** The valve. Arrow A: valve face fultoportula opening with two satellite pores. Arrow B: mantle fultoportula opening. Arrow C: sessile rimoportula. Arrow D: divided interfascicles. **Fig. 112.** The valve face/mantle junction. Arrow A: valve face fultoportula opening with two satellite pores. Arrow B: mantle fultoportula opening with two satellite pores. Arrow C. divided interfascicles. Arrow D: continuous cribra.



Figs 113–126. *Cyclotella meneghiniana* Kützing. **Figs 113–120.** LM photomicrographs of *C. meneghiniana* (morph A). Scale bar = 10 µm. **Figs 121–126.** SEM, external photomicrographs of *C. meneghiniana* (morph A). **Fig. 121.** Valve face of sub-types A1 without ornamentation. Arrow A: two valve face fultoportula openings. **Fig. 122.** Valve face of sub-types A2 with granules. Arrow A: striae extended to the mantle. **Fig. 123.** Valve face of sub-types A3 with star-like pattern created by delicate wrinkles. **Figs 124–126.** Valve face/mantle junction of *C. meneghiniana*. **Fig. 124.** Arrow A: interstriae with hyaline area. **Fig. 125.** Arrow A: interstriae with silica bridges. **Fig. 126.** Arrow A: fultoportula opening surrounded by three spinulae.



Figs 127–138. *Cyclotella meneghiniana* Kützing. **Fig 127–133.** LM photomicrographs of *C. meneghiniana* (morph B). Scale bar = 10 µm. **Figs 134–138.** SEM, external (**Figs 134–135**) and internal (**Figs 136–138**) photomicrographs of *C. meneghiniana* (morph B). **Fig. 134.** Valve face of sub-types B1 with star-like pattern created by delicate wrinkles. **Fig. 135.** Valve face of sub-types B2 without ornamentation. **Fig. 136.** Valve face. **Fig. 137.** Arrow A: stalked rimoportula opening. Arrow B: fultoportula opening. **Fig. 138.** Arrow A: additional fultoportula.



Figs 139–176. *Discostella pseudostelligera* (Hustedt) Houk et Klee. **Figs 139–172.** LM photomicrographs. Scale bar = 10 µm. **Figs 173–176.** SEM, external (**Figs 173–174**) and internal (**Figs 175–176**). **Fig. 173.** Concave valve face. Arrow A: rimoportula opening. Arrow B: hyaline ring. **Fig. 174.** Convex valve face. Arrow A: hyaline ring. Arrow B: fultoportula opening. **Fig. 175.** Valve face. Arrow A: divided interstriae. Arrow B: fultoportula opening with two satellite pores. Arrow C: stria. **Fig. 176.** Star-like pattern in the central part of the valve.

SEM observations: The valve face is externally undulate, with a concave (Fig. 173) or convex (Fig. 174) valve centre. The convex centre has a star-like pattern composed of one or two rows of areolae (Fig. 174). Shortened striae are present near the hyaline ring and there are a few separate areolae at the valve centre. The concave valve has a residual star-like pattern (Fig. 173). Both concave and convex valves have a hyaline ring between the valve centre and the striated marginal zone (Figs 173–174). Striae are composed of one or two rows of areolae with foramina; the interstriae can be elevated. The external fultoportula openings occur at the valve face/mantle junction, below every second to third striae (Fig. 174). One external slit-like rimoportula opening of rimoportula is visible (Fig. 173).

The internal openings of the mantle fultoportulae are small and tube-like with two satellites pores (Fig. 175). Interstriae are divided at the valve face/mantle junction (Fig. 175). One slit-like rimoportula opening is present (Fig. 175). The star-like pattern is composed of a single or double row of areolae (Fig. 176).

Distribution: *Discostella pseudostelligera* is a widespread species that has been reported from many different habitats, but mainly eutrophic ones. It has been recorded from the Río basin in Argentina (Guerrero & Echenique 2006), Lake Biwa in Japan (Tanaka 2007), the Danube in Hungary (Kiss & Genkal 1993), the Great Lakes in USA, the St. Lawrence River in USA (Reavie & Smol 1998, Reavie & Kireta 2015), rivers and lakes in Russia (Genkal & Popovskaya 2008, Genkal 2015) and a range of rivers in Germany (Houk et al. 2010) and Poland (Wojtal & Kwandrans 2006).

Discostella woltereckii (Hustedt) Houk et Klee (Figs 177–193)

Synonym: *Cyclotella woltereckii* Hustedt

Description

LM observations (Figs 177–188): Valve diameter: 4.0–7.3 µm; striae 28–30 in 10 µm, spines on the valve margin may be observed as bright points.

SEM observations: Externally, the valve face is flat with two different patterns: stellate, with large, almost circular or elongate puncta on the valve centre (Fig. 191); and dichotomous, with a narrow unpatterned central area (Figs 189–190).

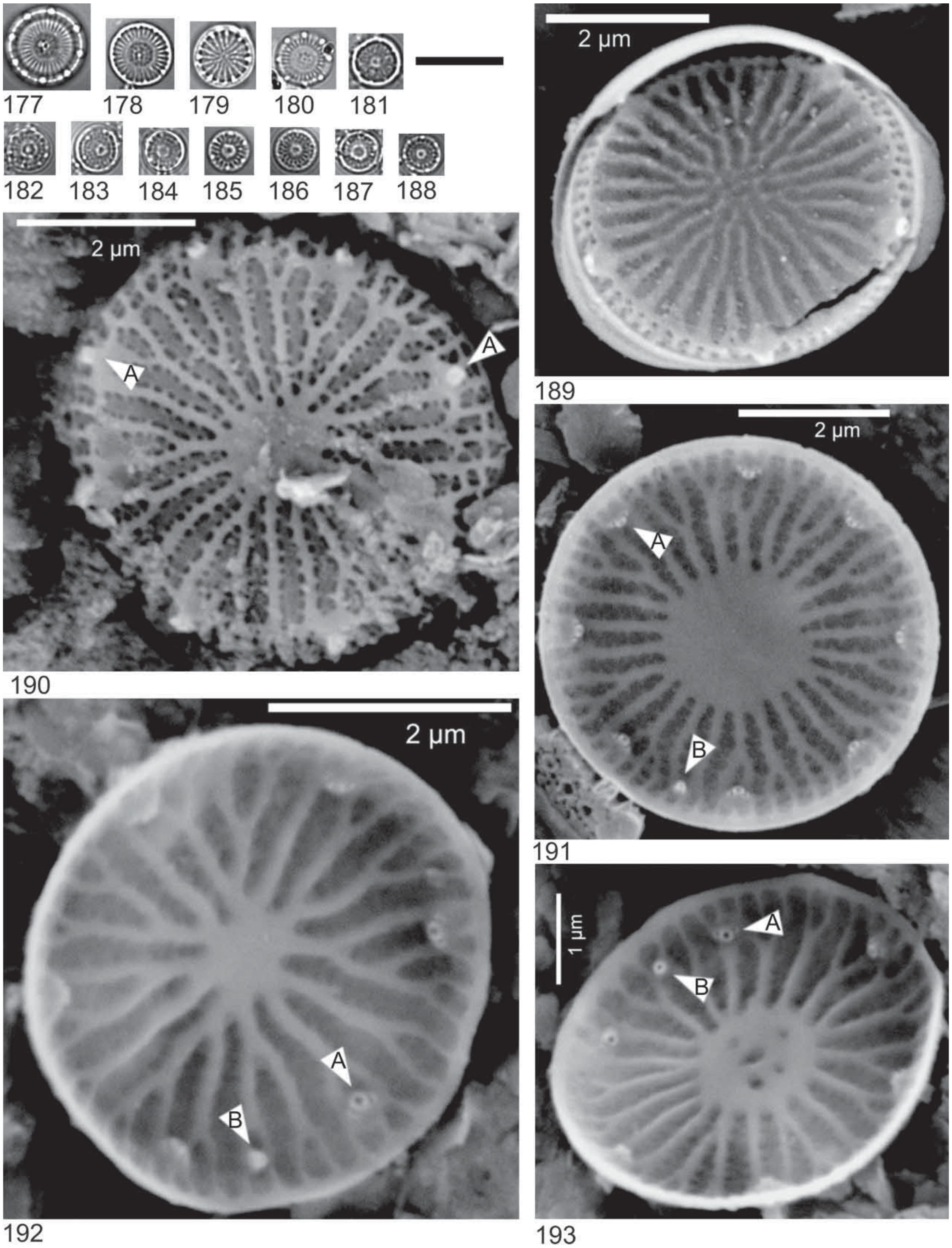
Costae branch in the marginal zone, with the small external fultoportula tubes lying between the costae, at the valve face/mantle junction (Figs 189–190). The external rimoportula opening is indistinct.

Internally the marginal fultoportula openings are small and tube-like, with two satellite pores (Figs 191–193). One sessile rimoportula is present at the valve face/mantle junction (Figs 191–193).

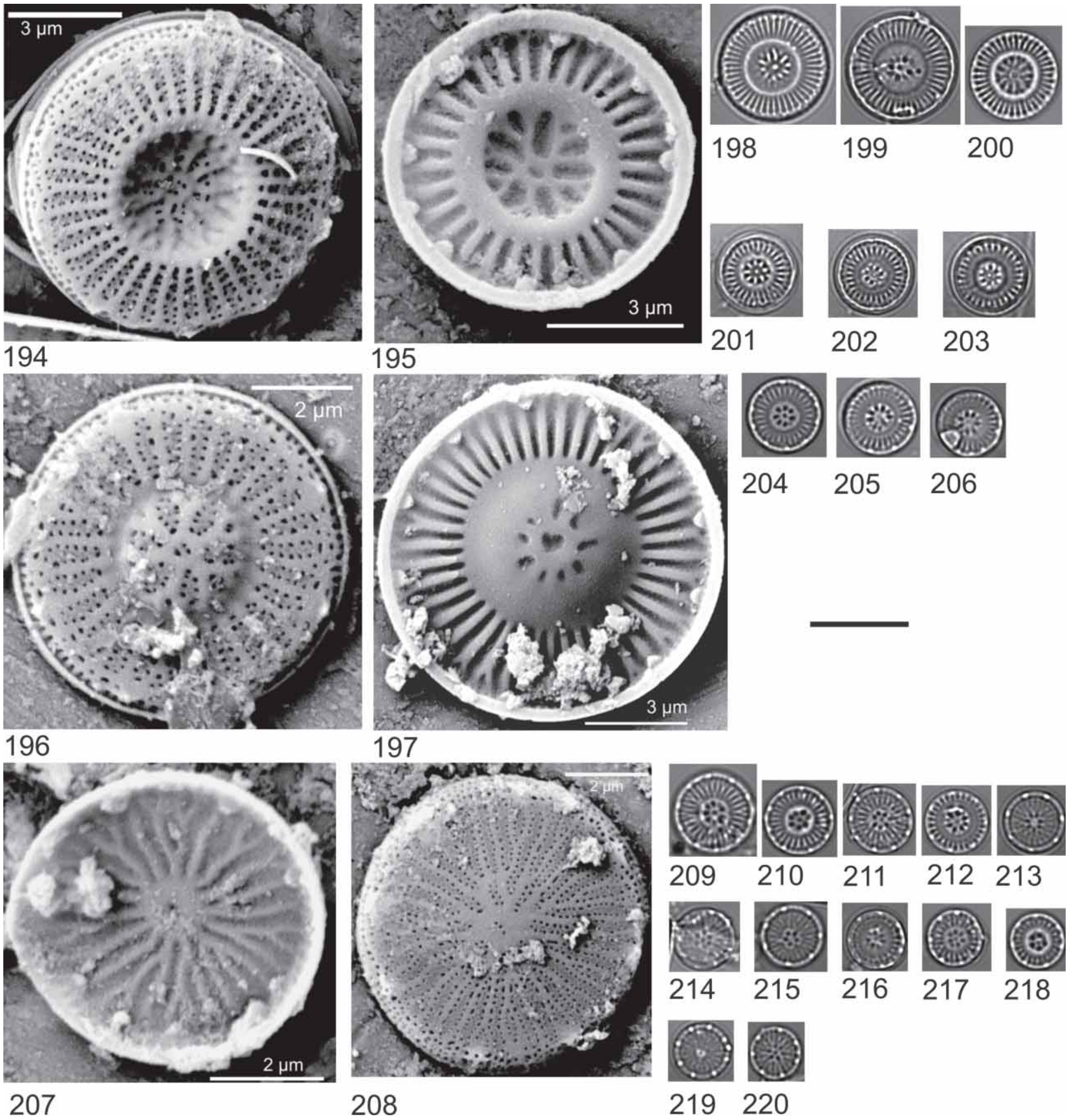
Distribution: *Discostella woltereckii* is known from its type locality, a pond in Java, Indonesia (Hustedt 1942), but has also been recorded from other areas, Tatara Pond, Argentina (Guerrero & Echenique 2006), Lake Kita in Japan (Tanaka 2007), and in a sediment core from a volcanic lake in Tanzania (Öberg et al. 2009). It has also been found in temperate ecosystems, in Poland (Wojtal et al. 2005, Wojtal & Kwandrans 2006), Russia and Hungary (Kiss & Genkal 1993, Genkal & Popovskaya 2008, Genkal 2015). Scheffler & Morabito (2003) found this species in eutrophic subalpine lakes.

Remarks on *D. woltereckii* and *D. pseudostelligera*: The first description of *Cyclotella woltereckii* (≡*D. woltereckii*) contained drawings of a valve with a dichotomous pattern (Hustedt 1942). However, in the *Atlas and Catalogue of the Diatom Types of Friedrich Hustedt* (Simonsen 1987), a valve of *C. woltereckii* is presented with a stellate pattern (Simonsen 1987, p. 400. fig. 7, 10, 11, 13 and 14). These microphotographs clearly show that the central area with the stellate pattern is narrow, the interstriae are divided, and marginal fultoportulae are present. In the case of *C. pseudostelligera* (≡*D. pseudostelligera*), Hustedt's original drawings (Hustedt 1939: 581, figs 1, 2) show that it differs from *C. woltereckii* in having a star-like pattern in its central part. In Simonsen's microphotographs (Simonsen 1987), the star-like patterns are different, with a more complex morphology than in *C. woltereckii*. In addition, there is a hyaline ring between the central area and the striated margin. The marginal fultoportulae are not always visible in LM and the interstriae are not as strongly divided as in *C. woltereckii,* or are only divided at the valve face/mantle junction. Photomicrographs in Kiss & Genkal (1993), Genkal (2015), Genkal & Popovskaya (2008) and Scheffler & Morabito (2003) have been labelled as *D. pseudostelligera*, however, in our opinion, the morphological features visible in these photographs are consistent with the description of *C. woltereckii* provided by Simonsen (1987).

Discostella woltereckii and *D. pseudostelligera* were described from different ecosystems; the first occurred in the tropics, the second in a temperate zone (Hustedt 1939, Hustedt 1942). Houk et al. (2010) provide a description of the ecology as a one of the distinguishing features of the two taxa. However, on the basis of our observations of the type material of *Cyclotella woltereckii* (≡*D. woltereckii*) (Figs 194–220), *C. pseudostelligera* (≡*D. pseudostelligera*) (Figs 221–225) and the material from the Łęczyca reservoirs, these two species co-occur in aquatic ecosystems with increased conductivity. Hevia-Orube et al. (2015) investigated the morphology and phylogeny of *D. pseudostelligera* and compared it with the molecular data for *D. woltereckii*. Their results show close similarity between these species; however, they admit that they had insufficient molecular data to separate or combine *D. pseudostelligera* and *D. woltereckii*.



Figs 177–193. *Discostella woltereckii* (Hustedt) Houk et Klee. **Figs 177–188.** LM photomicrographs. Scale bar = 10 µm. **Figs 189–193.** SEM, external **(Figs 189, 190)** and internal (**Figs 191–193**) photomicrographs. **Figs 189–190.** Valve face with dichotomous pattern. **Fig. 190.** Arrows A: mantle fultoportula openings. **Fig. 191.** Valve face with stellate pattern. Arrow A: mantle fultoportula opening. Arrow B: rimoportula opening. **Fig. 192.** Arrow A: mantle fultoportula opening with two satellite pores. Arrow B: rimoportula opening. **Figs 193.** Valve face with stellate pattern. Arrow A: mantle fultoportula with two satellite pores. Arrow B: rimoportula opening.



Figs 194–220. Type material of *Cyclotella woltereckii* (≡ *D. woltereckii*) Hustedt. **Figs 194–206.** *Discostella pseudostelligera* (Hustedt) Houk et Klee. **Figs 194–197.** SEM, external **(Figs 194, 196)** and internal **(Figs 195, 197)**. **Figs 198–206.** LM photomicrographs. Scale bar = 10 µm. **Figs 207–220.** *Discostella woltereckii* (Hustedt) Houk et Klee. **Figs 207, 208.** SEM, external (**Fig. 208**) and internal (**Fig. 207**). **Figs 209–220.** LM Scale bar = 10 µm.

Lindavia* aff. *balatonis (Pantocsek) Nakov et al. (Figs 226–250)

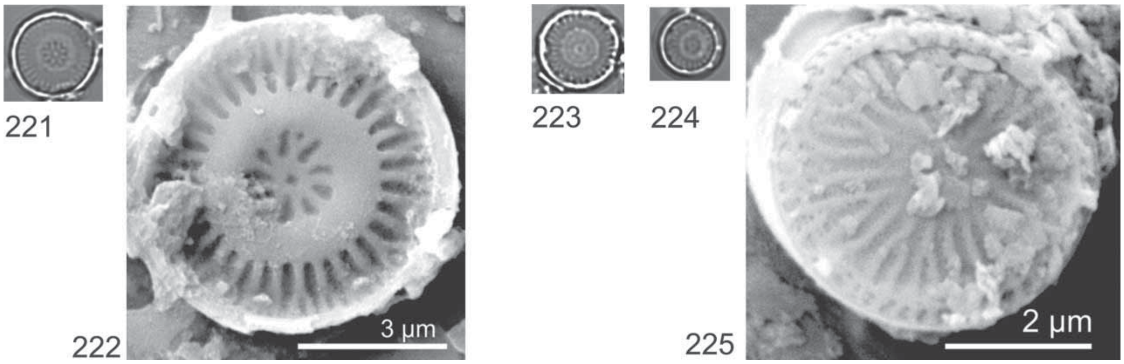
Synonyms: *Cyclotella balatonis* Pantocsek; *Puncticulata balatonis* (Pantocsek) Wojtal et Budzyńska in Budzyńska & Wojtal; *Handmannia balatonis* (Pantocsek) Kulikovskiy et Solak in Solak & Kulikovskiy.

Description

LM observations (Figs 226–245): Valve diameter: 9.4–14.4 µm; costae 6–7 in 10 µm, striae 18–20 in 10 µm.

SEM observations: The valve face is flat although the central part can be slightly undulate (Fig. 246). Striae are composed of 2–3 rows of loculate areolae with foramina (Fig. 246, 247). Interstriae may be divided near the margin (Figs 246, 247). One or two striae are shortened, with the external rimoportula openings at their ends (Fig. 247). Slit-like fultoportula openings are present on each costa at the valve face/mantle junction. Areolae (bigger puncta) and the fultoportula opening of the fultoportulae (smaller puncta) are visible in the central area (Fig. 248).

The internal openings of the mantle fultoportulae with two satellites pores are present on every costa (Fig. 250). Three to four thinner costae (interfascicles) are present between the costae, creating alveoli (Fig. 250). Thinner costae may be divided near the valve margin.



Figs 221–225. Type material of *C. pseudostelligera* (≡*D. pseudostelligera*) Hustedt. **Figs 221–222.** *Discostella pseudostelligera* (Hustedt) Houk et Klee. **Fig. 221.** LM Scale bar = 10 µm. **Fig. 222.** SEM, internal. **Figs 223–225.** *Discostella woltereckii*(Hustedt) Houk et Klee. **Figs 223, 224.** LM. Scale bar = 10 µm. **Fig. 225.** SEM, external.

From one to two sessile rimoportulae with radially or slightly obliquely positioned slits are present between the thicker costae (Fig. 250). At the valve centre, areolae with domed cribra are visible (Figs 249–250). There are different numbers of internal openings of fultoportulae with three satellites pores and cowlings between the areolae (Figs 249–250).

Distribution: *Lindavia balatonis* occurs mainly in pelagic or littoral zones of mesotrophic to eutrophic lakes, reservoirs or slow-running water ecosystems (Houk et al. 2010, Budzyńska & Wojtal 2011, Solak & Kulikovskiy 2013). *Lindavia* aff. *balatonis* from the Łęczyca reservoirs has similar ecological preferences to *Linadavia balatonis*.

Remarks: Pantocsek (1902) described *Cyclotella balatonis* from Balaton Lake. Håkansson (2002) established a new genus *Puncticulata.* However, this new genus did not include *C. balatonis*. A few years later, Budzyńska & Wojtal (2011) transferred *C. balatonis* to *Puncticulata*. Since *Puncticulata* was invalidly established in 2002, Solak & Kulikovskiy (2013) placed it to *Handmannia*. However, they did not transfer *P. balatonis* to the new genus. Afterwards, *P. balatonis* was transferred to the *Lindavia* by Nakov et al. (2015). The *Lindavia* was established on the basis of its main feature, which was the location of the openings of rimoportulae, both internal and external on the valve face (Nakov et al. 2015).

Stephanodiscus binatus Håkansson et Kling (Figs 251–266)

Description

LM observations (Figs 251–262): Valve diameter: 5.3–7.0 µm; interfascicles 15–18 in 10 µm, disorganized areolae can be observed at the valve centre.

SEM observations (Figs 263–266): SEM confirms the description of morphological features in the literature (Håkansson & Kling 1990, Houk et al 2014).

Distribution: *Stephanodiscus binatus* has been noted from oligotrophic to eutrophic lakes (Stoermer & Håkansson 1984, Håkansson & Kling 1990, Houk et al. 2014) and in iron ore post-mining reservoirs in Łęczyca.

Remarks: *Stephanodiscus binatus* can be described as cosmopolitan. It may be misidentified as *S. parvus* Stoermer & Håkansson or *S. minutulus* (Kützing) Cleve & Möller (Stoermer & Håkansson 1984), however, the areolae in the central part of the valve in *S. binatus* are larger and more distinctive than in the other two species (Håkansson & Kling 1990, Houk et al. 2014). This is the first record of *S. binatus* for Poland. Wojtal & Kwandrans (2006) described specimens of *S. minutulus* that were morphologically similar to *S. binatus* (Wojtal & Kwandrans 2006, figs 19.15, 19.17, 19.19). These specimens have thick interfascicles and very distinctive areolae. Furthermore, Reavie & Kireta (2015) showed specimens of '*S. parvus* var. 1' similar to *S. binatus* in the Coastal Laurentian Great Lakes (Reavie & Kireta 2015, pl. 4, figs 36, 37a, b, 39a, b, 40a, b.).

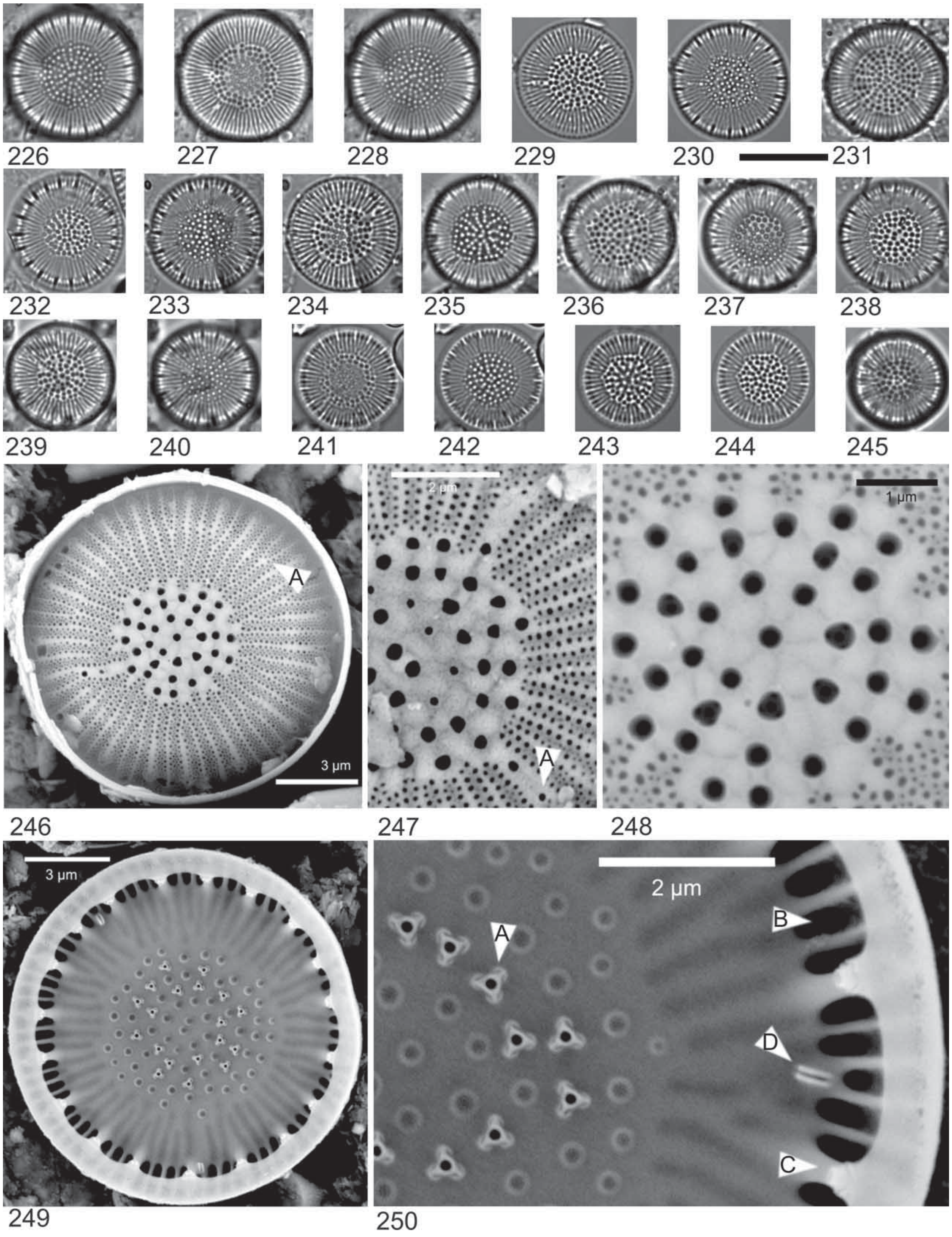
Stephanodiscus hantzschii Grunow in Cleve & Grunow (Figs 267–286)

Description

LM observations (Figs 267–281): Valve diameter: 8.0–16.6 µm; interfascicles 9–14 in 10 µm.
SEM observations (Figs 282–286): SEM confirms the morphological features given in the literature (Kling 1992, Håkansson 2002, Houk et al 2014).

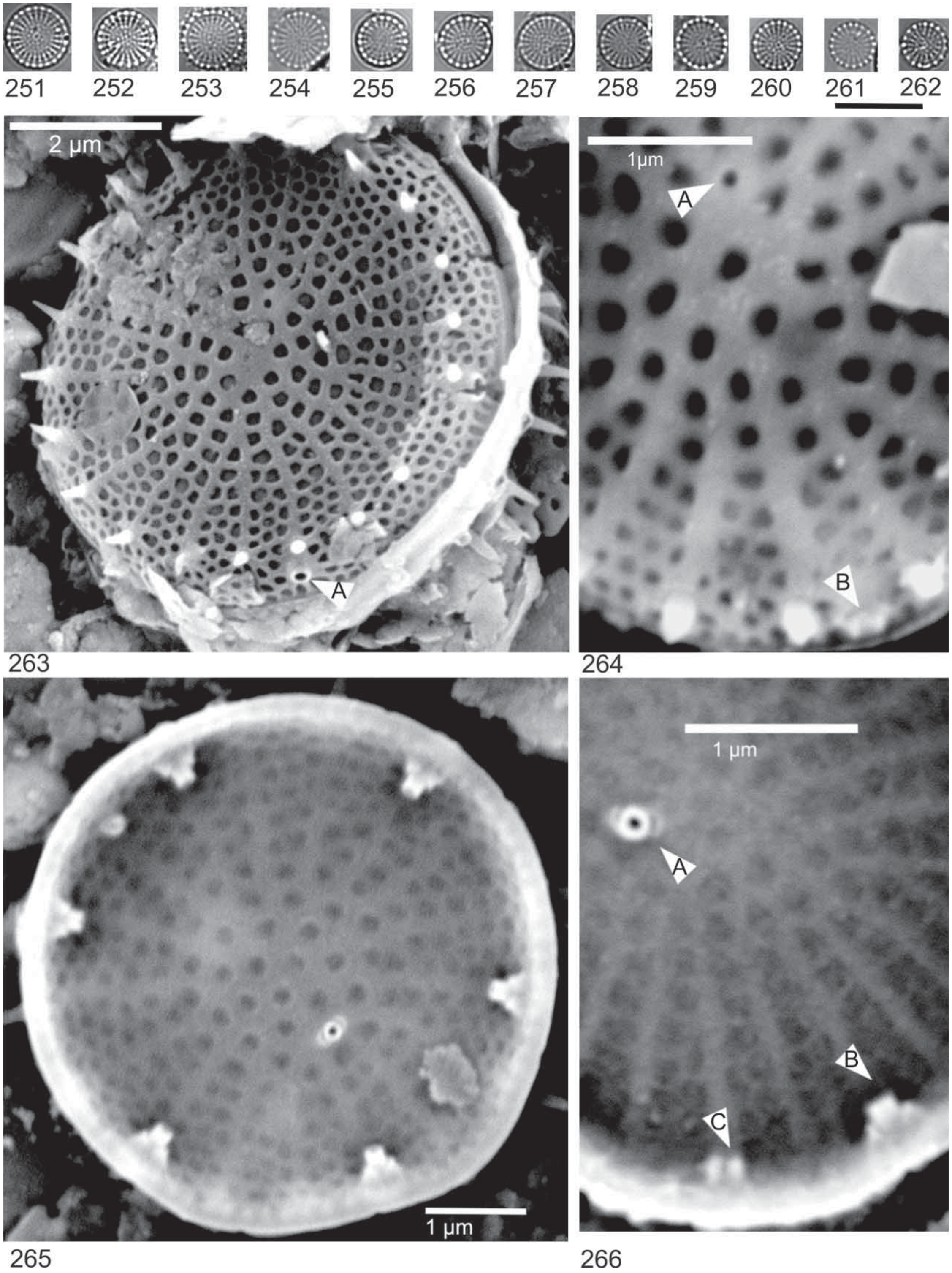
Distribution: *Stephanodiscus hantzschii* is a widespread species in eutrophic lakes, reservoirs, and slow-running aquatic ecosystems (Wojtal & Kwandrans 2006, Houk et al. 2010). Our research confirms that this species occurs more frequently in ecosystems with high human impact.

Remarks: The morphology of *S. hantzschii* varies widely. Håkansson & Bailey-Watts (1993) described five morphotypes. We also observed considerable morphological

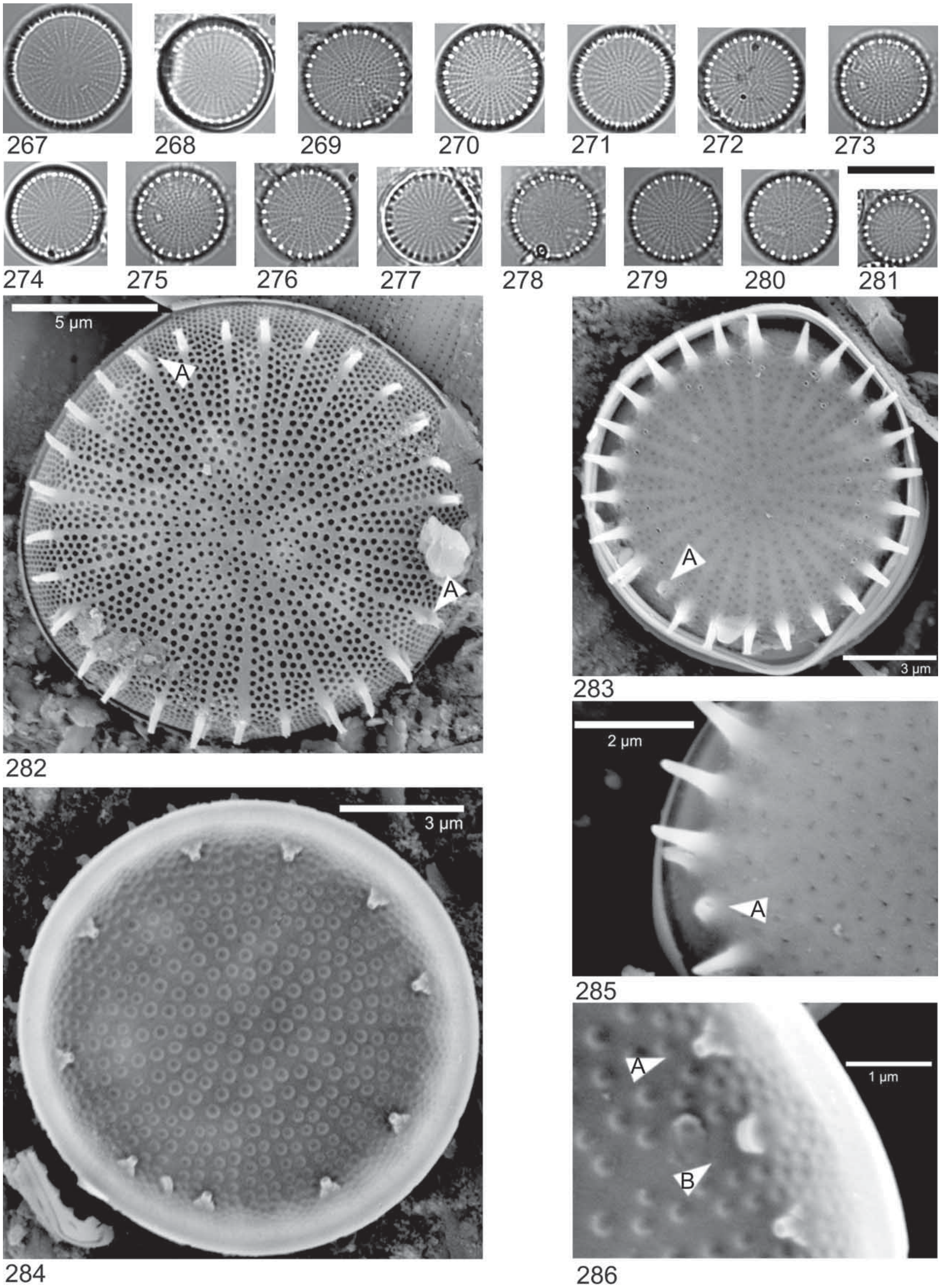


Figs 226–250. *Lindavia* aff. *balatonis.* **Figs 226–245.** LM. Scale bar = 10 µm. **Figs 246–250.** SEM, external (**Figs 246–248**) and internal (**Figs 249–250**). **Figs 246–248.** Valve face. **Fig. 246.** Valve face. Arrow A: divided interstriae. **Fig. 247.** Arrow A: valve face fultoportula opening. **Fig. 248.** Loculate areolae. **Fig. 249.** Valve face. **Fig. 250.** Valve face/mantle junction. Arrow A: valve face fultoportula opening with three satellite pores. Arrow B: alveolus. Arrow C: mantle fultoportula opening with three satellite pores. Arrow D: rimoportula opening.

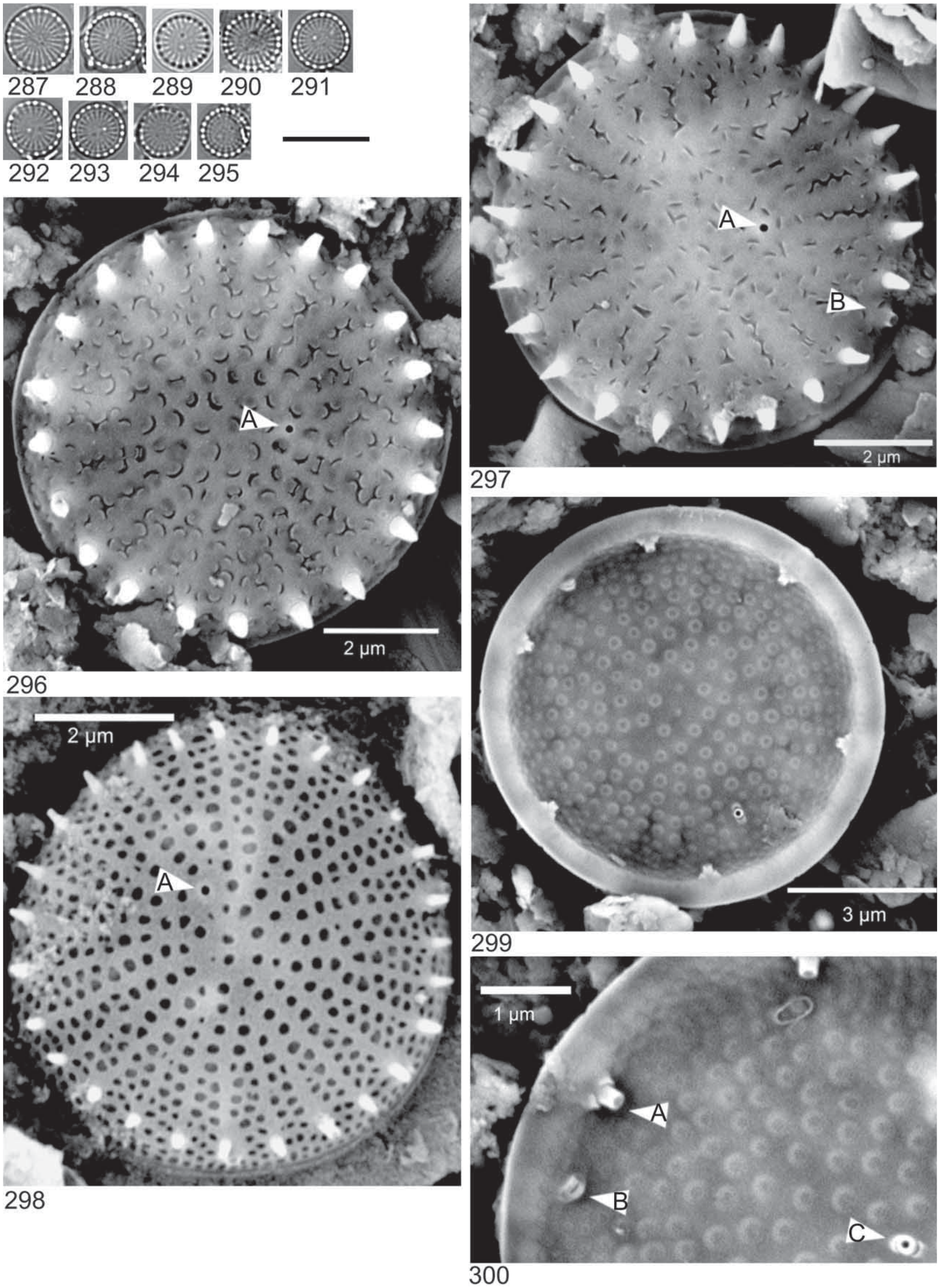
variability in specimens of *S. hantzschii*, i.e. with or without areola occlusions (Fig. 282); a similar specimen was shown by Houk et al. (2014, pl. 130, fig. 6). However, two features were the same in each morph: the absence of annulus and presence the tubular extension of the external opening of rimoportula (Kling 1992, Håkansson 2002)



Figs 251–266. *Stephanodiscus binatus* Håkansson et Kling. **Figs 251–262.** LM. Scale bar = 10 µm. **Figs 263–266.** SEM, external (**Figs 263–264**) and internal (**Figs 265–266**). **Figs 263–264.** Valve. **Fig. 263.** Arrow A: mantle fultoportula opening. **Fig. 264.** Arrow A:valve face fultoportula opening. Arrow B: rimoportula opening. **Fig. 265.** Valve face. **Fig. 266.** Valve face/mantle junction. Arrow A: valve face fultoportula opening with two satellite pores. Arrow B: mantle fultoportula opening with two satellite pores. Arrow C: rimoportula opening.



Figs 267–286. *Stephanodiscus hantzschii* Grunow in Cleve & Grunow. **Figs 267–281.** LM photomicrographs. Scale bar = 10 µm. **Figs 282–286.** SEM, external (**Figs 282–283, 285**) and internal (**Figs 284, 286**) photomicrographs. **Fig. 282.** Valve face without occlusion. Arrows A: interfascicles extend to the mantle where fultoportula tube opening is located. **Fig. 283.** Valve face with occlusion. Arrow A: rimoportula opening. **Fig. 284.** Valve face. **Fig. 285.** Valve face/mantle junction. Arrow A: rimoportula opening. **Fig. 286.** Valve face/mantle junction. Arrow A: mantle fultoportula opening with three satellite pores. Arrow B: rimoportula opening



Figs 287–300. *Stephanodiscus parvus* Stoermer et Håkansson. **Figs 287–295.** LM. Scale bar = 10 µm. **Figs 296–300.** SEM, external (**Figs 296–298**) and internal (**Figs 299–300**). **Fig. 296.** Valve face with slit-like ‘chinks’ occlusion. Arrow A: valve face fultoportula opening. **Fig. 297.** Valve face with wrinkled occlusion. Arrow A: valve face fultoportula opening. Arrow B: rimoportula opening. **Fig. 298.** Valve face with poorly occluded areolae. Arrow A: valve face fultoportula opening. **Fig. 299.** Valve face. **Fig. 300.** Valve face/mantle junction. Arrow A: mantle fultoportula opening with two satellite pores. Arrow B: sessile rimoportula labium. Arrow C: valve face fultoportula opening with two satellite pores.

Stephanodiscus parvus Stoermer et Håkansson (Figs 287–300)

Description:

LM observations (Figs 287–295): Valve diameter: 5.0–7.3 µm; interfascicles 14–16 in 10 µm, bright points on spines at the valve margin can be observed.

SEM observations (Figs 296–300): SEM confirms the description of morphological features in the literature (Stoermer & Håkansson 1984, Håkansson 2002, Houk et al. 2014).

Distribution: *Stephanodiscus parvus* is known from its type locality (Stoermer & Håkansson 1984). It is usually observed in eutrophic lakes and brackish rivers (Klee & Schmidt 1987, Liukkonen et al. 1997, Houk et al. 2014) as well as Łęczyca reservoirs.

Remarks: *Stephanodiscus parvus* can often be confused with *S. minutulus*. Håkansson (2002) pointed to two major features that separate these two species. In *S. minutulus* the valve face fultoportula has a heterotopic position and the valve face is undulate at the centre. On the other hand, *S. parvus* has a slightly eccentric fultoportula and the valve face is flat or slightly undulate. However, the position of the valve face fultoportula is not always precisely described; in some cases, it was not seen with LM (Wojtal & Kwandrans 2006). The presence of the valve centre undulation is not always visible in LM and some authors recognize differences in the undulation of these two species (Cruces et al. 2010). During this study, we only found specimens matching *S. parvus,* confirmed by SEM analyses.

Thalassiosira duostra Pienaar in Pienaar et Pieterse (Figs 301–315)

Description:

LM observations (Figs 301–311): Valve diameter: 10.6–18.6 µm; areolae 21–22 in 10 µm.

SEM observations: The valve face is non-plicate, areolae are loculate, with open, partial foramina and a fasciculate pattern. Silica overlapping the areolae is thicker at the valve centre. The areolae are elongated at the junction of the valve face and mantle. A single row of fultoportulae with short tubes is present at the margin of the valve. Rimoportula openings are tube like (Fig. 312).

Areolae on the valve face are rounded, with individual cribra (Fig. 315), whereas on the mantle, they are elongated with continuous cribra (Fig. 314). There are 11–14 mantle fultoportulae in 10 µm, each with four satellite pores (Figs 313–315), and usually 3–5 eccentrically located valve face fultoportulae, with four satellite pores (Fig. 314). From one to three sessile rimoportulae are located on the mantle (Figs 314–315).

Distribution: *Thalassiosira duostra* is known mainly from freshwater environments. It was first described from the eutrophic waters of the Vaal River (South Africa) (Pienaar & Pieterse 1990). In Europe, this species has been recorded from fresh or inland brackish waters in Poland from the Kobylanka stream, which is under anthropogenic pressure (Wojtal & Kwandrans 2006, Wojtal 2009); from eutrophic and polluted lakes and rivers in Hungary (Szabó et al. 2004, Kiss et al. 2012); from the Ebro estuary in Spain with increased conductivity and pH (Pérez et al. 2009) and from a eutrophic reservoir in Brazil (Torgan et al. 2006). It has also been recorded from coastal waters in China (Li et al. 2013).

Remarks: *Thalassiosira duostra* can be described as cosmopolitan, but has rarely been recorded. Our research confirms that it occurs under specific environmental conditions, i.e. running and standing eutrophic aquatic ecosystems with increased conductivity and pH (Pérez et al. 2009, Wojtal 2009, Kiss et al. 2012). Based on the original description of Pienaar & Pieterse (1990), later reports (Wojtal 2009, Kiss et al. 2012, Li et al. 2013) and recent studies, the morphology of *T. duostra* can be described as follows: the valve face is non-plicate, 7–27 µm in diameter. Areolae are loculate with a fasciculate pattern, 10–31 in 10 µm, opening externally by foramina, internally covered by individual cribra over the valve face, and continuous cribra on the mantle. There are 2–6 valve face fultoportulae with four satellite pores. There are from one to three rimoportulae on the mantle, next to a ring of fultoportulae (5–14 in 10 µm) with four satellite pores.

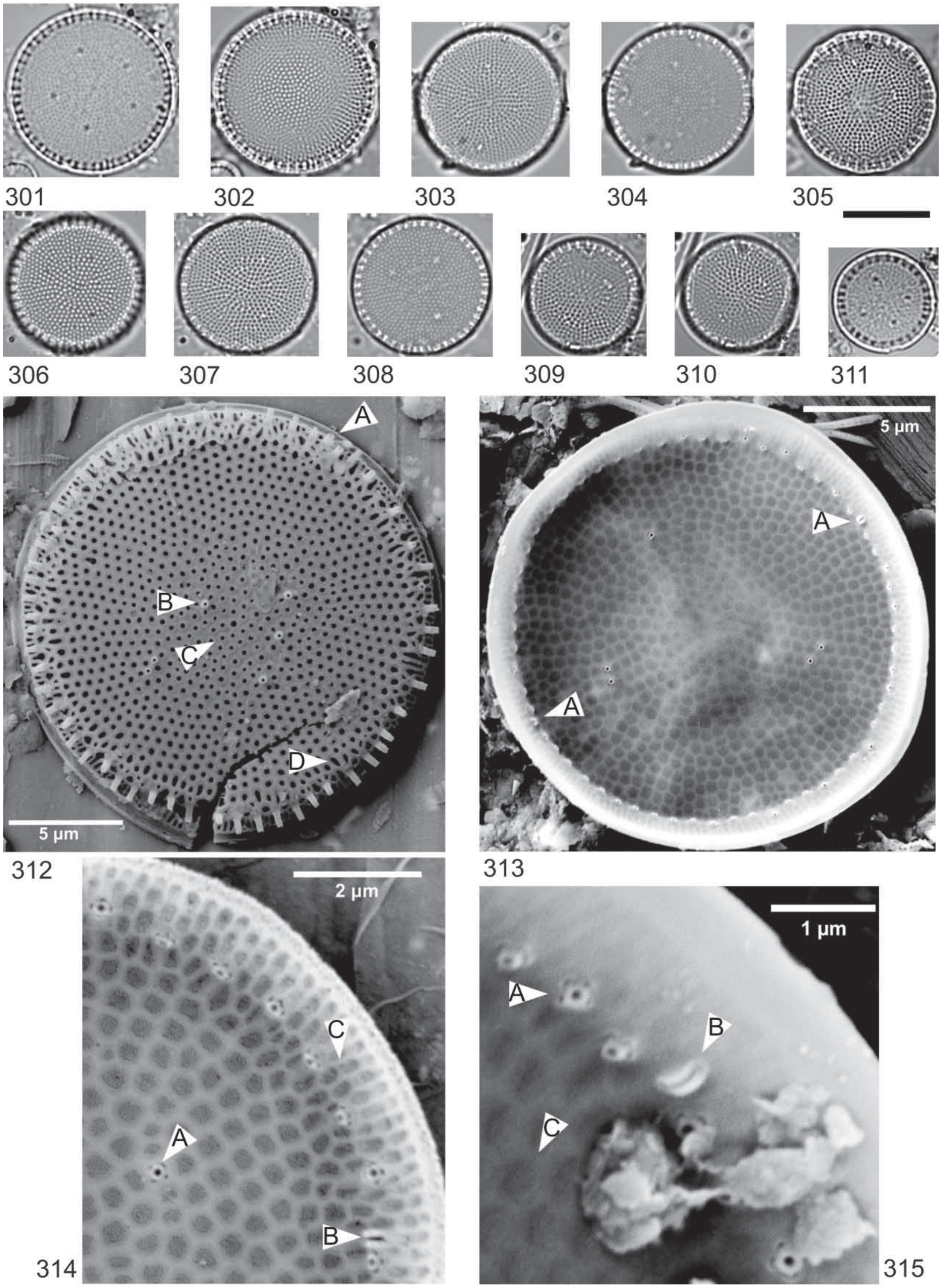
Conclusions

Post-mining reservoirs are unique aquatic ecosystems in which particular environmental conditions can be observed. This type of ecosystem provides an opportunity to observe the morphological diversity of species, at both the generic and infraspecific levels. The results of morphological and ecological analyses on the post-mining reservoirs in the city of Łęczyca led to the following conclusions:

Generally, *Aulacoseira* species exhibit two valve types, linking and separation, the morphology of which can be very different. Descriptions of new *Aulacoseira* species should, therefore, include descriptions of both linking and separation valves;

The diversity of valve morphology of *C. meneghiniana*, particularly the valve face morphology, interstria structure, and other features may be related to specific environmental conditions;

Based on SEM studies, *D. woltereckii*, occurs in both tropical and temperate regions. *Discostella woltereckii* was present in eutrophic reservoirs with a conductivity range of 553–865 µS cm^{-1} and pH of 7.47–8.80. The distinction between *D. woltereckii* and *D. pseudostelligera*, based



Figs 301–315. *Thalassiosira duostra* Pienaar in Pienaar et Pieterse. **Figs 301–311.** LM. Scale bar = 10 µm. **Figs 312–315.** SEM, external (**Fig. 312**) and internal (**Figs 313–315**). **Fig. 312.** Valve face. Arrow A: rimoportula opening. Arrow B: valve face fultoportula opening. Arrow C: areolae with foramina. Arrow D: elongate areolae at the valve face/mantle junction. **Figs 313–315.** Valve. **Fig. 313.** Arrows A: rimoportula openings. **Fig. 314.** Arrow A: valve face fultoportula opening with four satellite pores. Arrow B: rimoportula opening. Arrow C: mantle areolae covered by continuous cribra. **Fig. 315.** Arrow A: mantle fultoportula opening with four satellite pores. Arrow B: sessile rimoportula labium. Arrow C: areola with domed cribrum.

on environmental data, is insufficient. These two species can be observed in the same environment, such as post-mining reservoirs in Łęczyca, which are characterized by an increased concentration of ions.

Stephanodiscus binatus has a similar morphology to *S. parvus*. Based on the original description, references and recent studies, *S. binatus* occurs in mesotrophic and eutrophic environments, like *S. parvus*. This is the first record of *S. binatus* in Poland. *Stephanodiscus binatus* occurs in ecosystems with a wide range of trophy, from oligotrophic to eutrophic, but with increased pH and conductivity;

The morphological diversity of the rarely observed *T. duostra* has been supplemented with data from recent studies. On the basis of these studies and the ecological values of Van Dam et al (1994), *T. duostra* can be classified as: freshwater alkaliphilic, occurring mostly in eutrophic α-mesosaprobic water bodies.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Oświadczenie o współautorstwie

Oświadczam, że mój wkład w przygotowanie oryginalniej pracy polegał na: przygotowaniu koncepcji pracy, zaplanowaniu prac w terenie, identyfikacji taksonomicznej, opracowaniu wyników z wykorzystaniem metod matematycznych. Udział w dyskusji i interpretacji uzyskanych wyników i w przygotowaniu ostatecznej wersji manuskryptu. Swój udział w przygotowanie pracy oceniam na 45%.

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Katedra Algologii i Mykologii

Oświadczam, że mój wkład w przygotowanie oryginalniej pracy polegał na: przygotowaniu koncepcji pracy, zaplanowaniu i realizacji prac w terenie, identyfikacji taksonomicznej, przygotowanie dokumentacji fotograficznej. Udział w analizie i interpretacji uzyskanych wyników. Pracowałem również na ostateczną wersją manuskryptu. Swój udział w przygotowanie pracy oceniam na 50%.

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Critical multi-stranded approach for determining the ecological values of diatoms in unique aquatic ecosystems of anthropogenic origin

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ABSTRACT

Background: The ecological state of surface waters is typically assessed by a multi-aspect approach based on a determination of its chemical and physical parameters, by hydromorphology and the use of indicator organisms such as benthic diatoms. By assigning ecological indicator values, it is possible to create diatom indices which serve as the basic tool in assessing the ecological status of surface waters. These ecological indicator values are set according to classification systems, such as the *Van Dam, Mertens & Sinkeldam (1994)* system, which classifies species of diatoms according to seven different ecological factors. However, recent studies on the autecology of diatoms have shown the need to verify and establish new ecological indicator values. To this end, aquatic ecosystems are good environments to observe the range of tolerance of benthic diatoms to environmental conditions due to their unique physical and chemical parameters. The aim of the present study was to propose the establishment of new, or altered, ecological indicator values, according to the *Van Dam, Mertens & Sinkeldam (1994)* classification, of species of diatoms characteristic of three post-mining aquatic ecosystems.

Methods: In total, 36 species were identified that were characteristic of three waterbodies: a salt aquatic complex (water outflow, a drainage ditch and a pond), mined iron ore reservoirs and a mined lignite reservoir. Their ecological indicator values were specified using OMNIDIA software, and the environmental conditions prevailing in the studied ecosystems were determined. Of the 36 characteristic species, 16 lacking at least one assigned ecological indicator value were analyzed further. The analysis identified three groups of selected characteristic species which showed a correlation, or lack of such, to the tested physical and chemical parameters.

Results: Based on this multistage study of the autecology of characteristic diatoms, comprising an analysis of environmental conditions, literature analysis and reference ecological indicator values of other species, it is proposed that 32 ecological indicator values be established or adjusted for 16 species, and that *Planothidium frequentissimum* be excluded from water quality assessments.

Subjects Aquaculture, Fisheries and Fish Science, Freshwater Biology, Ecohydrology

Keywords Diatoms, Ecological values, Post-mining reservoirs

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INTRODUCTION

Diatoms (Bacillariophyta) are one of the main biotic elements used in the biological assessment of the ecological state of surface waters (Water Framework Directive, *European Union, 2000*). Due to the fact that many countries are obliged to continually engage in biomonitoring, there is a clear need to develop flawlessly functioning methods based on the standardized use of diatoms as bioindicators (*Kahlert et al., 2016*; *Poikane, Kelly & Cantonati, 2016*; *Szczepocka & Żelazna-Wieczorek, 2018*). Diatom indices and ecological systems based on the bioindication values of particular diatom species, derived from various environmental parameters, constitute a fundamental tool in the biological assessment of environments. Diatom indices have been commonly used to assess flowing and standing water for over 20 years (*Kelly et al., 2008*; *Harding & Taylor, 2014*; *Szczepocka et al., 2014*; *Hutorowicz & Pasztalenic, 2014*; *Holmes & Taylor, 2015*; *Żelazna-Wieczorek & Nowicka-Krawczyk, 2015*; *Kolada et al., 2016*).

Currently, many countries use the OMNIDIA program (*Lecointe, Coste & Prygiel, 1993*) as a biological assessment tool. Its latest version (version 6.0.6) allows the calculation of 18 diatom indices, and the determination of seven environmental parameters for eight ecological systems. However, the specific ecological indicator values of many of the species given in the OMNIDIA database are absent or have not been updated in response to recent research. To complete these missing values, and to verify existing ones, further studies are needed of the ecological optima and tolerance of diatom species in different types of aquatic ecosystems.

Due to their specific environmental conditions, post-mining reservoirs represent an extremely valuable source of information for the study of ecological diatom tolerance ranges. Some studies of these environments have been performed, but these have addressed diatom paleoecology and their role as indicators of past climatic or environmental change (*De Haan et al., 1993*; *Rakowska, 1996*; *Thomas & John, 2006*; *Sienkiewicz & Gąsiorowski, 2016*). Until now, the autecology of diatoms in post-mining reservoirs has rarely been studied (*Van Landingham, 1968*; *De Haan et al., 1993*; *Rakowska, 1996*; *Ferreira da Silva et al., 2009*; *Luís et al., 2009*, *2016*; *Sienkiewicz & Gąsiorowski, 2016*).

The present study examines the diatom assemblages present in three post-mining reservoirs of various geological origins. Due to variations in their environmental parameters, these bodies of water serve as specific and unique habitats for the development of these algae. The diatom assemblages quickly adapt to the currently prevailing conditions, which is manifested in the presence of taxa characteristic of these specific parameters. Considering their large share of the assemblage, the index values of the assemblages constitute the most important component in the calculation of diatom indices. These species are therefore of the greatest importance for surface water biomonitoring.

The aim of the present study was to identify the species of diatoms characteristic of the three studied types of post-mine reservoirs. Following this, taxa that did not have at least one ecological indicator value specified in the OMNIDIA database, according to the environmental parameters given by *Van Dam, Mertens & Sinkeldam (1994)*, were identified. New ecological indicator values were proposed based on the relationship between the occurrence of the individual species and certain selected physical and chemical parameters, or existing ones were verified.

The *Van Dam, Mertens & Sinkeldam (1994)* ecological system is one of the main systems on which the OMNIDIA program is based. It describes the ecological indicator values of diatoms according to pH, salinity, nitrogen uptake metabolism, oxygen requirement, saprobity, trophic state and moisture aerophily. These values play a key role in calculating diatom indices, and hence need to be kept up to date to enable accurate routine biomonitoring.

MATERIALS AND METHODS

Study area

The study was performed on three waterbodies created through exploration for mineral deposits or were formed by the closure of mines. All three are located in the Łódzkie and Wielkopolskie voivodeships, Central Poland.

The first complex of waterbodies—Pełczyska (PE), is situated in the village of Pełczyska, between Łódź and Łęczyca (Łódzkie voivodeship) (Fig. 1). As the local area is characterized by the presence of salt deposits, numerous wells were sunk in the eighteenth century to obtain brine. Currently, salt water flows out of one of them. This area has been studied by biologists and hydrobiologists since the 1960s (*Olaczek, 1963*; *Pliński, 1966*, *1969*, *1971a*, *1971b*, *1971c*, *1973*; *Żelazna-Wieczorek, 1996*, *2002*; *Żelazna-Wieczorek, Olszyński & Nowicka-Krawczyk, 2015*; *Żelazna-Wieczorek & Olszyński, 2016*). The waterbodies chosen for our research form the PE hydrological complex located in the vicinity of farmland; it comprises the salt water outflow, a drainage ditch and a pond, which acts as the receiver of the water.

The second complex of waterbodies—Łęczyca (LE), urban reservoirs located within the city of Łęczyca (Łódzkie voivodeship) (Fig. 1). The reservoirs were created following the flooding the open-cast iron ore mine in the 1990s. This area is rich in syderite deposits, which are accompanied by other minerals. The complex consists of three connected reservoirs: two are directly connected to each other (LEP1 and LEP2), and the third (LEP3) is connected to LEP2 via a water drainage ditch (*Olszyński & Żelazna-Wieczorek, 2018*). All three are located in an area with houses, garden plots and partly-wooded areas.

The third waterbody—Bogdałów reservoir (BO), created by the flooding of an opencast brown coal mine. It is located in the village of Bogdałów (Wielkopolskie voivodeship) in an area rich in lignite deposits (Fig. 1). Lignite from quaternary deposits was exploited since 1977 until 1991 to a depth of 50 m. Due to the specific construction of the open-pit area, being characterized by the thickest layer of poorly permeable boulder clay in the region. This pit was later transformed into a storage site for quarried rocks in Koźmin. Finally, in 1993/1994, the drainage and runoff of surface waters were blocked to form a reservoir with a depth of about 12 m surrounded by forest (*Gabryś-Godlewska et al., 2004*; *Gadomska et al., 2007*; *Orlikowski & Szwed, 2009*; *Kasztelewicz, 2011*).

Figure 1 **Location of sampling points in the Łódzkie and Wielkopolskie voivodships, Poland.** (A) Pełczyska (PE). (B) Łęczyca (LE). (C) Bogdałów (BO). Full-size DOI: 10.7717/peerj.8117/fig-1

Samples

Samples of benthic diatoms from sediments and water samples were collected quarterly (once in any season) from each hydrological waterbodies. Analysis of all water samples (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, $Fe^{2+/3+}$, Mn^{3+}) involved flame absorption spectrophotometry SpectrAA 300 (Varian, Palo Alto, CA, USA) (detection limit is 0.05 mg/L) and UV-vis spectrophotometry S.330 (Marcel, Poland) in the case of NH_4^+ (d.l. is 0.001 mg/L) and PO_4^{3-} (d.l. is 0.01 mg/L). SO_4^{2-} was determined by the gravimetric method (PN-C-04566-09), Cl^- by Mohr's method (PN-ISO 9297). The chemical analyses were performed in the Laboratory of the Department of Geology at the Faculty of Geographical Sciences, University of Lodz and the Laboratory of Computer and Analytical Techniques at the Faculty of Biology and Environmental Protection, University of Lodz. The water temperature, pH and electric conductivity were measured in situ (Elmetron CP-401 and CC-401 devices). The following sampling points were established:

Pełczyska (51°58′34.47″N; 19°14′21.11″E)—outflow (D.PESB) (4 samples, both water and benthic), ditch (D.PEDB) (one water and four benthic samples) and pond (D.PEPB) (4 samples, both water and benthic); samples were collected quarterly from July 2013 to March 2014;

Łęczyca (52°3′5.30″N; 19°11′50.24″E)—reservoir 1 (D.LEP1), reservoir 2 (D.LEP2) and reservoir 3 (D.LEP3), samples were collected quarterly from March 2014 to December 2015 (six water and eight benthic samples from each reservoirs);

Bogdałów (52°2′51.29″N; 18°35′51.49″E)—reservoir (D.BOZB), samples were collected quarterly from March 2015 to December 2016 (eight samples, both water and benthic).

In total, 44 benthic samples were collected. The permanent slides were prepared according to *Żelazna-Wieczorek (2011)*. To obtain pure diatom frustule the material was chemically treated using a H_2SO_4 and $H_2Cr_2O_7$. The cleaned diatom precipitate was mounted on permanent slides using Naphrax® synthetic resin.

Qualitative and quantitative analysis of diatoms was performed using a Nikon Eclipse 50i light microscope (LM) under 1000× magnification (plan oil-immersion objective 100×/1.25): the diatoms were identified and counted for up to 500 valves in each permanent slide. Light photomicrographs were taken with an OPTA-TECH digital camera.

When diatoms were difficult to identify using LM they were subjected to scanning electron microscope (SEM) analysis using a Phenom ProX (gold layer of 8 and 20 nm, at 10 kV, low vacuum mode) at the Laboratory of Microscopy Imaging and Specialist Biological Techniques, Faculty of Biology and Environmental Protection, University of Lodz.

Data processing and statistical analysis

The average percentage (AP) for a given species was determined based on the percentage contribution (%) of the species in the samples tested for a given hydrological object (*Żelazna-Wieczorek, 2011*). Species whit AP ≥5% for each hydrological object were identified as dominant.

The incidence was determined according to the *Tümpling & Friedrich (1999)* coefficient according to the range values: 100–75% euconstant taxa (EC), 75–50% constant taxa (CN), 50–25% accessory taxa (AC) and 25–1% accidental taxa (AD) (*Tümpling & Friedrich, 1999*).

Multidimensional scaling analysis (nMDS) based on Bray–Curtis similarity coefficients was used to identify natural groupings of samples. The results are given as a 3D diagram in which the degree of similarity is represented as the distances between particular points (samples), with greater distances indicating a lower degree of similarity. The reliability of the ordering of the assemblage is represented by the stress value, which reflects how well the ordination summarizes the observed distances among the samples. A three-dimensional presentation, whose stress value is lower, is likely to be more satisfactory than a two-dimensional one (*Clarke & Gorley, 2015*).

The Shade Plot analysis, based on the Bray–Curtis similarity coefficient, was used to identify the diatom species that have the strongest influence on the similarities between the samples demonstrated in the nMDS analysis. Shade Plot analysis compares two data matrices with each other and then groups them on two levels, according to the similarity of the samples and the factors affecting their similarity, that is, diatom species. The results are represented graphically by shading individual cells: the intensity of the shading indicates the degree of the influence of a given factor (species) on the position of its sample within a given similarity cluster. The range of the shading was determined on the basis of $\log(x + 1)$ (x—number of valves).

The SIMPER analysis was used to determine the characteristic species distinguishing the studied ecosystems. This method examines the participation of each variable in the overall similarity between groups of samples, thus indicating the species with the greatest influence on the degree of similarity, or dissimilarity, between particular samples and hydrological objects. This analysis is also based on the Bray–Curtis similarity coefficient;

however, unlike the nMDS method, in which one trial is compared to all the other samples, the SIMPER analysis compares a single sample to each subsequent sample (*Żelazna-Wieczorek, 2011*). The results indicate the species which most strongly differentiated a sampled site from the others, and to what extent. A species was regarded as being characteristic of the studied ecosystem if it was characterized by a mean dissimilarity ≥2 according to the SIMPER analysis, and a higher mean abundance greater in one ecosystem than the other.

In total, 19 physical and chemical parameters of water were measured in the studied ecosystems. The results of the correlation analysis found 15 physical and chemical parameters indicating an environmental conditions. The parameters were subjected to principal component analysis (PCA) to determine which had the strongest effect on the selected species.

Using the information from the OMNIDIA database, the environmental conditions for each sampling point were determined according to *Van Dam, Mertens & Sinkeldam (1994)* (Table S1). Following this, the percentage share of diatom species included in each ecological indicator value class was indicated. For species found to be characteristic of the studied ecosystems, classes of ecological indicator values were assembled. Taxa which had at least one value of 0 (unknown) were selected for further analysis.

The selected ecological indicator values according to *Van Dam, Mertens & Sinkeldam (1994)* were verified, or new ones established, for the species found to be characteristic of the studied ecosystems according to three premises: previous literature reports about ecological indicator values of those species, chemical and physical conditions analysis, and the classification of the environmental conditions according to *Van Dam, Mertens & Sinkeldam (1994)*.

The analyses were performed using PRIMER 7.0.13 (nMDS, Shade Plot, SIMPER), OMNIDIA 6.0.6 and STATISTICA 13 (PCA), software.

RESULTS

Chemical analysis of water samples

The mean values and range of all tested parameters are given in Table 1.

The PE hydrological complex was characterized by elevated values of electric conductivity, reaching as high as 9230 µS cm^{-1}. The pH changed with the direction of water outflow: a slightly acidic reaction was observed in the outflow and an alkaline one in the pond. Due to the geological profile of the region, the water flowing out of the well contained a high concentration of chloride ions, whose gradient decreased with the flow of water through the ditch to the pond. In addition, higher concentrations of the cations Mg^{2+}, Ca^{2+}, Na^{+} and K^{+} were observed compared to other ecosystems, as well as the anions HCO_3^-, PO_4^{3-} and SO_4^{2-}.

The K^{+} concentration is acknowledged parameter coming from agricultural activity, in particular animal husbandry, or municipal wastes (*Macioszczyk & Dobrzyński, 2002*).

Each of the sampling points in the PE complex was characterized by different chemical parameters, resulting in differences between the habitats. The highest electrolytic conductivity was noted in the outflow, which was mainly influenced by the concentrations

Table 1 Physical and chemical parameters in the examined sampling sites. The minimum, maximum and mean values.

	Pełczyska (PE)									**Łeczyca (LE)**									**Bogdałów (BO)**		
	Outflow (D.PESB)			**Ditch (D.PEDB)**			**Pond (D.PEPB)**			**L1 (D.LEP1)**			**L2 (D.LEP2)**			**L3 (D.LEP3)**			**Reservoir (D.BOZB)**		
	min	**max**	**avar.**	**min**	**max**	**avar.**	**min**	**max**	**avar.**	**min**	**max**	**avar.**	**min**	**max**	**avar.**	**min**	**max**	**avar.**	**min**	**max**	**avar.**
pH	6.6	7.7	7.0	6.4	6.4	6.4	7.8	9.6	8.7	7.9	8.6	8.2	7.5	8.6	8.2	7.5	8.8	8.3	7.9	8.3	8.1
Conductivity (μS cm^{-1})	4,450	9,230	6,699	5,170	5,170	5,170	2,645	5,150	3,646	657	865	743	558	836	728	472	778	680	505	734	623
T (°C)	6.8	13.9	9.1	7.8	7.8	7.8	1.1	17.3	7.1	1.6	20.6	11.1	0.6	21.1	11.1	2.2	15.2	9.2	4.7	22.7	11.4
HCO_3^- (mg L^{-1})	345	744	475	610	610	610	284	451	352	211	339	260	168	275	210	183	290	241	174	369	270
$CO_2^{(HCO3-)}$ (mg L^{-1})	124	268	171	220	220	220	102	163	126	76	122	94	61	99	75	66	105	87	63	133	96
Cl^- (mg L^{-1})	1,585	2,976	2,426	1,006	1,006	1,006	685	1,524	1,053	57	93	78	60	93	76	67	106	78	41	52	45
N_{NH4} (mg L^{-1})	0.02	1.63	0.45	0.15	0.15	0.15	0.00	0.36	0.11	0.02	0.71	0.22	0.03	0.53	0.26	0.00	1.16	0.32	0.00	0.07	0.02
NH_4^+ (mg L^{-1}])	0.03	2.09	0.71	0.19	0.19	0.19	0.00	0.46	0.14	0.03	0.55	0.22	0.03	0.54	0.28	0.00	0.90	0.31	0.00	0.09	0.03
PO_4^{3-} (mg L^{-1})	0.60	12.46	3.73	8.57	8.57	8.57	0.85	9.10	3.99	0.22	0.53	0.36	0.05	0.57	0.41	0.09	0.57	0.34	0.27	0.45	0.34
P_{PO4} (mg L^{-1})	0.20	4.11	1.23	2.83	2.83	2.83	0.28	3.00	1.32	0.07	0.17	0.12	0.02	0.19	0.13	0.03	0.19	0.11	0.09	0.15	0.12
SO_4^{2-} (mg L^{-1})	176	198	188	165	165	165	151	197	176	71	147	107	67	122	101	63	91	75	110	147	123
S_{SO4} (mg L^{-1})	58.8	66.2	62.9	55.1	55.1	55.1	50.3	65.7	58.7	23.5	49.0	35.8	22.3	40.8	33.8	21.0	30.3	25.1	37.9	49.2	41.5
COLOR (mgPt dm^{-3})	25	160	81	140	140	140	50	120	78	10	60	27	9	60	27	12	60	31	4	10	6
Mn^{3+} (mg L^{-1})	0.17	0.51	0.36	0.14	0.14	0.14	0.03	0.25	0.13	0.00	0.05	0.02	0.01	0.03	0.02	0.01	0.07	0.03	0.00	0.01	0.00
$Fe^{2+/3+}$ (mg L^{-1})	0.25	0.41	0.32	0.25	0.25	0.25	0.05	0.24	0.11	0.03	0.27	0.09	0.00	0.13	0.04	0.01	0.07	0.03	0.01	0.09	0.02
Mg^{2+} (mg L^{-1})	39.5	48.7	45.0	35.9	35.9	35.9	25.6	37.0	32.1	11.6	19.1	15.2	10.3	18.3	15.1	9.5	15.3	12.5	9.6	15.7	11.7
Ca^{2+} (mg L^{-1})	171.7	216.2	195.2	165.0	165.0	165.0	75.8	139.8	121.1	58.0	143.6	89.0	59.5	117.6	79.1	52.6	86.4	65.5	77.7	117.6	86.3
Na^+ (mg L^{-1})	500.7	1537.4	1227.1	453.3	453.3	453.3	277.3	681.8	455.9	15.6	40.4	30.9	20.3	42.4	28.3	22.0	42.4	33.0	24.0	43.1	32.6
K^+ (mg L^{-1})	8.5	124.8	42.4	109.8	109.8	109.8	58.6	68.8	63.5	4.5	9.5	7.2	5.1	9.3	7.3	5.6	10.7	8.2	0.1	3.6	1.5

of Cl^-, Na^+ and HCO_3^- ions. The maximum concentration of HCO_3^- ions was recorded in Pełczyska outflow in March 2014 (D.PESB.250314); in the other locations, it did not exceed 410 mg L^{-1}.

Low concentrations of K^+ ions were observed throughout the entire studied PE complex; however, maximum values were recorded in the locations characterized by the highest HCO_3^- ion content. The highest concentration of Ca^+ ions of all ecosystems was recorded in the outflow. The ditch represented an intermediate section between the PE sampling points. However, as it is susceptible to periodic drying, limited chemical data was collected from this habitat and hence it was not possible to assess its chemical and physical nature.

The lowest electrolytic conductivity was found in the pond, which displayed lower concentrations of Cl^-, Na^+ and, to a lesser degree, HCO_3^-. The pH of the water never dropped below 8, except in one case in March 2014. In the pond, the concentration of K^+ remained relatively unchanged, which could be related to the fact that the reservoir was also a receiver of waters flowing from the surrounding arable fields. The pond was also characterized by the lowest concentration of Ca^{2+} and Mg^{2+}. In the summer periods, a significant reduction in the water table level and occasional drying of the reservoir were noted.

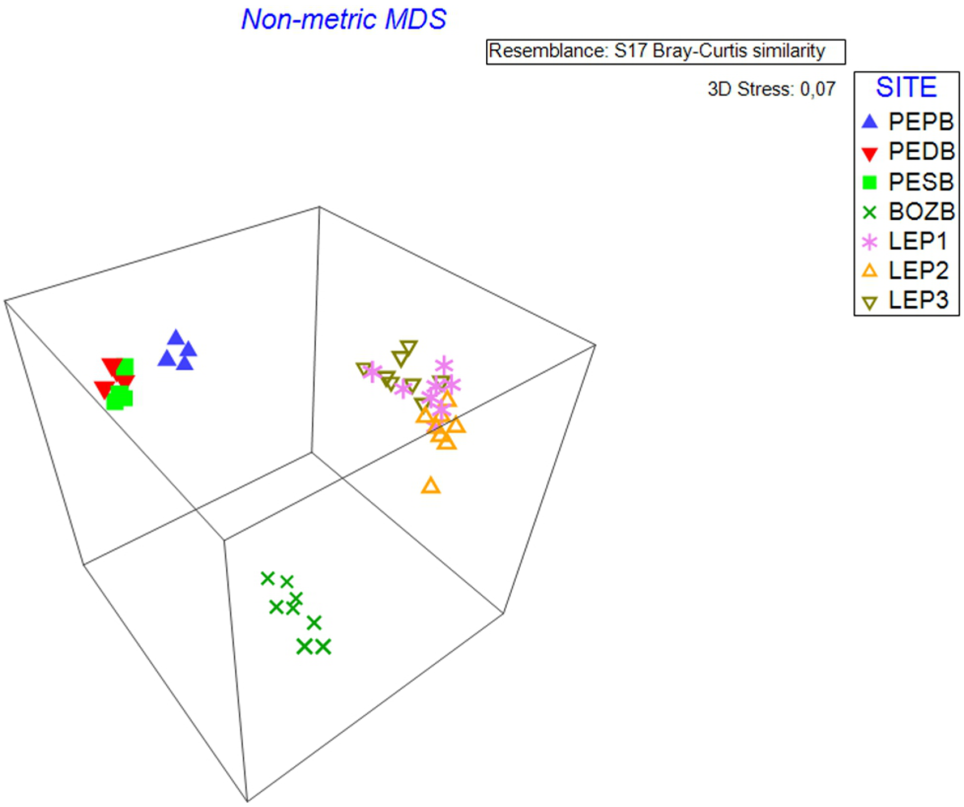

Figure 2 **nMDS 3D analysis.** The diagram shows three distinct clouds of samples which coincide with the three hydrological objects. Full-size DOI: 10.7717/peerj.8117/fig-2

The urban reservoirs in Łęczyca (LE) were characterized by a slightly alkaline water reaction, which was similar in all reservoirs during the course of the study. No elevated concentrations of $Fe^{2+/3+}$ and Mn^{3+} ions were observed. The content of SO_4^{2-} anions was not higher than in other waterbodies studied. The concentration of HCO_3^- ions was lower than that observed in BO and PE. No significant differences in chemical and physical parameters were observed between the individual sampling points constituting LE.

The Bogdałów (BO) reservoir was characterized by an alkaline reaction. It's K^+, Cl^- and NH^{4+} ion content was the lowest of the studied ecosystems.

Diatom samples

A total of 381 diatom taxa were identified in 44 benthic samples: 139 in PE, 192 in LE and 188 in BO. The dominant species in PE were *Navicula veneta*, and *Nitzschia frustulum*, in LE *Cyclostephanos dubius* and *Stephanodiscus hantzschii*, in BO *Achnanthidium minutissimum*, *Pantocsekiella ocellata* and *Mastogloia smithii*. In the examined ecosystems, the most commonly identified classes were accidental (PE-84; LE-111; BO-86), accessory (PE-25; LE-35) and euconstant taxa (BO-39) (Fig. S1).

nMDS analysis

nMDS analysis (stress level = 0.07) identified the variation between samples for each studied hydrological object (Fig. 2). The samples taken from BO constitute a separate

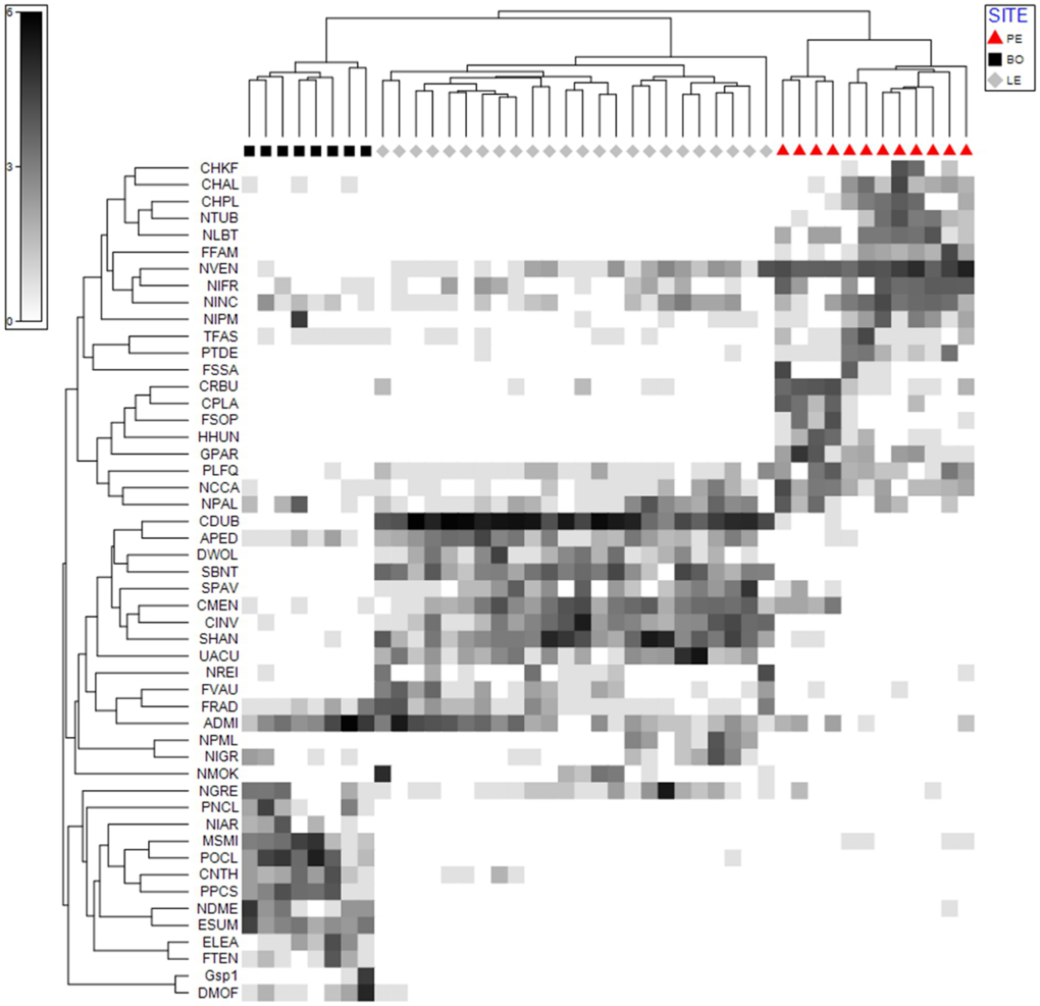

Figure 3 Shade Plot analysis. The diagram shows the strength of the factor (taxon) affecting the similarity between the samples. Upper dendrogram—samples divided according to hydrological object. Left dendrogram—50 taxa of diatoms which have the strongest influence on the similarity between the samples. Full-size DOI: 10.7717/peerj.8117/fig-3

cloud, with the samples demonstrating high similarity with each other, whereas the samples of D.LEP1, D.LEP2 and D.LEP3 constitute a distinct group, with no clear differentiation into individual reservoirs. In the case of PE, the pond group (D.PEPB) was found to be clearly distinct from the others.

Shade plot

Shade plot analysis identified 50 species which had the strongest influence on the degree of similarity, or non-similarity, between the samples in the studied ecosystems. Of these taxa, the 11 that most strongly influenced the similarity between the samples in at least two ecosystems were *Navicula veneta* (NVEN), *Navicula cincta* (NCCA), *Navicula gregaria* (NGRE), *Nitzschia frustulum* (NIFR), *Nitzschia inconspicua* (NINC), *Nitzschia palea* (NPAL), *Planothidium frequentissimum* (PLFQ), *Amphora pediculus* (APED), *Cyclotella meneghiniana* (CMEN), *Fragilaria radians* (FRAD) and *Achnanthidium minutissimum* (ADMI) (Fig. 3).

Table 2 Characteristic species according to SIMPER analysis for studied waterbodies.

Pełczyska complex PE	Łęczyca reservoirs LE	Bogdałów reservoir BO
Chamaepinnularia krookiformis (AC)	*Achnanthidium minutissimum* (EC)	*Achnanthidium minutissimum* (EC)
Chamaepinnularia plinskii (CN)	*Amphora pediculus* (EC)	*Diatoma moniliformis* (EC)
Cocconeis placentula (CN)	*Cyclostephanos dubius* (EC)	*Encyonopsis subminuta* (EC)
Craticula buderi (EC)	*Cyclostephanos invisitatus* (EC)	*Mastogloia smithii* (EC)
Craticula halophila (EC)	*Cyclotella meneghiniana* (EC)	*Nitzschia dissipata* var. *media* (EC)
Fragilaria famelica (EC)	*Navicula gregaria* (EC)	*Pantocsekiella ocellata* (EC)
Fragilaria sopotensis (CN)	*Navicula moskalii* (AC)	*Pantocsekiella pseudocomensis* (EC)
Gomphonema parvulum (EC)	*Nitzschia palea* (EC)	
Hippodonta hungarica (CN)	*Stephanodiscus binatus* (EC)	
Navicula cincta (EC)	*Stephanodiscus hantzschii* (EC)	
Navicula veneta (EC)	*Stephanodiscus parvus* (EC)	
Nitzschia frustulum (EC)		
Nitzschia inconspicua (EC)		
Nitzschia liebethruthii (EC)		
Nitzschia palea (CN)		
Nitzschia perminuta (EC)		
Nitzschia tubicola (CN)		
Planothidium delicatulum (EC)		
Planothidium frequentissimum (EC)		
Tabularia fasciculata (EC)		

Note:
EC, euconstant taxa; CN, constant taxa; CN, accessory taxa (*Tümpling & Friedrich, 1999*).

SIMPER analysis

SIMPER analysis allowed 36 species characteristic of the tested hydrological objects to be distinguished (Table 2). In addition, two species were found to be characteristic of two different ecosystems: *Achnanthidium minutissimum* for LE and BO and *Nitzschia palea* for PE and LE.

Ecological analysis based on OMNIDIA software

The ecological analysis of diatom assemblages based on data obtained from the OMNIDIA program database, indicated the following:

- pH requirements: while alkaliphilic species predominate in PE (63%), a large percentage in D.PEPB are unknown (24%) or neutrophilic species (23%). The LE reservoirs were dominated by alkalibiontic (45%) and alkaliphilic (24%) organisms. In D.LEP1, 25% of species were unknown. BO was dominated by alkaliphilic (39%) and neutrophilic (29%) species, and 26% of species were unknown (Fig. S2);

- Salinity: the PE complex was characterized by the occurrence of halophilic (43%), oligohalobous (30%) and mesohalobous species (16%); the greatest proportion of the mesohalobous species were found in D.PESB (28%). The LE reservoirs were dominated by oligohalobous (44%) and halophilic species (42%). BO was dominated by oligohalobous (43%), halophobe (24%) and unknown species (23%) (Fig. S2);

- Nitrogen uptake: the most common species in the PE complex were N-autotrophic tolerant (39%) followed by unknown (25%). The largest percentage of unknown species (28%) was recorded in D.PEPB and D.PEDB. In the LE reservoirs, the most common groups of species were N-autotrophic (57%) and unknown (25%). In BO, 51% species were unknown, 24% were N-autotrophic tolerant and 22% N-autotrophic sensitive (Fig. S2);
- Oxygen requirements: in PE, the largest groups of species were low oxygen (30%), unknown (27%) and moderate oxygen (24%). In LE, oxybiontic species were most common (43%) followed by unknown (25%). In BO, unknown (46%) and polyoxybiontic species (42%) predominated (Fig. S2);
- Sensitivity to saprobity: in PE, the largest group of taxa were α-meso-polysabrobe (28%) and unknown (23%). In D.PEPB, the most abundant was α-meso-polysabrobe (34%) followed by β-mesosaprobe (31%) and unknown (27%). LE primarily included taxa from the α-mesosaprobe group (47%) and unknown (23%). In BO, unknown (34%), β-mesosaprobe (31%) and oligosaprobe taxa (28%) predominated (Fig. S2);
- Trophic status: in PE, the largest group of diatoms were eutrophic (50%) and unknown taxa (25%), LE had the highest percentage (61%) of eutrophic species but also unknown (15%) and hypereutrophic (13%) were present. In BO, the most abundant species were unknown (42%), indifferent (19%) and meso-eutrophic (16%) (Fig. S2);
- Moisture aerophily: in PE, the largest group was aquatic to aerophilic (56%), representing 66% of species in D.PESB, 61% in D.PEDB, and 42% in D.PEPB. The second largest group was unknown (23%), constituting 32% of taxa in D.PEPB. In LE, 37% of the species were aquatic (24% of taxa in D.LEP2), 54% were occasionally aerophilic and 22% were unknown. In BO, the predominant groups of species were unknown (44%) and aquatic to aerophilic (33%) (Fig. S2).

Characteristic species: OMNIDIA and PCA analysis

The analysis of species characteristic of the tested ecosystems, determined according to *Van Dam, Mertens & Sinkeldam (1994)*, identified 16 taxa classified as 0 in at least one category (Table 3). The next step determined the percentage contribution of each of these species classified as class 0 for the ecological parameters defined by *Van Dam, Mertens & Sinkeldam (1994)* at each sampling point (Table S2).

The PCA was performed to find the relationships between the abiotic parameters and the characteristic species ($n = 36$) (Fig. 4). The Eigenvalues Plot method given eigenvalues above 1%, showed that 12 factors account for 83.2% of the total variance. The first two factors account for 31.3% of the total variance. Based on the PCA analysis for of the 16 characteristic taxa mentioned above and physical and chemical parameters, the following relationships were demonstrated:

- Group A: *Chamaepinnularia krookiformis*, *Chamaepinnularia plinskii*, *Nitzschia liebethruthii* and *Planothidium delicatulum* demonstrate a negative correlation with pH and a positive correlation with a decrease in the concentrations of HCO_3^-, Ca^{2+} and $Fe^{2+/3+}$ (Fig. 4).

- Group B: *Craticula buderi*, *Planothidium frequentissimum* and *Navicula cincta* did not demonstrate any relationship with any water parameters (Fig. 4).

- Group C: *Navicula moskalii*, *Cyclostephanos invisitatus*, *Stephanodiscus parvus*, *S. binatus*, *Diatoma moniliformis*, *Nitzschia dissipata* var. *media*, *M. smithii*, *Pantocsekiella pseudocomensis* and *Encyonopsis subminuta* demonstrated a negative correlation with a decrease in electrolyte conductivity, as well as with the concentrations of K^+, Mg^{2+}, Na^+, SO_4^{2-}, Cl^-, PO_4^{3-} and Mn^{3+} and water pigments (Fig. 4).

DISCUSSION

Verification and establishing new ecological indicator values is a key step in standard biomonitoring procedure (*Szczepocka & Żelazna-Wieczorek, 2018*). To specify ecological indicator values or establish new ones we have determined characteristic species. For these species we performed analysis of environmental condition of the ecosystem where they were noted, previous published data and co-occurring species.

Planothidium delicatulum (PTDE) (Figs. 5A–5E)

Planothidium delicatulum is a euconstant taxon for PE and an accidental taxon for LE. Its mean percentage share in PE was 2%, and constituted 5% in D.PESB.

Planothidium delicatulum does not currently have one ecological indicator value (oxygen requirements) according to *Van Dam, Mertens & Sinkeldam (1994)*.

This species was more abundant in environments such as D.PESB, which was also characterized by the highest concentration of Cl^- (up to 2976 mg L^{-1}), elevated electrolytic conductivity, and decreased K^+ concentration. The pH of the water in which this species was observed did not exceed 7.

Planothidium delicatulum was mainly recorded in salty and brackish environments with neutral or slightly alkaline conditions (*Campeau, Pienitz & Héquette, 1999*; *Gell et al., 2005*; *Caballero et al., 2013*; *Yamamoto, Chiba & Tuji, 2017*; *Van de Vijver, Wetzel & Ector, 2018*).

Based on our findings, we suggest changing the following ecological indicator values in the *Van Dam, Mertens & Sinkeldam (1994)* classification for *Planothidium delicatulum*:

- pH requirements: 3 (neutrophilic) (changing from 5 to 3);
- salinity: 5 (brackish-marine) (changing from 4 to 5).

Chamaepinnularia krookiformis (CHKF) (Figs. 5F–5I) and *Chamaepinnularia plinskii* (CHPL) (Figs. 5J–5M)

In 2016, *Chamaepinnularia krookiformis* was divided into two separate taxa: *Chamaepinnularia krookiformis* and *Chamaepinnularia plinskii* (*Żelazna-Wieczorek & Olszyński, 2016*). Both species were very often recorded together in the same ecosystem. However, the publications which identified *Chamaepinnularia krookiformis* often do not

Table 3 **Characteristics species with classification of ecological indicators values by *Van Dam, Mertens & Sinkeldam (1994)*.**

Species	Code	Moisture aerophity	Nitrogen uptake	pH requirements	Oxygen requirements	Salinity	Saprobity	Trophic state
Achnanthidium minutissimum	ADMI	3	2	3	1	2	2	7
Amphora pediculus	APED	3	2	4	2	2	2	5
Chamaepinnularia krookiformis	CHKF	3	0	3	0	3	1	0
Chamaepinnularia plinskii	CHPL	0	0	0	0	0	0	0
Cocconeis placentula	CPLA	2	2	4	3	2	2	5
Craticula buderi	CRBU	0	0	0	0	0	0	0
Craticula halophila	CHAL	2	2	4	2	4	3	5
Cyclostephanos dubius	CDUB	1	2	5	2	3	3	5
Cyclostephanos invisitatus	CINV	0	0	0	0	2	0	5
Cyclotella meneghiniana	CMEN	2	3	4	5	3	4	5
Diatoma moniliformis	DMOF	0	0	0	0	0	0	0
Encyonopsis subminuta	ESUM	0	0	3	1	1	1	1
Fragilaria famelica	FFAM	3	1	4	1	2	1	3
Fragilaria sopotensis	FSOP	1	2	4	1	2	2	4
Gomphonema parvulum	GPAR	3	3	3	4	2	4	5
Hippodonta hungarica	HHUN	3	2	4	3	2	2	4
Mastogloia smithii	MSMI	3	0	4	0	4	2	0
Navicula cincta	NCCA	0	0	0	0	2	0	7
Navicula gregaria	NGRE	3	2	4	4	3	3	5
Navicula moskalii	NMOK	0	0	0	0	0	0	0
Navicula veneta	NVEN	3	2	4	4	3	4	5
Nitzschia dissipata var. *media*	NDME	0	0	4	0	2	0	0
Nitzschia frustulum	NIFR	3	4	4	3	3	2	5
Nitzschia inconspicua	NINC	3	3	4	3	3	3	5
Nitzschia liebethruthii	NLBT	0	0	5	0	4	0	0
Nitzschia palea	NPAL	3	4	3	4	2	5	6
Nitzschia perminuta	NIPM	3	1	4	1	2	1	2
Nitzschia tubicola	NTUB	2	3	4	4	3	5	6
Pantocsekiella ocellata	POCL	1	1	4	1	1	1	4
Pantocsekiella pseudocomensis	PPCS	0	0	0	0	0	0	0
Planothidium delicatulum	PTDE	3	1	5	0	4	5	3
Planothidium frequentissimum	PLFQ	0	2	4	3	2	4	7
Stephanodiscus binatus	SBNT	0	0	0	0	0	0	0
Stephanodiscus hantzschii	SHAN	2	3	5	4	2	4	6
Stephanodiscus parvus	SPAV	0	0	5	0	2	0	6
Tabularia fasciculata	TFAS	3	2	4	3	4	3	5

provide appropriate photographic documentation or photos of individual specimens (*Witkowski, 1994*; *Bąk, Witkowski & Lange-Bertalot, 2006*; *Wojtal, 2009*; *Peszek et al., 2015*). Currently available documentation is insufficient to determine whether *Chamaepinnularia krookiformis* and *Chamaepinnularia plinskii* are both present simultaneously in a given environment or whether just one of these species exists.

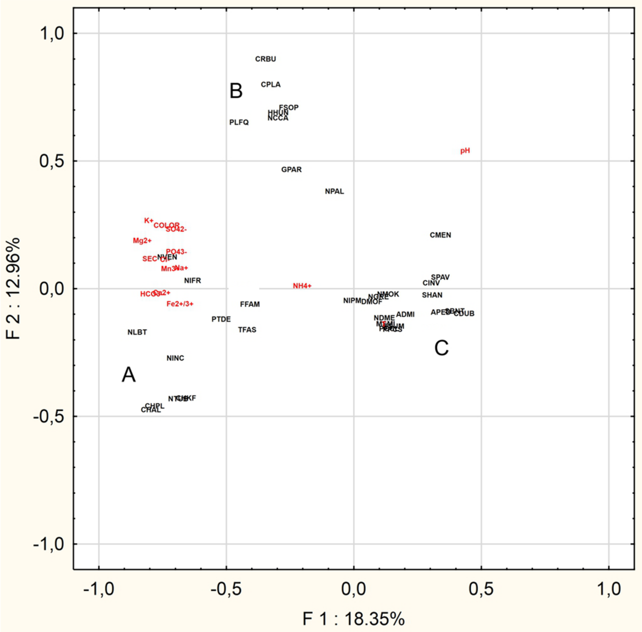

Figure 4 **Principal Component Analysis.** The diagram presents three groups of species A, B and C, whose occurrence can be correlated with selected physical and chemical factors.

Full-size DOI: 10.7717/peerj.8117/fig-4

Chamaepinnularia krookiformis is an accessory taxon for PE (a constant taxon for D.PEDB), *Chamaepinnularia plinskii* is a constant taxon for PE (a euconstant taxon for D.PEDB). The mean share of *Chamaepinnularia krookiformis* was 1.6% in all PE samples, 4% in D.PEDB; for *Chamaepinnularia plinskii*, this amounted to 2.7% in PE, 6% in D.PEDB.

Currently, *Chamaepinnularia krookiformis* lacks three assigned ecological indicator values. For PE, it constitutes 6% of the unknown group in nitrogen uptake, 6% in oxygen requirements and 6% in trophic state (respectively for D.PEDB: 16%, 16% and 15%). *Chamaepinnularia plinskii* has no assigned ecological indicator values and represents 26% of the unknown group for pH requirements, 19% for salinity, 12% for nitrogen uptake, 10% for oxygen requirements, 14% for saprobity, 11% for trophic state and 10% for moisture (respectively for D.PEDB: 60%, 25%, 24%, 23%, 32%, 23% and 28%).

The conditions of the environments in which both species have been recorded indicate that they are class 3 with regard to pH range (neutrophilic). Both species were the most abundant in locations subjected to periodic drying and characterized by high concentrations of chloride ions (up to 1006 mg L^{-1}) indicating a brackish environment (*Żelazna-Wieczorek, Olszyński & Nowicka-Krawczyk, 2015*).

On the basis of our findings and those of previous studies (*Krammer & Lange-Bertalot, 1986*; *Krammer, 1992*; *Witkowski, 1994*; *Bąk, Witkowski & Lange-Bertalot, 2006*; *Wojtal, 2009*; *Peszek et al., 2015*; *Żelazna-Wieczorek & Olszyński, 2016*),

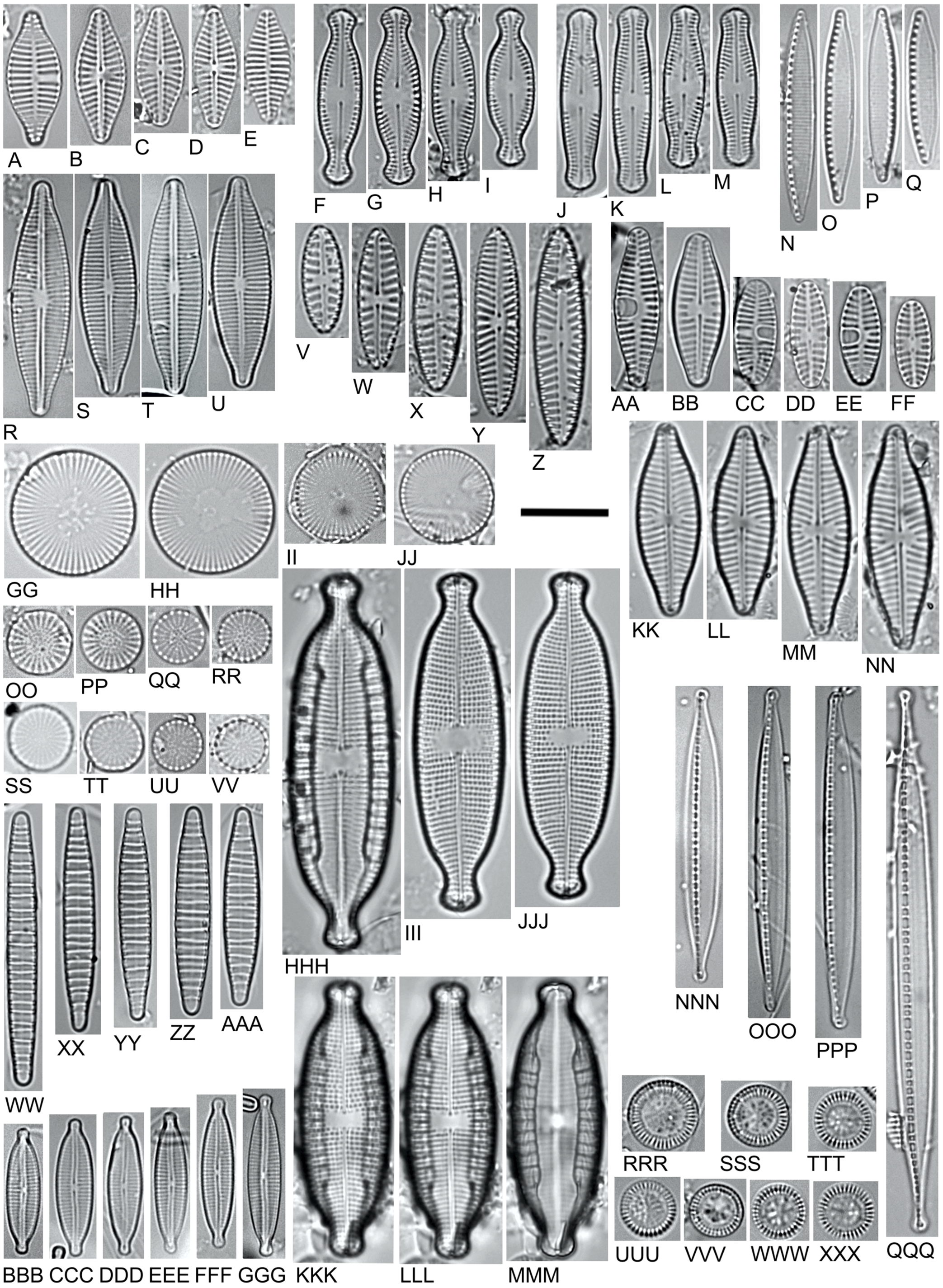

Figure 5 **LM microphotographs of characteristic diatom species.** (A–E). *Planothidium delicatulum*. (F–I) *Chamaepinnularia krookiformis*. (J–M). *Chamaepinnularia plinskii*. (N–Q) *Nitzschia liebethruthii*. (R–U) *Craticula buderi*. (V–Z). *Navicula cincta*. (AA–FF) *Planothidium frequentissimum*. (GG–JJ). *Cyclostephanos invisitatus*. (KK–NN). *Navicula moskalii*. (OO–RR). *Stephanodiscus binatus*. (SS–VV). *S. parvus*. (WW–AAA). *Diatoma moniliformis*. (BBB–GGG). *Encyonopsis subminuta*. (HHH–MMM). *Mastogloia smithii* (KKK–MMM. Same specimen, different focal plane). (NNN–QQQ) *Nitzschia dissipata* var. *media*. (RRR–XXX) *Pantocsekiella pseudocomensis*. Scale bar = 10 μm.

Full-size DOI: 10.7717/peerj.8117/fig-5

according to *Van Dam, Mertens & Sinkeldam (1994)* ecological indicator system we propose:

established ecological indicator values for *Chamaepinnularia plinskii*

- pH requirements: 3 (neutrophilic);
- salinity: 4 (mesohalobous);
- trophic state: 5 (eutrophic);
- moisture aerophily: 4 (aerophilic);
- saprobity: 4 (α-meso-polysabrobe);

for *Chamaepinnularia krookiformis*

- trophic state: 5 (eutrophic);

and the following changes for *Chamaepinnularia krookiformis*

- salinity: from 3 to 4 (mesohalobous);
- moisture aerophily: from 3 to 4 (aerophilic);
- saprobity: from 2 to 4 (β-mesosaprobe to α-meso-polysabrobe).

Due to the specific conditions and locations of the studied objects, they were exposed to large fluctuations in the inflow of organic matter, mainly from runoff from arable fields and pollution caused by animal grazing. These impurities were manifested as elevated concentrations of K^+ ions. Therefore, our results suggest that classifying *Chamaepinnularia krookiformis* as an oligosaprobe is inappropriate. Further tests are needed to determine the optimum occurrence of these species in areas subjected to organic matter loads.

Nitzschia liebethruthii (NLBT) (Figs. 5N–5Q)

Nitzschia liebethruthii is a euconstant taxon for PE. Its means percentage share was 4% in the PE samples, and 10% in the D.PEDB samples. It was most numerous in the sample from November 2013 (19%). This species has two specific ecological indicator values. The ecological indicator value analysis for PE found *Nitzschia liebethruthii* to represent 17% of the unknown group in nitrogen uptake, 16% in oxygen requirements, 18% in saprobity, 17% in trophic state and 21% in moisture (respectively for D.PEDB: 37%, 37%, 43%, 36% and 49%).

Nitzschia liebethruthii occurred in environments subjected to periodic drying with a pH close to 7 and high concentration of chloride ions.

This species was noted in environments with increased salinity, electrolytic conductivity and high pH value (*Rumrich, Lange-Bertalot & Rumrich, 2000*; *Witkowski, Lange-Bertalot & Metzeltin, 2000*; *Lange-Bertalot et al., 2017*; *Földi et al., 2018*).

We propose established new ecological indicator values according to *Van Dam, Mertens & Sinkeldam (1994)* assigned to *Nitzschia liebethruthii*:

- trophic state: 5 (eutrophic);
- moisture aerophily: 4 (aerophilic);

and following changes:

- pH requirements: from 5 to 3 (alkalibiontic to neutrophilic);
- saprobity: from 2 to 4 (β-mesosaprobe to α-meso-polysabrobe).

Craticula buderi (CRBU) (Figs. 5R–5U)

Craticula buderi is a euconstant taxon for PE and an accidental taxon for LE. Its mean percentage share was 4% in all samples for PE, and 12% for D.PEPB. This species has no recorded ecological indicator values. The ecological indicator value analysis for the PE found *Craticula buderi* to constitute 19% of the unknown group in pH requirements, 28% in salinity, 16% in nitrogen uptake, 16% in oxygen requirements, 16% in saprobity, 19% in trophic state and 15% in moisture (respectively for D.PEPB: 53%, 73%, 46%, 46%, 46%, 54% and 41%).

Although *Craticula buderi* was classified into group B (PCA), it was found to be most abundant in environments with an elevated concentration of Cl^- ions, ranging from 685 to 1090 mg L^{-1}, (all samples from D.PEPB and one sample from D.PEDB in which the concentration of chloride ions was 1006 mg L^{-1}). However, relative abundance was lower in the D.PEPB sample, which was characterized by a chloride ion content of over 1500 mg L^{-1}. Interestingly. The concentration of K^+ ions exceeded 100 mg L^{-1} at Cl^- concentrations below 1500 mg L^{-1}; therefore, it is possible that the decline of this species could be related to the concentration of K^+ ions alone. Our observations indicate that the population of *Craticula buderi* from D.PEPB favors a concentration of chloride ions from 500 to 1006 mg L^{-1} which coincides with a K^+ ions concentration from 50 to 70 mg L^{-1}.

Craticula buderi is widespread throughout the world and recognized as cosmopolitan (*Rumrich, Lange-Bertalot & Rumrich, 2000*; *Lange-Bertalot, 2001*; *Bahls, 2009*; *Soltanpour-Gargari, Lodenius & Hinz, 2011*; *Żelazna-Wieczorek, 2011*; *Cichoń, 2016*). This species was found to be dominant in environments characterized by increased electrolytic conductivity and alkaline water (*Holmes & Taylor, 2015*). *Holmes & Taylor (2015)* place *Craticula buderi* in the Bad water quality class. Their recorded values of diatom indices indicate that the environment was eutrophic.

We therefore propose the following classes of ecological indicator values according to *Van Dam, Mertens & Sinkeldam (1994)* for *Craticula buderi*:

- pH requirements: 4 (alkaliphilic);
- trophic state: 5 (eutrophic);
- salinity: 4 (mesohalobous);
- moisture aerophily: 3 (aquatic to aerophilic);

and following change:

- sabrobity: from 2 to 4 (β-mesosaprobe to α-meso-polysabrobe).

Navicula cincta (NCCA) (Figs. 5V–5Z)

Navicula cincta is a euconstant taxon for PE, a constant taxon for LE and an accessory taxon for BO. The mean percentage share of this species for PE is 3%, of which 7% was found in D.PEPB samples.

Currently this species has been assigned two ecological indicator values. The ecological indicator values analysis for the PE found *Navicula cincta* to constitute 24% of the unknown group in pH requirements, 13% in nitrogen uptake, 12% in oxygen requirements, 13% in saprobity, and 12% in moisture (respectively for D.PEPB: 28%, 25%, 25%, 26% and 23%).

An analysis of the physical and chemical data and the variability of occurrence did not show any clear relationships between environmental parameters and the percentage share of *Navicula cincta* in the tested samples. This lack of dependence is also confirmed by the PCA analysis.

Navicula cincta has been recorded in various types of ecosystems, although mainly in eutrophic ones with high conductivity. It also tolerates elevated levels of organic matter. This species was also observed in habitats subjected to periodic drying (*Lange-Bertalot & Genkal, 1999*; *Rumrich, Lange-Bertalot & Rumrich, 2000*; *Witkowski, Lange-Bertalot & Metzeltin, 2000*; *Lange-Bertalot, 2001*; *Żelazna-Wieczorek, 2011*; *Wojtal, 2013*; *Lange-Bertalot et al., 2017*). However, several new species from the group *Navicula cincta* s.l. have been described, and it can be assumed that each of these individual species in this group may be associated with narrower optimal ecological conditions (*Cantonati et al., 2016*).

Based on our present findings, and those of previous studies, in our opinion that it is not appropriate to classify *Navicula cincta* as an oligohalobous species with regard to salinity: it has been recorded in fresh (*Żelazna-Wieczorek, 2011*; *Wojtal, 2013*), brackish (*Żelazna-Wieczorek, Olszyński & Nowicka-Krawczyk, 2015*; *Żurek et al., 2018*) and salt waters (*Witkowski, Lange-Bertalot & Metzeltin, 2000*). We propose the following ecological indicator value according to *Van Dam, Mertens & Sinkeldam (1994)* for *Navicula cincta* s.l.:

- moisture aerophily: 3 (aquatic to aerophilic).

Shade Plot analysis found that the presence of *Navicula cincta* s.l. can falsely indicate high similarity between samples from different environments, thus distorting the results of any environmental analysis. Therefore, with regard to the unclear taxonomic status of *Navicula cincta* s.l. and the current lack of knowledge regarding its activities, we recommend this taxon be excluded from the biological assessment of surface water quality.

Planothidium frequentissimum (PLFQ) (Figs. 5AA–5FF)

Planothidium frequentissimum is a euconstant taxon for PE and LE. The mean percentage of this species for PE is 2.7%, of which 5% was found in D.PEPB samples. It was most numerous in the D.PEDB.301113 sample (19%). The species has currently six established ecological indicator values. The ecological indicator values analysis for PE found

Planothidium frequentissimum to constitute 12% of the unknown group in moisture aerophily (for D.PEPB 14% and D.PESB: 20%).

No relationship was observed between percentage share of *Planothidium frequentissimum* and the changes in chemical and physical parameters in the tested samples. This lack of relationship was confirmed by PCA analysis.

Planothidium frequentissimum is an eurytopic species that occurs globally in a variety of habitat types, from natural springs to rivers in urban areas with high levels of pollution. Its value as an indicator is low, as confirmed by the Shade Plot analysis, which found it to significantly affect the degree of similarity observed between samples from different environments (*Siver et al., 2005*; *Levkov et al., 2007*; *Żelazna-Wieczorek, 2011*; *Kulikovskiy, Lange-Bertalot & Kuznetsova, 2015*; *Szczepocka, Nowicka-Krawczyk & Kruk, 2018*).

Recently *Planothidium frequentissimum* was divided into several different species. In studied samples we observe several species which belong to *Planothidium frequentissimum* s.l. (Figs. 5AA–5FF) (*Wetzel et al., 2019*). A light microscope (LM) is still used to identify species in ecological research and biological assessment of aquatic ecosystems. Due to the likeness of the basic morphological features of the newly described species observed in LM, especially in the case of *Planothidium frequentissimum* s.s. and *Planothidium straubianum*, distinguishing them will be difficult or limited, which may lead to errors in the assessment. We therefore recommend that *Planothidium frequentissimum* s.l. be excluded from the biological assessment of surface water quality.

Cyclostephanos invisitatus **(CINV) (Figs. 5GG–5JJ)**

Cyclostephanos invisitatus is a euconstant taxon for LE. Its percentage share for LE was 4.8%. *Cyclostephanos invisitatus* currently has two ecological indicator values assigned. The ecological indicator values analysis for LE found it to constitute 24% of the unknown group in pH requirements, 17% in nitrogen uptake, 18% in oxygen requirements, 19% in saprobity, and 20% in moisture.

Cyclostephanos invisitatus occurs in diverse environments, however, it is most frequently reported in aquatic ecosystems subjected to high human impact, alkaline and high conductivity (*Reavie & Smol, 1998*; *Yang et al., 2005*; *Wojtal & Kwandrans, 2006*; *Kiss et al., 2012*; *Houk, Klee & Tanaka, 2014*; *Reavie & Kireta, 2015*; *Olszyński & Żelazna-Wieczorek, 2018*).

We therefore propose that the following classes of ecological indicator values according to *Van Dam, Mertens & Sinkeldam (1994)* be established for *Cyclostephanos invisitatus*:

- pH requirements: 4 (alkaliphilic);
- moisture aerophily: 1 (aquatic).

Navicula moskalii **(NMOK) (Figs. 5KK–5NN)**

Navicula moskalii is an accessory taxon for LE. Its mean percentage share for LE was 1.5%. Its incidence was greatest in sample D.LEP1.250315 (26%).

Navicula moskalii has no assigned ecological indicator values. The ecological indicator value analysis for LE found it to constitute 5% of the unknown group in pH requirements, 7% in salinity, 5% in nitrogen uptake, 5% in oxygen requirements, 5% in saprobity, 6% in trophic state and 5% in moisture. The greatest occurrence of *Navicula moskalii* was observed in samples with the highest concentrations of Ca^{2+} (143.6 mg L^{-1}), HCO_3^- (338.6 mg L^{-1}), SO_4^{2-} (146.9 mg L^{-1}) and with high Mg^{2+} content.

Navicula moskalii was observed in a number of ecosystems (*Metzeltin & Witkowski, 1996*; *Lange-Bertalot, 2001*; *Żelazna-Wieczorek, 2011*; *Noga et al., 2016*; *Lange-Bertalot et al., 2017*), particularly in eutrophic waters with an elevated level of Ca^{2+} and HCO_3^- ions. *Żelazna-Wieczorek (2011)* report a significant number of *Navicula moskalii* in springs with high levels of eutrophication, however with Ca^{2+}, SO_4^{2-}, HCO_3^- and Mg^{2+} concentrations lower than those in the LE samples.

We therefore propose that the following classes of ecological indicator values according to *Van Dam, Mertens & Sinkeldam (1994)* be established for *Navicula moskalii*:

- pH requirements: 4 (alkaliphilic);
- salinity: 2 (oligohalobous);
- trophic state: 7 (indifferent).

Stephanodiscus binatus **(SBNT) (Figs. 5OO–5RR)**

Stephanodiscus binatus is a euconstant taxon for LE. Its percentage share for LE was 4.3%. *S. binatus* has no recorded ecological indicator values. The ecological indicator value analysis for LE found it to constitute 25% of the unknown group in pH requirements, 47% in salinity, 18% in nitrogen uptake, 18% in oxygen requirements, 20% in saprobity, 29% in trophic state and 21% in moisture.

The largest percentage share of *S. binatus* was recorded in the spring months and the lowest in autumn. Its abundance was found to be elevated in December 2014 and 2015; the same samples demonstrated the highest concentrations of Ca^{2+}, Mg^{2+} and the highest pH (above 8).

Stephanodiscus binatus has been recorded in various water ecosystems ranging from oligotrophic to eutrophic; however, all are characterized by elevated pH value (*Stoermer & Håkansson, 1984*; *Håkansson & Kling, 1990*; *Houk, Klee & Tanaka, 2014*; *Olszyński & Żelazna-Wieczorek, 2018*).

We therefore propose that the following classes of ecological indicator values according to *Van Dam, Mertens & Sinkeldam (1994)* be established for *S. binatus*:

- pH requirements: 4 (alkaliphilic);
- salinity: 2 (oligohalobous);

Stephanodiscus parvus **(SPAV) (Figs. 5SS–5VV)**

Stephanodiscus parvus is a euconstant taxon for LE and an accidental taxon for PE. Its percentage share for LE was 2.4%. It was most abundant in the D.LEP3.260714 sample (22%). This species has three assigned ecological indicator values. The ecological indicator values analysis for LE found the taxon to constitute 9% of the unknown group in nitrogen uptake, 9% in oxygen requirements, 10% in saprobity, and 10% in moisture.

Stephanodiscus parvus is noted mainly in eutrophic hypereutrophic ecosystems with elevated electrolytic conductivity. It is also a good indicator of waters with a strong anthropogenic impact (*Reavie & Smol, 1998*; *Reavie & Kireta, 2015*; *Olszyński & Żelazna-Wieczorek, 2018*; *Reavie & Cai, 2019*).

Based on our findings and literature data, we propose the following change in ecological indicator values according to *Van Dam, Mertens & Sinkeldam (1994)* for *S. parvus*:

- pH requirements: from 5 to 4 (alkalibiontic to alkaliphilic).

Diatoma moniliformis (DMOF) (Figs. 5WW–5AAA)

Diatoma moniliformis is a euconstant taxon for BO. Its mean percentage share for BO was 3.9%. It currently has no assigned ecological indicator values. According to the ecological indicator values analysis for BO, this taxon constituted 10% of the unknown group in pH requirements, 11% in salinity, 8% in nitrogen uptake, 8% in oxygen requirements, 9% in saprobity, 9% in trophic state and 8% in moisture.

Diatoma moniliformis was found in 87.5% of samples from BO. Interestingly, it constituted 28% of the share in one sample from December 2016 (D.BOZB.091216); however, its share was below 2% in the previous season, and was not higher than 1–2% in the other samples from December 2016. The chemical and physical characteristics of D.BOZB.091216 did not differ significantly from those of the other samples.

This species is also found in fresh and salt water, as well as the Baltic and arctic areas with high conductivity (*Potapova & Snoeijs, 1997*; *Rumrich, Lange-Bertalot & Rumrich, 2000*; *Levkov et al., 2007*; *Pniewski & Sylwestrzak, 2018*).

One of the factors that influences the abundance of *D. moniliformis* is the water temperature. Studies indicate that temperatures above 10–15 °C (*Potapova & Snoeijs, 1997*; *Pniewski & Sylwestrzak, 2018*) are associated with population growth. However, populations have been observed in freshwater streams and lakes in arctic areas, in which the temperature of the water is below 10 °C (*Antoniades, Douglas & Smol, 2005*). Population growth was also observed at 4.7 °C in sample D.BOZB.091216; therefore, low temperature may have an influence on the abundance of this species.

Encyonopsis subminuta (ESUM) (Figs. 5BBB–5GGG)

Encyonopsis subminuta is a euconstant taxon in BO, where its mean percentage share was 4.1%. Presently, *E. subminuta* has been assigned five ecological indicator values. Ecological indicator values analysis for BO found it to constitute 7% of the unknown group in nitrogen uptake and 8% in moisture.

Encyonopsis subminuta was found to be most abundant in sample D.BOZB.041115. The sample was also characterized by an elevated concentration of $Fe^{2+/3+}$ ions and the lowest pH value. In subsequent samples, when the concentration of Fe ions dropped, the abundance of *E. subminuta* also decreased.

Encyonopsis subminuta is regarded as a cosmopolitan taxon, occurring in the temperate and boreal zone. It is most abundant in oligo- to mesotrophic waters with electrolytic conductivity between 190 and 250 µS cm^{-1} (*Krammer, 1997*; *Noga et al., 2014*; *Novais et al., 2014*; *Feret, Bouchez & Rimet, 2017*).

Encyonopsis subminuta may be sensitive to the concentration of Fe ions; however, the increase of these ions is associated with a drop in pH. Our research confirms that the optimal pH for population size is close to 7.

Mastogloia smithii (MSMI) (Figs. 5HHH–5MMM)

Mastogloia smithii is a euconstant taxon for BO. Its mean percentage share for BO was 6.3%. it was found in greatest numbers in D.BOZB.300615 (22%) and D.BOZB.261016 (15%). *M. smithii* has been assigned four ecological indicator values. The ecological indicator values analysis for the BO found this species to constitute 13% of the unknown group in nitrogen uptake, 14% in oxygen requirements and 15% in trophic state.

The environment in BO regarding salinity was classified according to *Van Dam, Mertens & Sinkeldam (1994)* as oligohalobus (43% species); however, 7% of the mesohalobous species were represented by one species: *M. smithii*.

This species is recorded in fresh, brackish and salt water (*Witkowski, Lange-Bertalot & Metzeltin, 2000*; *Busse & Snoeijs, 2003*; *Weckström & Juggins, 2005*; *Martinez-Goss & Evangelista, 2011*; *Lange-Bertalot et al., 2017*). Its presence in environments with varying degrees of salinity may suggest that this does not have an significant influence on population size.

Based on our present findings and literature data, we propose the following change in the ecological indicator values according to *Van Dam, Mertens & Sinkeldam (1994)* for *M. smithii*:

- salinity: from 4 to 3 (mesohalobous to halophilic).

Nitzschia dissipata var. *media* (NDME) (Figs. 5NNN–5QQQ)

Nitzschia dissipata var. *media* is a euconstant taxon for BO. Its mean percentage share for BO was 3.8%, and the highest proportion (20%) was found in D.BOZB.041115. *Nitzschia dissipata* var. *media* has been assigned two ecological indicator values. Ecological indicator values analysis for BO found it to constitute 9% of the unknown group in nitrogen uptake, 10% in oxygen requirements, 11% in saprobity, 11% in trophic state and 9% in moisture.

The increase in occurrence of *Nitzschia dissipata* var. *media* is associated with an increase in the level of $Fe^{2+/3+}$, similar to *Encyonopsis subminuta*. In addition, it was found in the ecosystem, that is, BO, with the lowest concentrations of ions indicative of the presence of organic pollutants in the environment, such as K^+ and NH_4^+.

Although *Nitzschia dissipata* var. *media* is found sporadically, it is commonly found in oligo- to mesotrophic waters with a pH between 7 and 8 (*Krammer & Lange-Bertalot, 1997*; *Van de Vijver, Frenot & Beyens, 2002*; *Antoniades, Douglas & Smol, 2005*; *Żelazna-Wieczorek, 2011*; *Lange-Bertalot et al., 2017*).

Table 4 Selected 16 characteristic species with the new or altered (bold) ecological indicator values according to *Van Dam, Mertens & Sinkeldam (1994)*.

Species	Code	Moisture aerophity	Nitrogen uptake	pH requirements	Oxygen requirements	Salinity	Saprobity	Trophic state
Chamaepinnularia krookiformis	CHKF	**4**	0	3	0	**4**	**4**	**5**
Chamaepinnularia plinskii	CHPL	**4**	0	**3**	0	**4**	**4**	**5**
Craticula buderi	CRBU	**3**	0	**4**	0	**4**	**4**	**5**
Cyclostephanos invisitatus	CINV	**1**	0	**4**	0	2	0	5
Diatoma moniliformis	DMOF	0	0	0	0	0	0	0
Encyonopsis subminuta	ESUM	0	0	3	1	1	1	1
Mastogloia smithii	MSMI	3	0	4	0	**3**	2	0
Navicula cincta	NCCA	**3**	0	0	0	2	0	7
Navicula moskalii	NMOK	0	0	**4**	0	**2**	0	**7**
Nitzschia dissipata var. *media*	NDME	0	0	4	0	2	**2**	0
Nitzschia liebethruthii	NLBT	**4**	0	**3**	0	4	**4**	**5**
Pantocsekiella pseudocomensis	PPCS	0	0	0	0	0	0	0
Planothidium delicatulum	PTDE	3	1	**3**	0	**5**	5	3
Planothidium frequentissimum	PLFQ	0	2	4	3	2	4	7
Stephanodiscus binatus	SBNT	0	0	**4**	0	**2**	0	0
Stephanodiscus parvus	SPAV	0	0	**4**	0	2	0	6

Based on our findings and literature data, we propose the following ecological indicator values according to *Van Dam, Mertens & Sinkeldam (1994)* for *Nitzschia dissipata* var. *media*:

- saprobity: 2 (β-mesosaprobe)

Pantocsekiella pseudocomensis (PPCS) (Figs. 5RRR–5XXX)

Pantocsekiella pseudocomensis is a euconstant taxon for BO. Its mean percentage share for BO was 4.4%. It was most abundant in D.BOZB.250315 (9%) and in D.BOZB.220616 (10%). *Pantocsekiella pseudocomensis* has not been assigned any ecological indicator values according to *Van Dam, Mertens & Sinkeldam (1994)*. The ecological indicator values analysis for BO found it to represent 22% of the unknown group in pH requirements, 25% in salinity, 10% in nitrogen uptake, 11% in oxygen requirements, 18% in saprobity, 12% in trophic state and 13% in moisture.

The greatest amount of *Pantocsekiella pseudocomensis* was found in samples characterized by the highest levels of ammonium ions. Its percentage share was lowest in samples with the lowest water temperature, apart from D.BOZB.250315.

Currently, *Pantocsekiella pseudocomensis* is assigned to the *Pantocsekiella comensis* complex, with *Pantocsekiella comensis* and *Pantocsekiella costei*. In our opinion that assigning ecological indicator values for particular species of the *Pantocsekiella comensis* complex is unjustified at the current state of knowledge, and that all species within the complex should be assigned the same provisional ecological indicator values until their individual properties are better understood (*Houk, Klee & Tanaka, 2010*; *Kistenich et al., 2014*; *Duleba et al., 2015*).

CONCLUSIONS

The water ecosystems created in the post-mining areas create a complex of conditions that are not found in other natural ecosystems, and the benthic diatom species present in such environments are very often present in higher numbers than in other habitats. The specific hydro-geological conditions prevailing in the post-production reservoirs provide a unique opportunity to observe interspecies differences and intra-species variability, allowing for the verification or isolation of new taxa and a greater insight into their autecology (*Żelazna-Wieczorek & Olszyński, 2016*; *Olszyński & Żelazna-Wieczorek, 2018*).

The identification of species characteristic of the studied ecosystems may foster further growth of ecological research and increase the reliability of surface water quality assessment, as such knowledge is needed to verify their ecological indicator values, and hence calculate diatoms indices with greater accuracy.

Ecological indicator values as set out by *Van Dam, Mertens & Sinkeldam (1994)* are utilized in many ecological works describing the ecological conditions of the studied ecosystems. These ecological indicator values form the basis for calculating diatoms indices describing the ecological state of surface waters. It is therefore necessary to constantly update and establish new ecological indicator values for particular diatom species.

Many authors who describe new species, or encounter existing species in new ecosystems, regularly propose updates for individual ecological indicator values. However, these findings, may not be introduced and updated in the OMNIDIA program for a number of years. The OMNIDIA system is used by state institutions in many countries around the world to assess surface water quality (*Campeau, Pienitz & Héquette, 1999*; *Rumrich, Lange-Bertalot & Rumrich, 2000*; *Witkowski, Lange-Bertalot & Metzeltin, 2000*; *Gell et al., 2005*; *Potapova & Ponader, 2008*; *Wojtal & Sobczyk, 2012*; *Caballero et al., 2013*; *Żelazna-Wieczorek & Olszyński, 2016*; *Yamamoto, Chiba & Tuji, 2017*; *Lange-Bertalot et al., 2017*; *Földi et al., 2018*; *Van de Vijver, Wetzel & Ector, 2018*). Clearly, if these assessments are based on incomplete or outdated data, assessments of aquatic environments may be fraught with error.

The present study used three principles to identify proposed changes in the classification of ecological indicator values for characteristic species according to the *Van Dam, Mertens & Sinkeldam (1994)* system, or to establish new values which were previously absent: the analysis of environmental conditions prevailing in the studied ecosystems, the analysis of relevant literature data, and references to the ecological indicator values of other species (Table 4). This mode of research can serve as a model for updating databases used to assess surface water quality.

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The authors declare that they have no competing interests.

Author Contributions

- Rafał M. Olszyński performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Ewelina Szczepocka analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
- Joanna Żelazna-Wieczorek conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

Data Availability

The following information was supplied regarding data availability:

Raw data is available in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.8117#supplemental-information.

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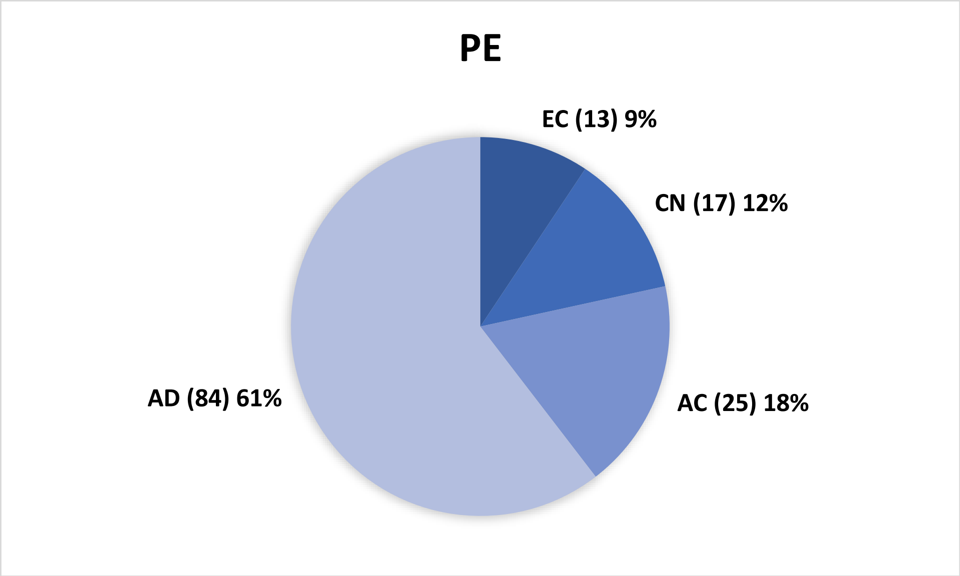
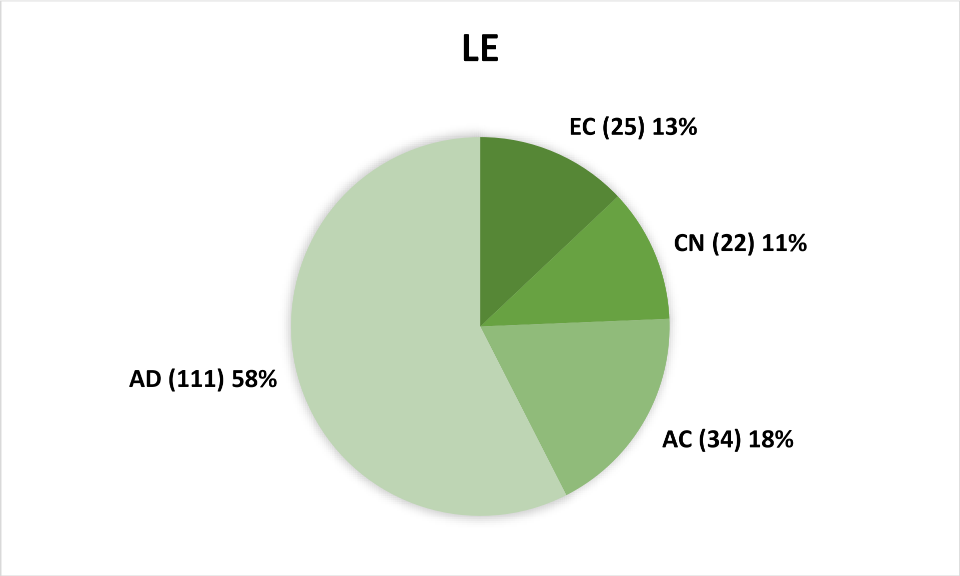
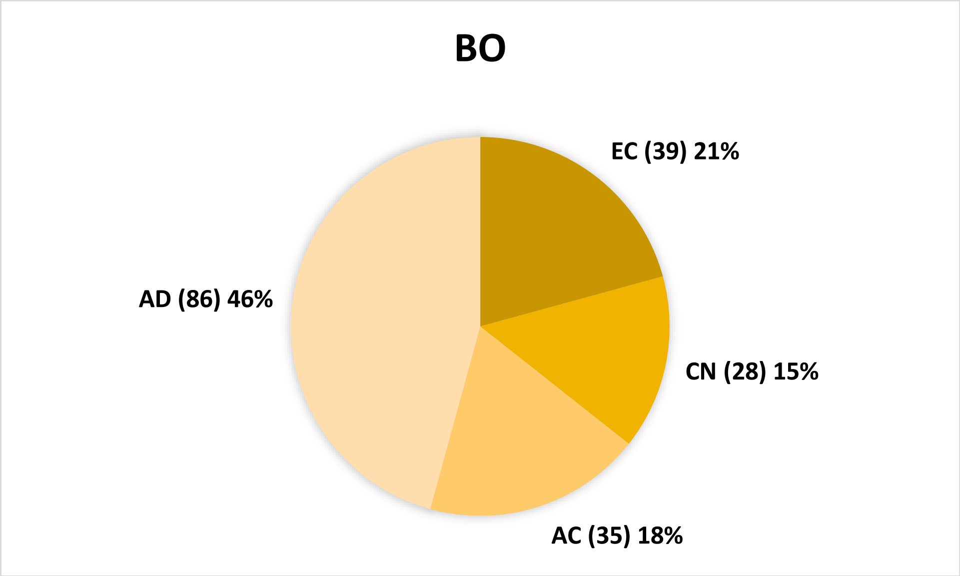
Załącznik 4.1: Percentage share of diatoms in individual classes of prevalence according to the Tümpling & Friedrich factor (1999). doi.org/10.7717/peerj.8117/supp-1.

PE – Pełczyska complex; LE – Łęczyca reservoir; BO – Bogdałów reservoir; EC – euconstant taxa, CN – constant taxa; AC – accessory taxa; AD – accidental taxa. The number of species is shown in brackets.

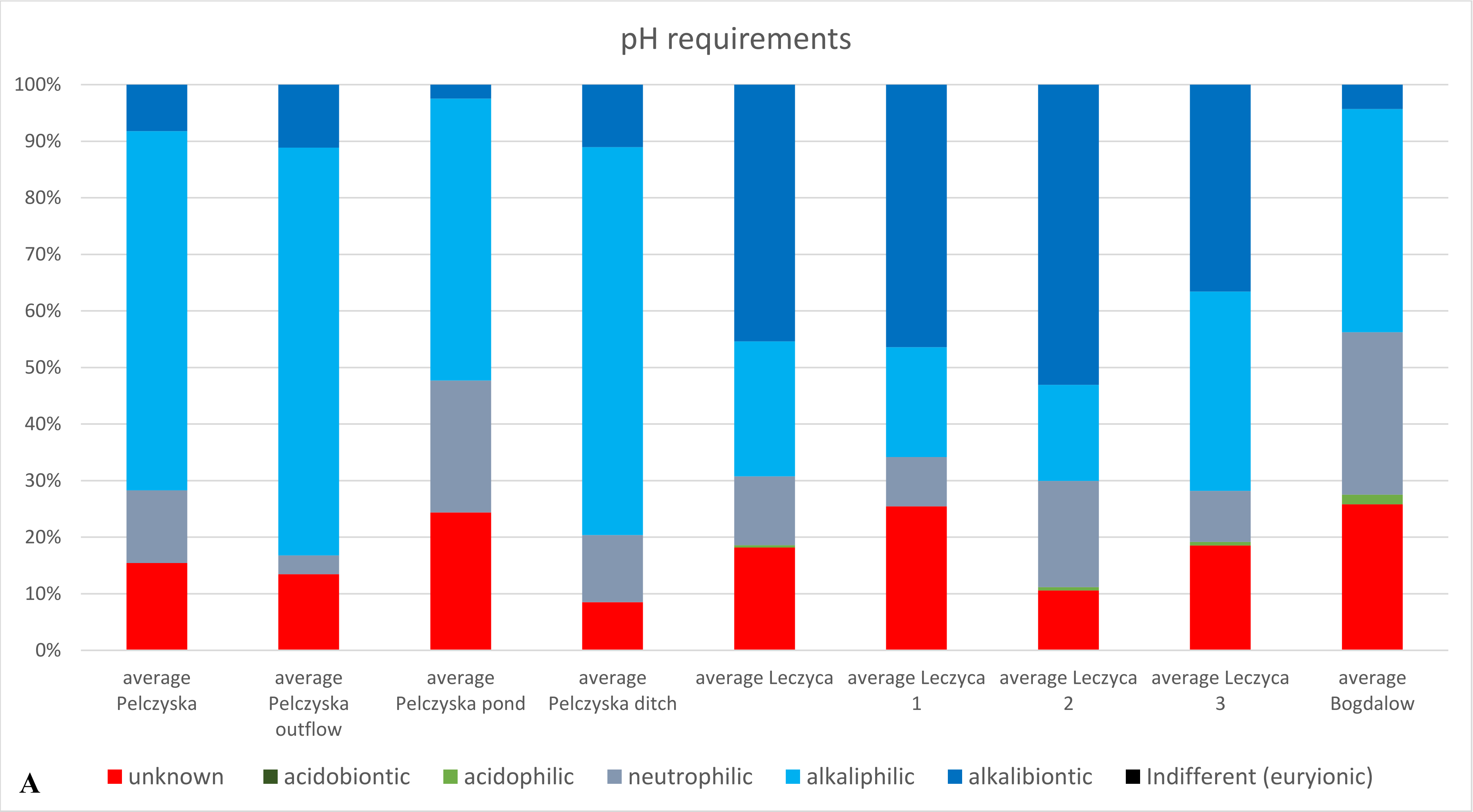

PE
EC (13) 9%
CN (17) 12%
AC (25) 18%
AD (84) 61%

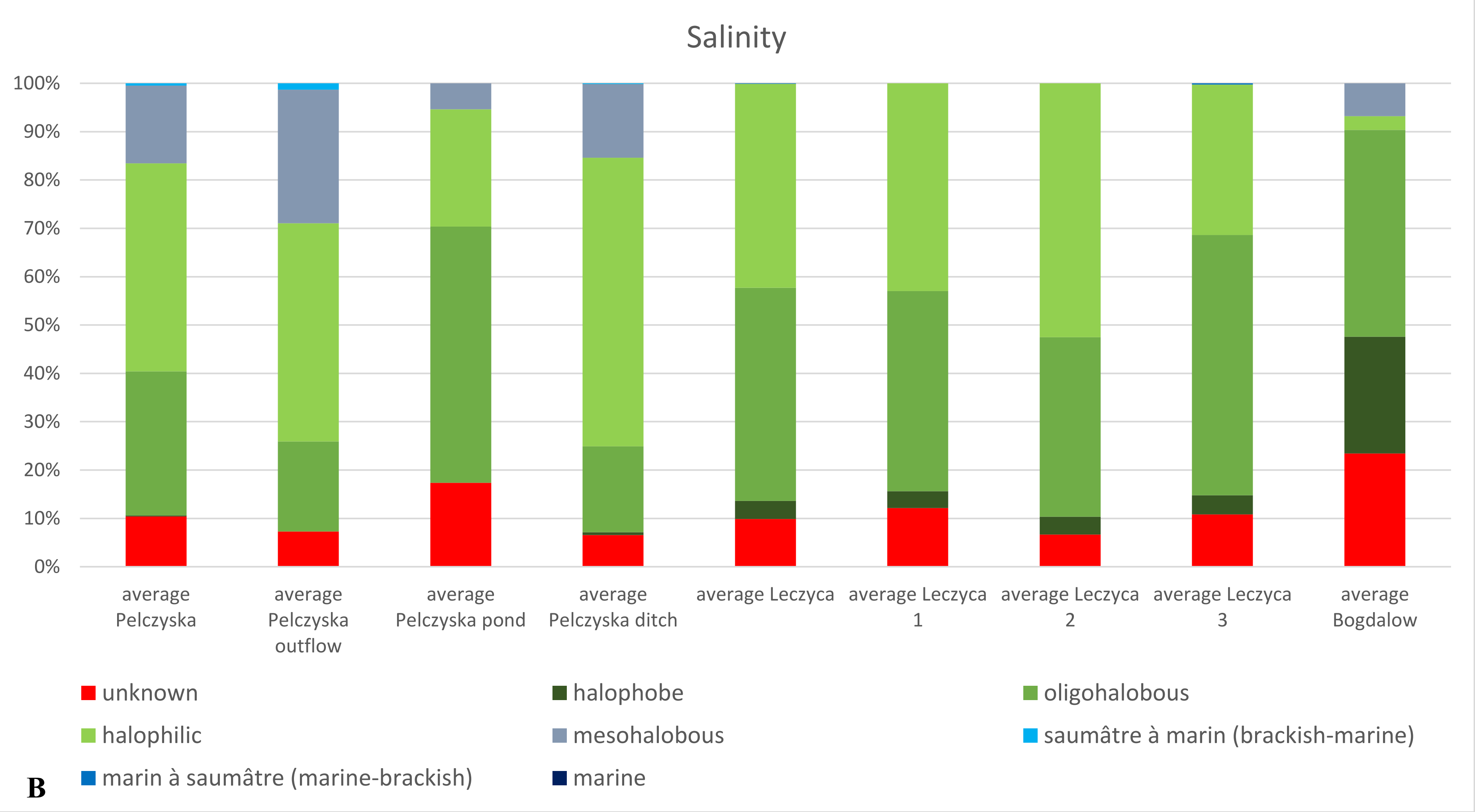

LE
EC (25) 13%
CN (22) 11%
AC (34) 18%
AD (111) 58%

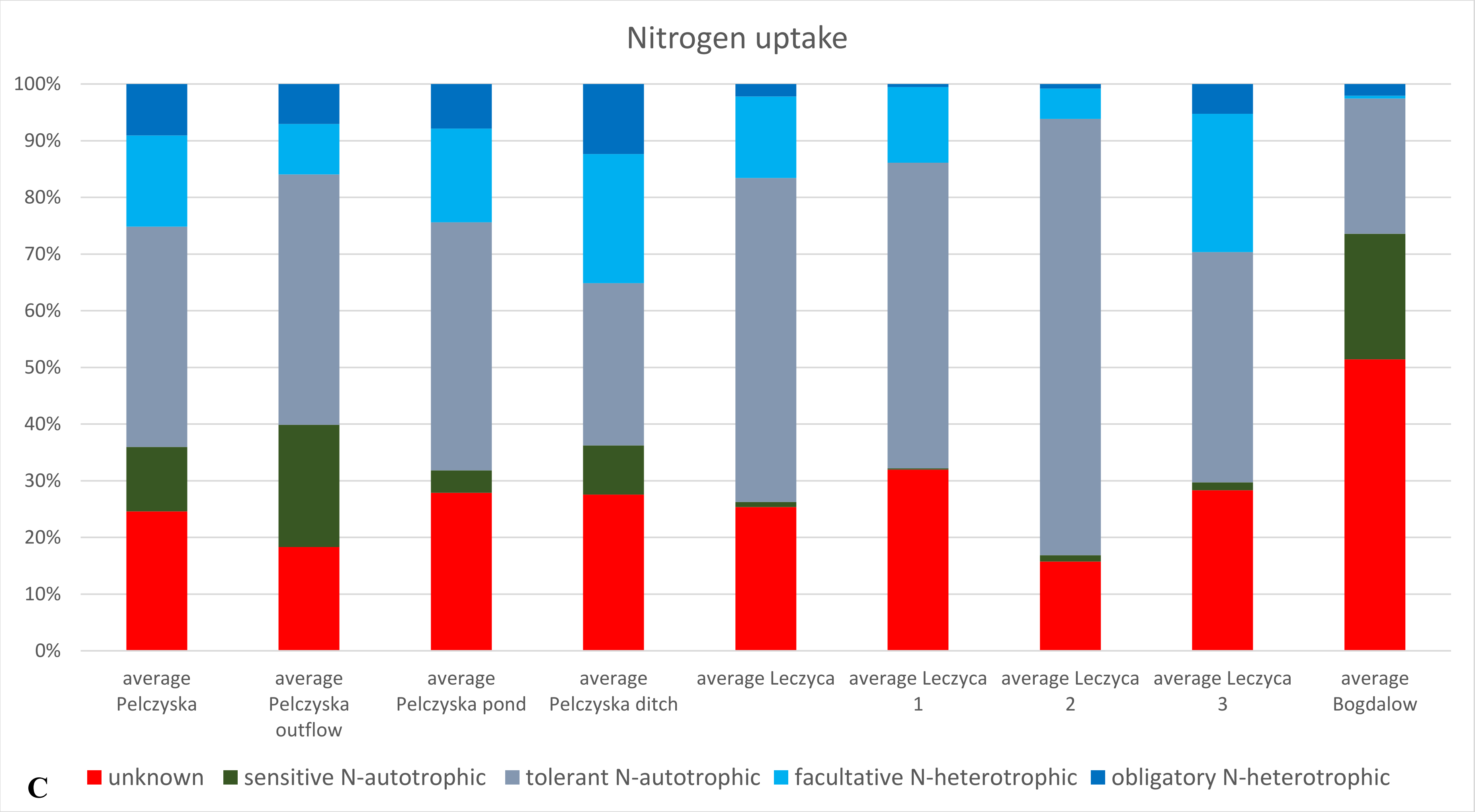

BO
EC (39) 21%
CN (28) 15%
AC (35) 18%
AD (86) 46%

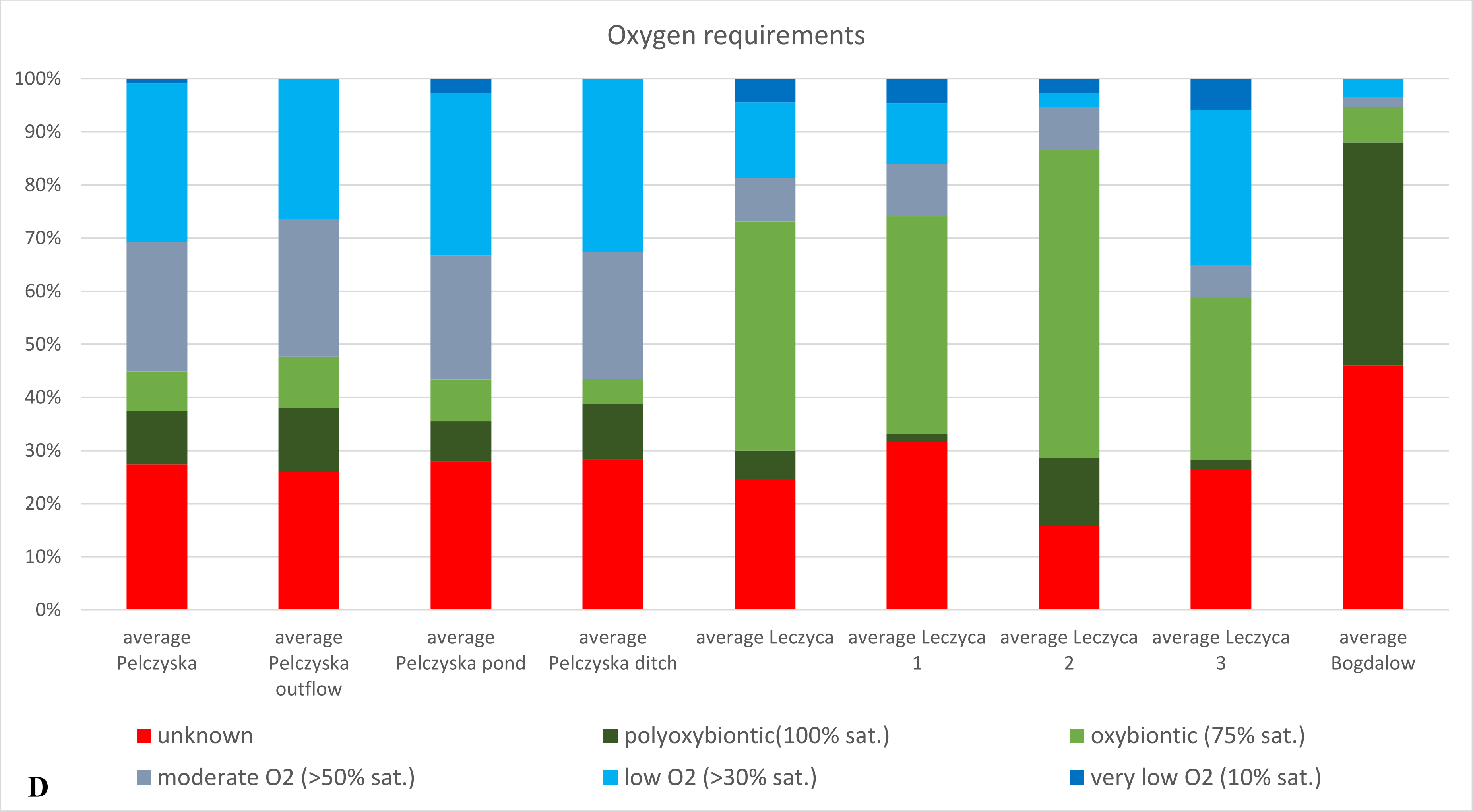
Załącznik 4.2: Average percentages of diatom species in individual classes of ecological values according to Van Dam, Mertens & Sinkeldam (1994) based on OMNIDIA 6.0.6 software. doi.org/10.7717/peerj.8117/supp-2.

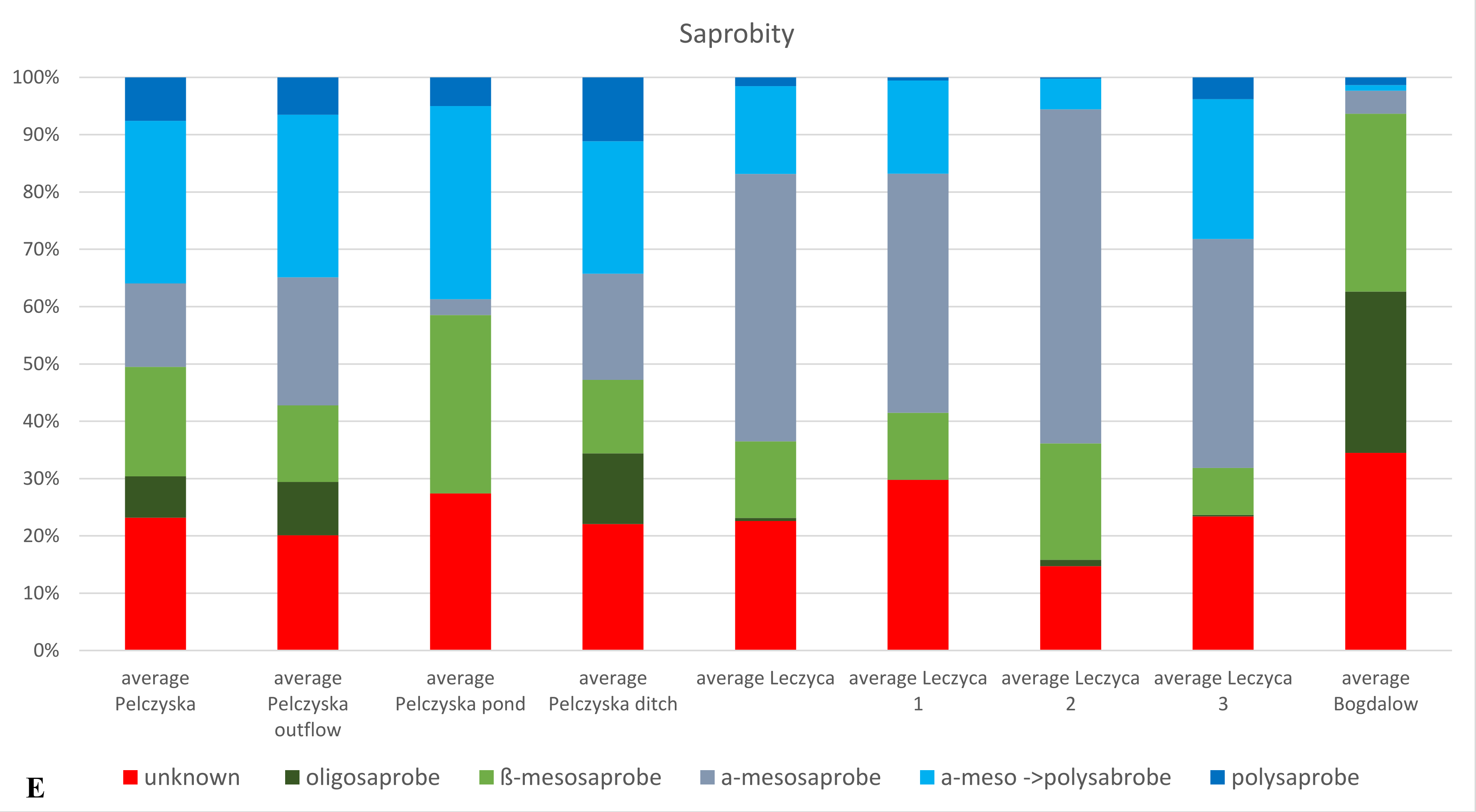
A. pH requirements. B. Salinity. C. Nitrogen uptake. D. Oxygen requirements. E. Saprobity. F. Trophic state. G. Moisture aerophily

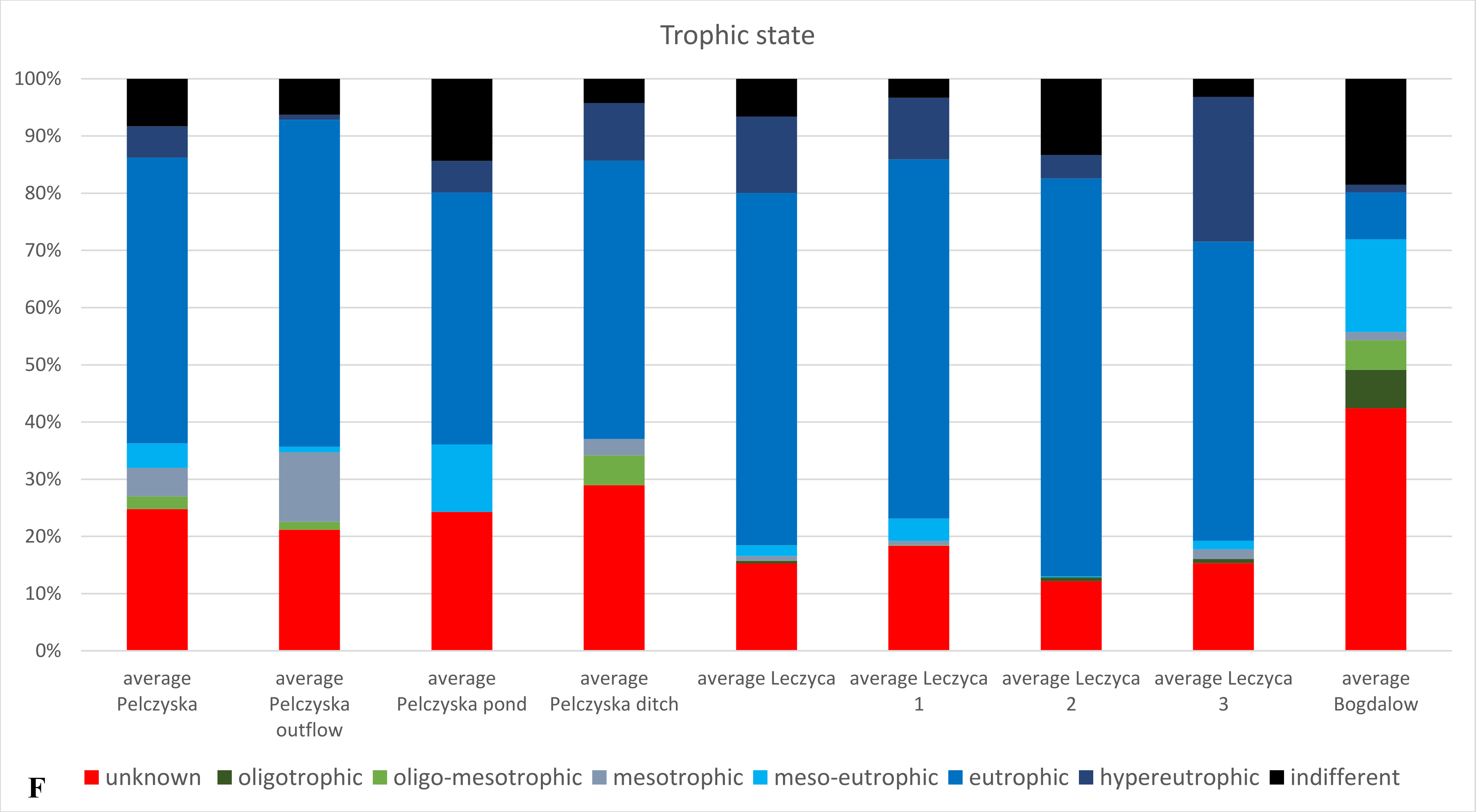

pH requirements
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
average Pelczyska
average Pelczyska outflow
average Pelczyska pond
average Pelczyska ditch
average Leczyca
average Leczyca 1
average Leczyca 2
average Leczyca 3
average Bogdalow
A
unknown
acidobiontic
acidophilic
neutrophilic
alkaliphilic
alkalibiontic
Indifferent (euryionic)

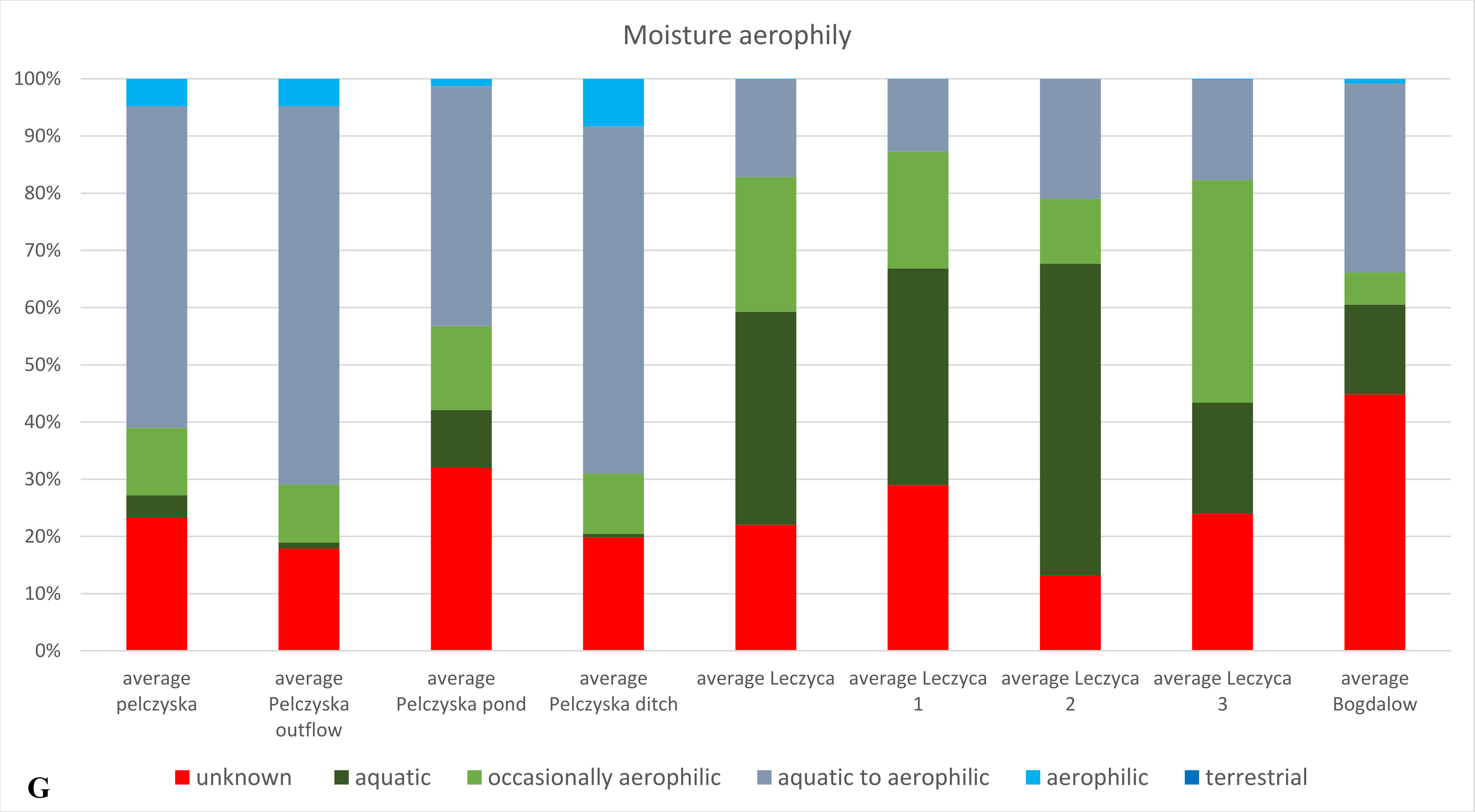

Salinity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
average Pelczyska
average Pelczyska outflow
average Pelczyska pond
average Pelczyska ditch
average Leczyca
average Leczyca 1
average Leczyca 2
average Leczyca 3
average Bogdalow
unknown
halophobe
oligohalobous
halophilic
mesohalobous
saumâtre à marin (brackish-marine)
marin à saumâtre (marine-brackish)
marine
B


Nitrogen uptake
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
average Pelczyska
average Pelczyska outflow
average Pelczyska pond
average Pelczyska ditch
average Leczyca
average Leczyca 1
average Leczyca 2
average Leczyca 3
average Bogdalow
C
unknown
sensitive N-autotrophic
tolerant N-autotrophic
facultative N-heterotrophic
obligatory N-heterotrophic


Oxygen requirements
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
average Pelczyska
average Pelczyska outflow
average Pelczyska pond
average Pelczyska ditch
average Leczyca
average Leczyca 1
average Leczyca 2
average Leczyca 3
average Bogdalow
unknown
polyoxybiontic(100% sat.)
oxybiontic (75% sat.)
moderate O2 (>50% sat.)
low O2 (>30% sat.)
very low O2 (10% sat.)
D


Saprobity
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
average Pelczyska
average Pelczyska outflow
average Pelczyska pond
average Pelczyska ditch
average Leczyca
average Leczyca 1
average Leczyca 2
average Leczyca 3
average Bogdalow
unknown
oligosaprobe
ß-mesosaprobe
a-mesosaprobe
a-meso ->polysabrobe
polysaprobe
E


Trophic state
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
average Pelczyska
average Pelczyska outflow
average Pelczyska pond
average Pelczyska ditch
average Leczyca
average Leczyca 1
average Leczyca 2
average Leczyca 3
average Bogdalow
unknown
oligotrophic
oligo-mesotrophic
mesotrophic
meso-eutrophic
eutrophic
hypereutrophic
indifferent
F


Moisture aerophily
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
average pelczyska
average Pelczyska outflow
average Pelczyska pond
average Pelczyska ditch
average Leczyca
average Leczyca 1
average Leczyca 2
average Leczyca 3
average Bogdalow
G
unknown
aquatic
occasionally aerophilic
aquatic to aerophilic
aerophilic
terrestrial

Załącznik 4.3: Classes of ecological indicators values by Van Dam, Mertens & Sinkeldam (1994). doi.org/10.7717/peerj.8117/supp-4.

		Class (*Van Dam et al. 1994*)							
		0	1	2	3	4	5	6	7
Ecological parameters	pH requirements	unknown	acidobiontic	acidophilic	neutrophilic	alkaliphilic	alkalibiontic	indifferent	
	Salinity	unknown	halophobe	oligohalobous	halophilic	mesohalobous	brackish-marine	marine-brackish	marine
	Nitrogen utake	unknown	sensitive N-autotrophic	tolerant N-autotrophic	facultative N-autotrophic	obligatory N-autotrophic			
	O_2 requirements	unknown	polyoxybiontic	oxybiontic	moderate	low O_2	very low O_2		
	Saprobity	unknown	oligosaprobe	β-mesosaprobe	a-mesosaprobe	a-meso -> polysaprobe	polysaprobe		
	Trophy state	unknown	oligotrophic	oligo-mesotrophic	mesotrophic	meso-eutrophic	eutrophic	hypereutrophic	indifferent
	Moisture aerophily	unknown	aquatic	occasionally aerophilic	aquatic to aerophilic	aerophilic	terrestial		

Załącznik 4.4: Percentage of characteristic species in the unknown (total) class according to Van Dam, Mertens & Sinkeldam (1994) at each sampling point. doi.org/10.7717/peerj.8117/supp-4.

Percentage of individual characteristic species constituting at least 10% of the ’unknown’ class at each sampling point.

	pH	**Salinity**	**Nitrogen uptake**	**Oxygen requirements**	**Saprobity**	**Trophic state**	**Moisture aerophily**
D.PESB.	total 33%	total 44%	total 34%	total 48%	total 30%	total 22%	total 43%
	CHPL (19%) NCCA (11%)	CHPL (33%) CHKF (10%)	CHPL (11%) NCCA (10%)	PTDE (25%)	CHPL (11%)	CHPL (10%)	PLFQ (20%)
D.PEDB.	total 92%	total 25%	total 82%	total 82%	total 80%	total 74%	total 85%
	CHPL (60%) NCCA (32%)	CHPL (25%)	NLBT (37%) CHPL (25%) CHKF (16%)	NLBT (37%) CHPL (16%) CHKF (16%)	NLBT (43%) CHPL (32%)	NLBT (365) CHPL (23%) CHKF (15%)	NLBT (49%) CHPL (285)
D.PEPB.	total 81%	total 73%	total 78%	total 78%	total 79%	total 60%	total 84%
	CRBU (53%) NCCA (27%)	CRBU (73%)	CRBU (46%) NCCA (25%)	CRBU (46%) NCCA (25%)	CRBU (46%) NCCA (26%)	CRBU (54%)	CRBU (41%) NCCA (23%) PLFQ (14%)
D.LEP1.	total 76%	total 79%	total 67%	total 68%	total 71%	total 54%	total 78%
	CINV (36%) SBNT (23%) NMOK (16%)	SBNT (56%) NMOK (21%)	CINV (29%) SBNT (19%) NMOK (14%)	CINV (29%) SBNT (19%) NMOK (14%)	CINV (31%) SBNT (20%) NMOK (14%)	SBNT (36%) NMOK (17%)	CINV (32%) SBNT (22%) NMOK (14%)
D.LEP2.	total 39%	total 44%	total 35%	total 35%	total 28%	total 23%	total 44%
	SBNT (27%) CINV (11%)	SBNT (44%)	SBNT (18%)	SBNT (18%)	SBNT (20%)	SBNT (23%)	SBNT (22%) SPAV (11%) CINV (11%)
D.LEP3.	total 57%	total 41%	total 50%	total 53%	total 60%	total 29%	total 57%
	SBNT (25%) CINV (24%)	SBNT (41%)	SBNT (17%) CINV (15%) SPAV (13%)	SBNT (17%) CINV (16%) SPAV (14%)	SBNT (20%) CINV (18%) SPAV (15%)	SBNT (29%)	SBNT (20%) CINV (17%) SPAV (15%)
D.BOZB.	total 33%	total 36%	total 47%	total 44%	total 38%	total 47%	total 38%
	PPCS (22%) DMOF (10%)	PPCS (25%) DMOF (11%)	MSMI (13%) PPCS (10%)	MSMI (14%) PPCS (11%) NDME (10%)	PPCS (18%) NDME (11%)	MSMI (15%) PPCS (12%) NDME (11%)	PPCS (13%)

Załącznik 4.5: Raw data. doi.org/10.7717/peerj.8117/supp-5.

Number - numbers of valves in the samples "+" - les than 3 valves in the sample N/A - not analysed	CODE	D.PEPB.040713	D.PEPB.301113	D.PEPB.190214	D.PEPB.250314	D.PEDB.040713	D.PEDB.301113	D.PEDB.190214	D.PEDB.250314	D.PESB.040713	D.PESB.301113	D.PESB.190214	D.PESB.250314	D.LEP1.250314	D.LEP2.250314	D.LEP3.250314	D.LEP1.260714	D.LEP2.260714	D.LEP3.260714	D.LEP1.011014	D.LEP2.011014	D.LEP3.011014	D.LEP1.031214	D.LEP2.031214	D.LEP3.031214	D.LEP1.250315	D.LEP2.250315	D.LEP3.250315	D.LEP1.300615	D.LEP2.300615	D.LEP3.300615	D.LEP1.041115	D.LEP2.041115	D.LEP3.041115	D.LEP1.101215	D.LEP2.101215	D.LEP3.101215	D.BOZB.250315	D.BOZB.300615	D.BOZB.041115	D.BOZB.101215	D.BOZB.210316	D.BOZB.220616	D.BOZB.261016	D.BOZB.091216
Achnanthes brevipes	ABRE					+	+	3	+	6	4	7	+																																
Achnanthidium affine	ACAF																							+																					
Achnanthidium exiguum	ADEG																		+	+		+									+														
Achnanthidium kranzii	ADKR																								+																				
Achnanthidium minutissimum	ADMI	5	8	7							3	+		+	55	8	6	66	5	12	34	4	+	35	+	20	216	3	13	49	10		13	5		26	3	72	15	3	14	360	27	12	102
Achnanthidium neomicrocephalum	ADNM																																					15	5	5		+	14	+	27
Achnanthidium saprophilum	ADSA															+																													
Amphipleura pellucida	APEL																																					+		29	5	3		+	+
Amphora cf. neglecta f. densestriata	AcfNDE														+									3	+					+		+	+	+	+										
Amphora cf. parallerlisteriata var. manguinii	AcfMPM													+																															
Amphora cf. vetula DE 5 P54 F12-13	AcfVTU																										+			+		+	+			19									
Amphora copulata	ACOP													5	+			+			7	+	22	13		+	+	+	+	+	+	+	+		+	9	+								
Amphora inariensis	AINA																+	+			+	6					+	4	+	+	7		+	+	+	3	+					+			+
Amphora indistincta	AMID															4	+			5																	+	+					3	+	
Amphora lange-bertalotii	AMLB																																					+		+			+		
Amphora lange-bertalotii var. tenuis	ALGT													+											+							+	+	+	+	+									
Amphora macedoniensis	AMCD																																						+						
Amphora minutissima	AMNU													+	+											+		+		+															
Amphora ohridana	AMOH																+				+		+						+	+		+	+									+		+	
Amphora ovalis	AOVA													+				+																											
Amphora ovalis var. tenuis	AOLT													+																															
Amphora pediculus	APED		+							+					22	19	3	11	8	20	24	7	4	78	+	5	6	5	5	30	23	+	17	9	+	28	4	8	+	+	+	+	+	5	
Amphora affinis	AAFI																												+																
Aneumastus balticus	ABLT																																					3	4	+	4	6	16	6	+
Aneumastus cf. rosettae	ANcfRO																																								+		+		
Aneumastus laetus	ALTU																																							+			+		
Aneumastus pseudotusculus	ANPT																																					+							
Aneumastus rostratus	ANRO																																					+	3	+	+		+	+	
Aneumastus stroesei	ANRO																																					5	6	+	10	+	6		+
Aneumastus tusculus	ANTU																																									+			
Anomoeoneis sphaerophora	ASPH	+	+	+				+		+	4									+		3			+			+		+	+			+			+								
Anomoeoneis sphaerophora f. sculpta	ASSC				+																																								
Asterionella formosa	AFOR																																					9		4	+	+	28	+	+
Aulacoseira pseudomuzzanensis	APMU													33	27	11	40	10	12	8	19	18	21	12	4	6	8	10	8	7	8	9	21	15	+	13	+								
Brachysira microcephala	BMIC																																											+	
Caloneis alpestris	CAPS																																					+			+			+	+
Caloneis amphisbaena	CAMP													+			3						+			3			+			+						+		+	+	+	+		+
Caloneis bacillum	CBAC													+												+										+		+							
Caloneis biconstrictoides	CBCT																																					+							
Caloneis cf. leptosoma	CAcfLE																																					+	+	+	+	+	3	+	+
Caloneis cf. meridionalis	CcfMER																																						+	+	8	+	+	+	+
Caloneis cf. silicula	CcfSIL																																								+				
Caloneis cf. thermalis	CcfTHE																																					+				+			
Caloneis fontinalis	CFON																																								+	+	+		+
Caloneis lancettula	CLCT														+																														
Caloneis schumanniana var. biconstricta	CSBI																																								+				
Caloneis silicula	CSIL													+			+								+	+					+		+	+							+	4	+	+	
Caloneis silicula var. gibberula	CSGB																																						+	+					
Caloneis sp. 1	CSP1																																					+	+		5				
Caloneis sp. 163/2-6 ID 5	CID5																																					+							
Caloneis sp. 2	CSP2																																							+					
Caloneis sp. 3	CSP3																																							+					
Chamaepinnularia krookiformis	CHKF							30	55	+			3																																
Chamaepinnularia plinskii	CHPL		+			27		32	45	6	6	20																																	
Cocconeis neothumensis	CNTH																				+									+			5			+		59	9	9	5	3	7	31	+
Cocconeis pediculus	CPED	4												28	+				+	+	+		+	+		+	+		+	9	6		+			+	+	+	+	+					
Cocconeis placentula	CPLA	44	39	27	4			4		+																																			
Cocconeis placentula var. lineata	CLNT													+	3	8	7	4	4	+	6	6	10	23		+	3	5	8	10	21	+	9	3	+	14	+	7	+	+	5	+		+	+
Cocconeis pseudothumensis	COPS									3																																			
Craticula ambigua	CAMB									+					+				+												+	+		+											
Craticula buderi	CRBU	32	61	47	50	+	+	+		3	5	+									+					4								+	4		+								
Craticula cuspidata	CRCU		+											+						+		+	+		+			+						7			8								
Craticula halophila	CHAL				+	3	6	4	75	11	5	29	9																											+		+		+	
Ctenophora pulchella	CTPU	+	+					+		5		3	4																																
Cyclostephanos dubius	CDUB	+	+											70	172	60	245	376	154	145	268	93	218	195	40	49	56	72	300	359	178	220	254	31	76	324	17								
Cyclostephanos invisitatus	CINV													34	27	31	17	3	31	72	8	50	21	4	15	+	+	14	9	+	17	52	22	7	202	+	5				+				
Cyclotella meneghiniana	CMEN	7	22	7	5									3	6	26	9	+	43	28	15	33	23	22	31		+	21	6	5	32	55	59	44	69	7	6			+				+	+
Cymatopleura elliptica	CELL													+																													+		
Cymatopleura solea	CSOL													+					3			+	+		+	+		+			+		+	3			+			+		+			
Cymbella aspera	CASP																				+			+																					
Cymbella compacta	CCMP																											+																	
Cymbella cymbiformis	CCYM													3																															
Cymbella dorsenotata	CDNO																																									+			+

Cymbella excisa	CAEX																																						+	14	4	+		+	+
Cymbella excisiformis	CAEX																									+																			
Cymbella helvetica	CHEL																							+																	+		+		+
Cymbella lanceolata	CLAN													+									+																						
Cymbella lange-bertalotii	CLBE																																					+	+	+		+			
Cymbella neocistula	CNCI																			+			+			+	3		+					+				+	+	6	6	4		+	
Cymbella sp. 1	CSP1																																							+					
Cymbella subleptoceros	CSLP														+																														
Cymbella vulgata	CVUL																																					+							
Cymbopleura amphicephala	CBAM																																					+				+			
Cymbopleura cf. subaequalis	CcfSAQ																																											+	
Cymbopleura frequens	CBFQ																																					3	8	+	+	6	+	+	+
Cymbopleura lata	CYBL																																						+	+	+	+	+	+	+
Denticula subtilis	DSUB					+	+	3		8		4	3																																
Diatoma moniliformis	DMOF																									+	+											3		+	5	7	+	+	160
Diatoma tenuis	DITE		+		+							+	+		+	+	+	+						3		+	9		+	+	+	+	+			+	+	3			+	6	+		+
Diploneis cf. parahinziae	DPHI																																								+	+			
Diploneis cf. separanda	DcfSEP																																					+	+	+	5	+	+	+	+
Diploneis cf. smithii	DcfSMI												+																																
Diploneis interrupta	DINT					+		8			26	5	4																																
Diploneis krammeri	DKRA							+			3	+																										+	+	+	+	+	+		+
Diploneis oculata	DOCU																																					+	+		4		+		
Diploneis separanda	DSEP																																					+	+	+		+		+	
Discostella pseudostelligera	DPST													+	12	5	8	+	4		5	5	8	9	+	+	6	+	+	4			2	1	1	1							4		
Discostella stelligera	DSTE																						+									6													
Discostella woltereckii	DWOL														25	5	+	6	+		+	5	28	15	+	+	+	15	5	5	+	15	85	2	25	1	5								
Encyonema aequilongum	EAEQ																																					+	+	+			+	+	
Encyonema cespitosum var. cespitosum	ECAS													+		+		+						3			+	+	+	+	5		+			+	+								7
Encyonema cespitosum var. comensis	ECCO																																										+		
Encyonema lange-bertalotii	ENLB																																									+			
Encyonema minutum	ENMI																			8														+											
Encyonema prostratum	EPRO													+							+			+						+						+									
Encyonema silesiacum	ESLE											+		4	3		+	+																	+										
Encyonema ventricosum	EVET														8	+							+	4	+	+	6		+	+		+	+	+		+									+
Encyonopsis minuta	ECPM																																					5	+	+	4		+	+	+
Encyonopsis subminuta	ESUM																																					13	6	83	9	10	17	24	24
Entomoneis paludosa	EPAL	+																																											
Eolimna cf. minima	EOcfMI				5																																								
Eolimna cf. tantula	EcfTAN		6																																										
Eolimna minima	EOMI													+																															
Eolimna subminuscula	ESBM															+																													
Epithemia frickei	EFRI													+																															
Epithemia adnata	EADN																																	+				+	+		+				+
Epithemia smithii	ESMI																																					+					+		
Epithemia sorex	ESOR																		4	+		+									20			7			3		+	+				+	
Epithemia sorex var. gracilis	ESGR																																					+			+				
Epithemia turgida	ETUR																																						+				+		+
Epithemia turgida var. granulata	ETGR																																									+			
Eucocconeis flexella	EUFL																																					+	+	+	+	+		+	+
Eucocconeis laevis	ELEA																																					63	3		+	13	+	8	+
Eunotia bilunaris	EBIL													+		+	+									+			+	+															
Fallacia monoculata	FMOC					+	5	+	+		+	+	3		+					+		+			+						+						+								
Fallacia pygmaea	FPYG	+	+	+	+					+		+	+							+																									
Fallacia sublucidula	FSLU	4		3			+				6																																		
Fistulifera pelliculosa	FPEL															+																													
Fragilaria binodis	FBNB	+																																											
Fragilaria capucina	FCAP																									14	+					+						+		+		+		12	7
Fragilaria capucina var. perminuta	FCPE																																												10
Fragilaria construens	FCON									6											+						+				+								+		+				
Fragilaria dilatata	FDIL																																						+	+		+		+	+
Fragilaria famelica	FFAM	+			+	7	5	8	5	+	7	9	74																																
Fragilaria gracilis	FGRA																																					+				9			
Fragilaria leptostauron	FLEP																																								+				
Fragilaria mesolepta	FMES													+	+	+	+	+			+					+	5			+						+									+
Fragilaria parasitica	FPAR																																							+	6		+		+
Fragilaria parasitica var. subconstricta	FPSC													6	+	+	+	+	+	+	+		+	+	+	+		+	+	+	+	+		+	+	+	+								
Fragilaria perminuta	FPEM																+																					+							
Fragilaria radians	FRAD													20	11	6	15	3		+	+	+	+	9		50	53		+	4	+	+	+	+	+	4	+	7	+	+	+	+	+	3	22
Fragilaria rumpens	FRUM																									+																			
Fragilaria sopotensis	FSOP	+	59	27	3					+	+																																		
Fragilaria subsalina	FSSA	72				+				40		+																																	
Fragilaria tenera	FTEN																																					50	+	+	4	6	+		+
Fragilaria vaucheriae	FVAU				+				+					9	34	5	8	8	+		+		4	8	+	18	41		7	+		+			+	+									
Frustulia creuzburgensis	FCRE									3	+		+																																
Frustulia vulgaris	FVUL																							+			+								+	+									
Geissleria decussis	GDEC																																					+	+	+	3		3		
Gomphonema acidoclinatum	GADC						+															+																							
Gomphonema acuminatum	GACU														+			+					+							+		+				+						+			+
Gomphonema angustatum	GANT															+																													

Gomphonema capitatum	GCAP																+																												
Gomphonema exilissimum	GEXL		+																																								+		
Gomphonema gracile	GGRA														+														+								+								
Gomphonema italicum	GITA		+																																										
Gomphonema micropus	GMIC													+		+	+	+	+	+		+	+	+			+		+							+	+								
Gomphonema minusculum	GMIS																													+															
Gomphonema minutum	GMIN														+									+		+			+										+		+	+			
Gomphonema olivaceum	GOLI														+	+		+								4	12		+	+	+		+	+	+	+			+			+			+
Gomphonema pala	GOPA			+				+				+		3	+	+					+		+	+		+	+		+	+	+		+	+		+									
Gomphonema parvulum	GPAR	8	+	114	50		+	11	9	6	+	7	+																																
Gomphonema parvulum var. parvulum	GPPA													+	+	4	+	3		+	+	+	+	4	+	+	8	3	+	+	4	+	3	+	+	6	+								
Gomphonema pseudoaugur	GPSA																													+															
Gomphonema pumilum	GPUM																				+			+									+								+				
Gomphonema sp. 1	Gsp1																																									+			98
Gomphonema subclavatum	GSCL		+		+																		+			+													+						
Gomphonema truncatum	GTRU													+																															
Gomphonema utae	GUTA	+	5		+	+	+																																						
Gomphonema vibrio	GVIB																																						+						
Gyrosigma acuminatum	GYAC												+	+	+		+	+		+				4		+	+		+	+						+									
Gyrosigma attenuatum	GYAT																																					+			+	+		+	+
Gyrosigma kuetzingii	GYKU											+																																	
Halamphora acutiuscula	HLAC							+		3	11	5	+																																
Halamphora aponina	HLAP										+	+																																	
Halamphora borealis	HABO										+		11																																
Halamphora oligotraphenta	HADT																																						12	+	+		+	+	+
Halamphora dominici	HDOM	5	7	11	20	+			+		+	3																																	
Halamphora luciae	HLUC							+			27																																		
Halamphora montana	HLMO																			3		4		+				+										+							
Halamphora sp. 2	HSP2													+	+		+										+		+						+										
Halamphora sydowii	HSYD											4																																	
Halamphora tenerrima	HTNR										+		3																																
Halamphora veneta	HVEN		+		8											+	+	+		+					+			+	+	+	+		+		+		+								
Hantzschia abundans	HABU	+				+	9	27	5	3	+	5	4																																
Hantzschia amphioxys	HAAM	4	+	+	+	+			+				+					+				+												+			+								
Hantzschia calcifuga	HCAL								+																							+													
Hantzschia spectabilis	HSPE		+																															+											
Hippodonta capitata	HCAP													29	+	4	27	3	5	+	+	6	33	3	+	23	+	5	25	+	21	+	+	+	+	+	8			+		+		+	+
Hippodonta hungarica	HHUN	+	31	5	46		+	+		+		5																																	
Karayevia clevei	KCLE										+																									+									
Karayevia kolbei	KAKO														+									+																					+
Karayevia ploenensis	KAPL										+										+								+				+												
Karayevia ploenensis var. gessneri	KAPG																																					+						+	
Lemnicola hungarica	LHUN		+	+										+	+	+	+	+	+	+		+	+	+	+			3	+		+	+	+	+	+										
Lindavia first division cell	Lkpp																																						+	+	+	+	15	+	
Lindavia balatonis	LBAL													3	4		+	+			5	+	3	+		+	+		+	+		+	8		+	3									
Lindavia radiosa	LRAD																																									+	11	+	+
Lindavia sp. 1	Lsp1																																					5	5	+	+	+	+	+	
Luticola cf. mutica	LcfMUT					+	+																																						
Luticola goeppertiana	LGOP					+	+																																						
Luticola mutica	LMUT	3							+	+	+	+																									+								
Luticola nivalis	LNIV																	+																											
Luticola paramutica	LPAR													+																					+										
Luticola pseudonivalis	LPNI																								+																				
Luticola sp. 1	LUSP1											+																																	
Luticola ventricosa	LVEN											+																																	
Mastogloia baltica	MBAL																																												+
Mastogloia elliptica	MELL									+		+																																	
Mastogloia lacustris	MLAC																																						+	+		+		+	+
Mastogloia smithii	MSMI									+	+	+	+																									4	121	19	22	+	28	84	3
Mayamaea atomus	MAAT																																				+								
Mayamaea atomus var. permitis	MAPE					+	+		5				+																														+		
Mayamaea lacunolaciniata	MLLC					8																																							
Melosira varians	MVAR														+		+						+			+	+		+			+										+			
Meridion circulare	MCIR													12	+			+					+			+			+			+	+		+										
Navicula antonii	NANT		+											+			+				+		+			+		+	+		+	5			+	+		4	+	5	18	10	9	3	+
Navicula capitatoradiata	NCPR														5	+	+	+		+	+		+	+	+	+	3	+	+	+	+	5	+	5	+	+	+								
Navicula cari	NCAR																+	+																											
Navicula cariocincta	NCCC																																	+		+									
Navicula cf. cryptocephala	NcfCRY																																	4											
Navicula cf. trivialis	NTRV															+																													
Navicula cincta	NCCA	67	21	6	22	8	4	4		6	7	+	5		+	3		+	+	5		21	+	+	7	+			+		+			+	+		5			+		+			+
Navicula cryptocephala	NCRY	3		+	9									3	+	+	+	+		+	+			+							+		+	12	+	+	3								
Navicula cryptotenella	NCTE													+	+	+		+	+	4	5	+	+	3	+	+	3	3	3	3	9	+	+	+	+	+	+	+	10	+	4	+	+	5	+
Navicula cryptotenelloides	NCTO																	3	+	+	+	6		+					+	+	+		+	+			+	5	12	10	7		20	37	21
Navicula digitoconvergens	NDGC			+	+																																								
Navicula erifuga	NERI			+																																									
Navicula gregaria	NGRE			4			+							+	+	3	3	+		+	+	6	9	+	3	+		6	+		+	3	+	11	+		222			24	23	6	30		+
Navicula hanseatica	NHTI		+	6	3																																								
Navicula ID 7	NID7										4		9																																

Navicula kefvingensis	NKEF					+	+	+		+	+	3	+																														
Navicula meulemansii	NMEU						+				5	3	+																														
Navicula moskalii	NMOK													+									21			141			25			5		+	3										
Navicula oblonga	NOBL													+																												+			
Navicula peregrina	NPRG					+				6	5	+																																	
Navicula radiosa	NRAD													5	+	+	+									+	+			+	+	+		+	+		+	+	7	14	4	+	+	+	+
Navicula reichardtiana	NRCH													+		28	+	+	+	+	+	+	+	3	+		8	+	+	3	+	+	+	4	+	+	3								
Navicula reinhardtii	NREI										+			66	6		27						3			24			+			+			+	+					+				
Navicula rhynchotella	NRHT				7									7	+	+	+	+	3	3	+		+	5	3	+	+	18	3	+	4	+	+	4	+	+	3								
Navicula salinarum	NSAL	20	7	9	22	4			5	+	3	+	4																																
Navicula salinicola	NSLC							+								+				+	+	3						5									3								
Navicula slesvicensis	NSLE				+							+		+	+	+	+	+		+							+		+			+	+	+	+	+	+								
Navicula sp.	NSP																																					+		+			+		
Navicula sp. 2	NSP2																																										+		
Navicula sp. 3	NSP3																																											+	
Navicula sp. 3 pel	NSP3P										4																																		
Navicula sp. 4 DE P52 F 14-22	NSP4														+			+	+			5	+														+								
Navicula subalpina	NSBN																																							8		+			
Navicula subrhynchocephala	NSRH																																										+	+	
Navicula tripunctata	NTPT													34	+	+	+	+			+		+	+	+	+	+		+	6	+		+		3	+	+		+	+	3	+	+	+	+
Navicula trophicatrix	NTCX																																							+	+				
Navicula upsaliensis	NUSA													8	+		6				+		+	+		+	+	+	+	+		+	+		+										
Navicula veneta	NVEN	66	42	40	41	54	40	141	88	30	181	58	86	55	+	8	7	+	+	8	+	11	11	+	3		+	9	+	+	+	+		6	+		16				+				
Navicula wiesneri	NWIE	3	4	9																																									
Navicymbula pusilla	NCPU												+																																
Neidium affine	NEAF															+																+													
Neidium binodis	NBID																																					+	+		+	+		+	
Neidium cf. juba	NEcfJU																																	+											
Neidium dubium	NEDU													+							+		+			+			+						+										
Neidium dubium morf. biconstrictum	NDUC																																						+	+	+	+	3		
Nitzschia acicularis	NACI															3			8	+		+																							
Nitzschia acidoclinata	NACD						6																																						
Nitzschia acuminata	NACM																					+																							
Nitzschia amphibia	NAMP	+		+							3				3	+		+		+	+		+	+	+	+			+	+	+	+	+	+		8	+								
Nitzschia archibaldii	NIAR																																						5	5	7	+	50		
Nitzschia calida	NICA																							+						+		+	+		4										
Nitzschia clausii	NCLA												+																																
Nitzschia commutata	NICO		+		+	+	+	3	+	+	4	3	3																													+			
Nitzschia dissipata	NDIS														+	+		+		+	+			+		+	+		+						+			3	+	+	17		+		
Nitzschia dissipata var. media	NDME												+																									+		113	11	11	24	+	11
Nitzschia frustulum	NIFR	40		9		48	45	33	30	11	43		44							+	+			10			+	6		8	3		6	7	+		+					+	3		+
Nitzschia gisela	NGIS						+	+	+			+	+																																
Nitzschia graciliformis	NIGF															6	21			+	3	+									3						+								
Nitzschia gracilis	NIGR																+		+	16	+	56			6			3			4			+					+	9	7	+			
Nitzschia heufleriana	NHEU																																									+			
Nitzschia homburgensis	NHOM																									+																			
Nitzschia inconspicua	NINC	5	8	+		71	34	32	25	21	5	32	29		+	4	3	+		10	+	7		3	7		+	21		+	4			+			11	3	+		12		+	4	+
Nitzschia intermedia	NINT																		3	4		10			11			6										+					+		
Nitzschia lacuum	NILA																																										+		
Nitzschia liebethruthii	NLBT	6	8		8	22	53	26	29		3	22	+																																
Nitzschia linearis	NLIN											+		10					7			+						3		+				+			+			10		20		+	
Nitzschia macilenta	NMCL																																								+				
Nitzschia microcephala	NMIC									+																														+	4		+		
Nitzschia nana	HNAN										+	3																																	
Nitzschia palea	NPAL	47	+	4	31	9			4				3		+	+	5		17	14	7	36	+	3	6	3	+	9	+	+	15		+	53	+	+	15			4		+	5	44	
Nitzschia paleacea	NPAE															4			+	+	+	+									13			14	+		+		+	7	+		16	+	+
Nitzschia perminuta	NIPM	+		+		44	11	20	+	3	7	9	+						+	+		+													+		+							97	
Nitzschia pumila	NPML																		7	13		53			+			3			17			9											
Nitzschia recta	NREC														+			+								+	+												+	+	12			+	
Nitzschia rectirobusta	NRBU																																									+			
Nitzschia rosenstockii	NRST																																					14	4	41	19	3		30	14
Nitzschia scalpelliformis	NISC										+																																		
Nitzschia sigmoidea	NZSG																																	+											
Nitzschia sinuata var. delognei	NSDE																																							+				+	
Nitzschia solgensis	NSOL																						+																						
Nitzschia subtilis	NISU															8		+	+										+		5				+										
Nitzschia supralitorea	NZSU																					+													+		3								
Nitzschia tabellaria	NTAB																			+																		+	+		5	+	+	+	
Nitzschia tenuis	NITE															+				4		8			15	+	11	31																	
Nitzschia tubicola	NTUB			+		28	24	21	58		3	3	4																																
Nitzschia umbonata	NUMB											+				+													+																
Nitzschia valdestriata	NIVA																																					7	+	5	4		3	12	+
Nitzschia vermicularis	NVER															3			+																										
Nitzschia vitrea var. salinarum	NVSA					+	11	23	4	5	3	7	+																																
Nitzschia vitrea var. vitrea	NIVI											+	+																										+						
Nitzschia subacicularis	NSUA																																	9											
Nupela cf. silvahercynia	NcfSHC										9																																		
Pantocsekiella ocellata	POCL																			+																		42	191	9	80	+	111	32	4
Pantocsekiella pseudocomensis	PPCS																																					51	28	9	17	+	60	30	+

Pinnularia bertrandii	PBTD				+	+		+	+	+	+																					+													
Pinnularia brebissonii	PBRE					+	+	+	3	+		+			+			+								+			+			+		+											
Pinnularia neomajor var. frequentis	PNFR															3				+					+	+	+		+		+	+		+											
Pinnularia rhomboelliptica var. inflata	PRHI													+								+				+								+											
Pinnularia viridiformis	PVIF													+											+																				
Pinnularia lundii	PLUN																																					+		+	8	+			
Placoneis anglica	PAGC																																										+		
Placoneis cf. clementispronina	PcfDMT																																					+	+	+	+		+	+	+
Placoneis nanoclementis	PNCL																																							7	84	19	5	+	+
Placoneis pseudoanglica	PPAN																																								10				
Placoneis sp. 1 (cf. placentula)	PcfPLC																																											+	
Planothidium delicatulum	PTDE	+	+	+	+	5	+	3	+	21		24	26							+				+																					
Planothidium dubium	PTDU																		+														+												
Planothidium engelbrechtii	PLEN										+																																		
Planothidium frequentissimum	PLFQ	9	45		24	3	+		3	5	9	6	23	14	+	5	5	+		5	+	+	+	+	+	4	+		7	+	+	+	+	+	+	+	+	+							
Planothidium lanceolatum	PTLA				+																																								
Planothidium minutissimum	PLMN																																					17	+	7	5	3	8	8	
Planothidium pericavum	PPCV				+			3	+		4		3																																
Planothidium rostratum	PRST																				+			+					+	+		+	3		+	+									
Platessa conspicua	PTCO														3	+	+	3		+	+	+		+	+					+			3	+		+			+						
Pleurosigma sp. 1	PLSP1										+		+																																
Prestauroneis protracta	PPRT				+							+	+	4	+								3			38			+			3			+							+	+	+	
Prestauroneis protractoides	PPRD																																									+			
Prestauroneis tumida	PTUD																																					+	+	+	3	+	+	+	
Psammothidium bioretii	PBIO																																					6				+	+	+	
Psammothidium subatomoides	PSAT																																					+							
Rhoicosphenia abbreviata	RABB													+	5	+	+	+			3		+	+		4	3		+	+	+	+	+		+										+
Rhopalodia acuminata	RACU									+		+																																	
Rhopalodia brebissonii	RBRE							+		+	+	+																																	
Rhopalodia constricta	RCON									+																																			
Rhopalodia gibba	RGIB																																					+	+	4	+		+	+	
Rhopalodia gibberula	RGBL										+																																		
Rhopalodia parallela	RPAR																																					+	37	34	4	+	+	+	+
Sellaphora bacillum	SEBA													+	+			+					+			+		+				+			+										
Sellaphora cf. joubaudii	ScfJOU	+																																											
Sellaphora pupula	SPUP																			+						+				+	+			+											
Sellaphora pupula "grooved lanceolate"	SPUP-g																																							+					
Sellaphora pupula 'spike'	SPUP-s																																						+	+			3	5	
Sellaphora pupula var. densistriata	SEPD																																					+	+		+			+	
Sellaphora sp. 1	SSP1																																											+	
Stauroneis cf. obtusa	ScfOBT																						+			+									+										
Stauroneis leguminopsis	SLGF							+			+																																		
Staurosira brevistriata	SBRV														+			+			+			+			+			2	+	+	+		+	7		11	7	+	4	+	6	9	+
Staurosira venter	SSVE	+																																											
Staurosirella pinnata	SRPI	+								7					+					+	+			+			+						+			+		+	+		+	+	+	+	
Stephanodiscus binatus	SBNT													18	48	47	25	4	19	5	7	9	73	18	44	34	25	76	21	7	13	20	19	3	32	3									
Stephanodiscus hantzschii	SHAN			+	+									7	23	162	18	+	30	83	21	22	6	15	45	46	5	15	10	3	34	110	21	218	61	10	161	+							
Stephanodiscus medius	SMED																																					4	+	+	+	3			
Stephanodiscus parvus	SPAV	+	+	7											+	13	4	+	115	22	43	10	10	4	20	+	+	12			3		11		42	4	3								
Surirella amphioxis	SAPH																															+													
Surirella angusta	SANG																									+	+																		
Surirella brebissonii	SBRE	+				6		+	3	3		+	+									+														+				+		+			
Surirella brebissonii var. kuetzingii	SBKU						+																																						
Surirella minuta	SUMI																														+														
Surirella ovalis	SOVI									+		+		+		+							+																						
Surirella parahelvetica	SUPH																																							+	+				
Surirella splendida	SSPL																											+																	
Surirella striatula	SSTR												+																																
Surirella subsalsa	SSSA					4		+	+	+	5	+	+																																
Surirella venusta	SVEN												+																																
Tabularia fasciculata	TFAS	4	+		+	+	+	+	+	24	4	72			+								+	+		+			+	+				+				+	+		+	+		+	+
Tabularia tabulata	TTAB												+																										+						
Thalassiosira duostra	TDUO																		7	+	+	7	+	+	+		+	+	+	+	+	+	3	+		+									
Tryblionella angustata	TANG																																							+	+	+		+	
Tryblionella constricta	TRCO			+				+			+	+										+	+									4								+	+	+			
Tryblionella debilis	TDEB	+				4	7	22	9	4	4	6	3	+		+		+											+		+									+				+	
Tryblionella hantzschiana	THAZ																																												
Tryblionella hungarica	THUN													+	+	3		+	6	3		+			+	+	+	+	+		6	+		3	+		+		+			+			
Ulnaria acus	UACU														11	13	+	3	10	4	23	3	+	5	244	5	23	134		+	13	+	15	9	7	8	10								
Ulnaria ulna	UULN													8	12	+	+	+			+		+	+		5	4		+	+	+	+		+	+	+	+	+	+	3	+	+		+	+

Parameter	Unit	Values																																											
pH		9,55	8,83	8,53	7,82	N/A	N/A	N/A	6,40	6,60	6,80	6,78	7,99	8,23	8,32	8,70	8,26	8,45	8,48	7,89	7,86	8,25	7,86	8,05	7,47	8,55	8,60	8,80	8,19	8,25	8,55	8,66	8,45	8,48	7,86	7,50	7,81	8,32	8,15	7,85	7,99	8,26	8,13	8,01	8,20
Conductivity	µS cm^{-1}	5150	3858	2645	2930	N/A	N/A	N/A	5170	9230	6376	6740	4450	865	836	732	748,0	754,2	732,0	657	558	553	766	716	778	712	625	472	825	787	715	649,0	754,2	754,0	723,5	792	706	556	734	650	711,2	570	505	591	669
T	ºc	17,3	1,1	3,2	6,8	N/A	N/A	N/A	7,8	13,9	8,5	7,2	6,8	8,8	8,5	8,7	21,3	20,9	21,6	15,0	16,1	15,2	1,6	0,6	2,2	10,2	10,1	10,3	20,6	21,1	4,8	5,9	6,2	5,1	5,5	5,2	5,7	8,8	21,0	10,3	5,3	8,8	22,7	9,8	4,7

HCO^{3-}	mg L^{-1}	283,7	451,4	320,3	353,8	N/A	N/A	N/A	610	405,7	344,7	405,7	744,2	286,7	274,5	280,6	N/A	N/A	N/A	210,5	203	183	274,5	216,6	289,8	338,6	167,8	234,9	234,9	192,2	219,6	N/A	N/A	N/A	216,6	204,4	241	247,1	173,9	302	247,1	369,1	253,2	297,4	269,3
$CO_2^{[HCO3-]}$	mg L^{-1}	102	163	112	128	N/A	N/A	N/A	220	146	124	146	268	103	99	101	N/A	N/A	N/A	76	72	66	99	78	105	122	61	85	85	69	79	N/A	N/A	N/A	78	74	87	89	63	109	89	133	91	99	92
Cl^-	mg L^{-1}	1524	912	1090	685	N/A	N/A	N/A	1006	2783	2359	2976	1585	57	60	74	N/A	N/A	N/A	77	69	68	82	71	106	78	93	73	93	82	83	N/A	N/A	N/A	78	84	67	52	46	44	42	47	42	46	41
N_{NH4}	mg L^{-1}	0,021	0	0,046	0,357	N/A	N/A	N/A	0,15	0,101	0,046	0,021	1,626	0,163	0,112	0,055	N/A	N/A	N/A	0,22	0,53	0,617	0,705	0,356	1,158	0,024	0,027	0	0,027	0,215	0,053	N/A	N/A	N/A	0,153	0,296	0,039	0,041	0	0,028	0,006	0	0,067	0,029	0
NH^{4+}	mg L^{-1}	0,026	0	0,059	0,459	N/A	N/A	N/A	0,193	0,13	0,6	0,026	2,094	0,21	0,144	0,071	N/A	N/A	N/A	0,283	0,54	0,795	0,547	0,277	0,899	0,031	0,034	0	0,034	0,277	0,068	N/A	N/A	N/A	0,198	0,382	0,05	0,053	0	0,036	0,007	0	0,087	0,02	0
PO_4^{3-}	mg L^{-1}	1,474	4,521	0,854	9,1	N/A	N/A	N/A	8,565	0,79	0,596	1,09	12,46	0,353	0,463	0,341	N/A	N/A	N/A	0,303	0,35	0,311	0,527	0,546	0,566	0,368	0,459	0,356	0,379	0,566	0,36	N/A	N/A	N/A	0,223	0,052	0,09	0,269	0,451	0,364	0,345	0,296	0,368	0,335	0,312
P_{PO4}	mg L^{-1}	0,486	1,492	0,282	3	N/A	N/A	N/A	2,825	0,261	0,197	0,36	4,11	0,116	0,153	0,113	N/A	N/A	N/A	0,1	0,1	0,103	0,174	0,18	0,187	0,121	0,151	0,118	0,125	0,187	0,119	N/A	N/A	N/A	0,074	0,017	0,03	0,089	0,149	0,12	0,134	0,098	0,121	0,123	0,112
SO_4^{2-}	mg L^{-1}	196,9	150,6	189,9	166,2	N/A	N/A	N/A	165	191,1	198,3	176,1	187,6	122,2	122,4	71	N/A	N/A	N/A	70,51	66,8	62,92	71,4	84,1	68,5	146,9	112,3	66,9	137,2	99,8	90,1	N/A	N/A	N/A	94,8	122,4	90,7	147,3	130,6	117,7	113,5	118,1	126,7	121,6	110,3
S_{SO4}	mg L^{-1}	65,7	50,3	63,4	55,5	N/A	N/A	N/A	55,1	63,8	66,2	58,8	62,6	40,8	40,8	23,7	N/A	N/A	N/A	23,5	22,3	21	23,8	28,1	22,9	49	37,5	22,3	45,8	33,3	30,1	N/A	N/A	N/A	31,6	40,8	30,3	49,2	43,6	39,3	37,9	39,4	42,3	41,5	38,5
COLOR	mgPt dm^{-3}	80	120	50	60	N/A	N/A	N/A	140	40	100	25	160	10	10	16	N/A	N/A	N/A	60	60	60	30	50	50	20	9	25	30	20	24	N/A	N/A	N/A	10	14	12	4	8	10	4	8	4	6	4
Mn^{3+}	mg L^{-1}	0,03	0,25	0,13	0,1	N/A	N/A	N/A	0,14	0,28	0,17	0,47	0,51	0,01	0,01	0,01	N/A	N/A	N/A	0,04	0,02	0,01	0,05	0,02	0,07	0,01	0,01	0,02	0,02	0,02	0,02	N/A	N/A	N/A	0	0,03	0,02	0,01	0,01	0	0	0	0	0	0
$Fe^{2+/3+}$	mg L^{-1}	0,24	0,05	0,05	0,11	N/A	N/A	N/A	0,25	0,33	0,3	0,253	0,41	0,04	0,02	0,02	N/A	N/A	N/A	0,03	0,01	0,01	0,27	0,04	0,07	0,04	0	0,03	0,07	0,13	0,01	N/A	N/A	N/A	0,06	0,02	0,02	0,01	0,03	0,09	0,02	0,01	0,01	0,01	0,01
Mg^{2+}	mg L^{-1}	37	34	31,9	25,6	N/A	N/A	N/A	35,9	48,7	39,5	48,1	43,8	19,1	18,3	15,3	N/A	N/A	N/A	12,6	10,3	9,5	13,3	14,5	11,4	18,9	16,4	14,9	15,6	15,1	13,6	N/A	N/A	N/A	11,6	16	10,5	15,7	11,9	11,9	11,6	10,1	9,6	11,3	11,1
Ca^{2+}	mg L^{-1}	139,8	138,3	130,6	75,8	N/A	N/A	N/A	165	216,2	171,7	211,7	181,3	104,5	98,1	66,2	N/A	N/A	N/A	66,7	61,2	52,6	82,5	75	86,4	143,6	117,6	65,7	78,6	63,3	58	N/A	N/A	N/A	58	59,5	64,1	117,6	78,6	77,7	79,2	91,3	84,4	79,6	82,1
Na^+	mg L^{-1}	681,8	401,8	462,6	277,3	N/A	N/A	N/A	453,3	1444	1537	1427	500,7	15,6	20,3	27,7	N/A	N/A	N/A	30,9	28	22	29,5	21,5	34	30,3	28,5	32,8	38,7	29,2	42,4	N/A	N/A	N/A	40,4	42,4	38,8	29,8	25,6	35,2	43,1	24,7	24	39,6	38,6
K^+	mg L^{-1}	58,6	68,8	67,6	59	N/A	N/A	N/A	109,8	8,5	14,8	21,5	124,8	7	7,7	7,8	N/A	N/A	N/A	8,4	9,3	10,5	8,9	8,2	10,7	9,5	8,5	8,3	5,1	5,2	6	N/A	N/A	N/A	4,5	5,1	5,6	3,6	1,7	1,6	1,9	0,09	0,8	1,2	1,4

IX. Streszczenie

Okrzemki (Bacillariophyta) od pierwszej połowy XX wieku są wykorzystywane jako organizmy bioindykacyjne w ocenie jakości wód powierzchniowych. Rozwój technik analiz mikroskopowych oraz badań w dziedzinie genetyki sprawił, że liczba opisywanych nowych gatunków tej grupy glonów w ostatnich latach szybko wzrasta. Prace taksonomiczne, w których opisywany jest nowy takson nie zawsze zawierają informację o jego autekologii. Takie dane pozyskiwane są dopiero wtedy, gdy takson ten jest notowany w innych ekosystemach, w różnych regionach świata. Ważnym zagadnieniem związanym z prawidłową i rzetelną oceną jakości wód powierzchniowych jest konieczność opisywania nowych taksonów wraz z ich autekologią oraz stałego aktualizowania danych dotyczących zakresów tolerancji wobec warunków środowiska wodnego okrzemek wykorzystywanych w naukowych podstawach biomonitoringu.

Program OMNIDIA jest jednym z głównych narzędzi wykorzystywanych do oceny jakości wód powierzchniowych. W swojej bazie danych program ten zawiera informacje o ponad 24 tysiącach gatunków okrzemek wraz z przypisanymi im ekologicznymi wartościami wskaźnikowymi, które są wykorzystywane podczas wyliczania indeksów okrzemkowych. Jednym z głównych systemów klasyfikacji ekologicznej stanowiącym część bazy danych programu OMNIDIA jest system Van Dama i współautorów z 1994 roku. System ten klasyfikuje taksony okrzemek w odniesieniu do siedmiu cech opisujących warunki środowiska wodnego. Pomimo, iż w ostatnich latach wielu autorów opisując nowe gatunki podaje również informacje o ich autekologii, dane te nie są na bieżąco aktualizowane w bazie OMNIDIA. Z tego powodu ocena stanu środowiska wodnego wykonana za pomocą tego programu może być obarczona błędem. Dlatego niezbędna jest wielowątkowa analiza autekologii okrzemek na podstawie której będzie można przypisywać lub ustanawiać nowe ekologiczne wartości wskaźnikowe dla okrzemek wykorzystywanych w ocenia jakości środowiska wodnego.

Ekosystemy wodne pochodzenia antropogenicznego, które powstały w wyniku eksploatacji kopalin mineralnych charakteryzują się specyficznymi oraz unikalnymi warunkami środowiskowymi. Panujące w nich warunki hydrogeochemiczne kształtują zbiorowiska okrzemek nie występujące w innych, naturalnego pochodzenia, ekosystemach wód powierzchniowych. Dlatego też, stanowią one doskonałe obiekty badań autekologii oraz taksonomii tej grupy glonów. Zbiorowiska okrzemek występujące w zbiornikach

poeksploatacyjnych badane były dotychczas w celu prześledzenia zmian klimatycznych zachodzących w czasie lub określenia różnorodności gatunkowej. Badania zbiorowisk okrzemek takich ekosystemów dają możliwości weryfikacji lub ustanowienia nowych ekologicznych wartości wskaźnikowych gatunkom dla nich charakterystycznym.

W celu zbadania różnorodności, zmienności międzygatunkowej i wewnątrzgatunkowej okrzemek, oraz ich autekologii wytypowano trzy obiekty hydrologiczne powstałe w wyniku działalności człowieka. Każdy z badanych obiektów wyróżniał się innymi warunkami środowiskowymi: kompleks hydrologiczny Pełczyska charakteryzował się wysokim stężeniem jonów chlorkowych oraz wysokim przewodnictwem elektrolitycznym wody; kompleks zbiorników w Łęczycy charakteryzował się wysokim odczynem wody oraz najniższym stężeniem jonów wodorowęglanowych; natomiast zbiornik Bogdałów charakteryzował się alkaicznym odczynem wody oraz najniższym stężenie jonów K^+, Cl^- i NH_4^+.

Zasadniczą częścią badań było (1) zbadanie zmienności wewnątrzgatunkowej i międzygatunkowej oraz (2) zbadanie autekologii okrzemek występujących w zbiornikach wodnych powstałych w wyniku górniczej działalności człowieka. W tym celu poddano analizie 62 próby okrzemkowe z wyznaczonych obiektów hydrologicznych i 31 prób wody w celu analizy parametrów fizycznych i chemicznych. Kompleks hydrologiczny Pełczyska poddany był dodatkowej analizie mającej na celu określenie zmian w strukturze zbiorowisk okrzemek w okresie 50 lat z wykorzystaniem prób archiwalnych. W pracy zastosowano następujące analizy matematyczne: analiza MDS, nMDS, HCA, Shade Plot, SIMPER oraz PCA. Do opracowania wyników analiz struktury zbiorowisk okrzemek oraz danych o parametrach fizycznych i chemicznych wykorzystano programy PRIMER 7.0.13 i STATISTICA 13. Autekologia okrzemek została opracowana na podstawie danych z programu OMNIDIA 6.0.6.

Szczegółowa analiza zbiorowisk okrzemek oraz danych chemicznych wody kompleksu hydrologicznego Pełczyska wykazała, iż w okresie 50 lat wraz ze spadającym stężeniem jonów chlorkowych zmieniła się struktura zbiorowisk okrzemek. Ponadto odnotowano w nim gatunki okrzemek typowe dla wód brakicznych i morskich. Zaproponowano rozszerzenie skali zasolenia, z 4 do 7 stopniowej, wykorzystywanej w programie OMNIDIA oraz ustalenie nowych ekologicznych wartości wskaźnikowych związanych z zasoleniem wody dla wybranych gatunków okrzemek: *Halamphora tenerrima*, *Parlibellus crucicula*, *Staurophora salina*, *Halamphora dominici, H. subsalina, Achnanthes brevipes*, *Navicula perminuta* i *Opephora mutabilis*.

Analiza zmienności międzygatunkowej w badanych ekosystemach, z wykorzystaniem technik mikroskopii świetlnej oraz skaningowej mikroskopie elektronowej, wykazała zróżnicowanie w budowie morfologicznej *Discostella woltereckii* oraz *D. stelligera* oraz potwierdziła występowanie obu gatunków w jednym środowisku. Dane te wzbogaciły wiedze o autekologii tych gatunków oraz zweryfikowały negatywnie informację o tym, iż cechą różnicująco oba gatunki jest środowisko, w którym występują.

Szeroka zmienności wewnątrzgatunkowa obserwowana w strukturze zbiorowisk okrzemek w badanych ekosystemach umożliwiła szczegółową analizę zmienności morfologicznej wybranych gatunków. Na podstawie szczegółowych obserwacji budowy morfologicznej okazów z materiału typowego oraz wybranych populacji gatunków okrzemek opisano dwa nowe dla nauki gatunki: *Chamaepinnularia plinskii* Żelazna-Wieczorek & Olszyński z kompleksu hydrologicznego Pełczyska oraz *Aulacoseira pseudomuzzanensis* Olszyński & Żelazna-Wieczorek z kompleksu zbiorników w Łęczycy.

Specyficzne warunki środowiskowe panujące w badanych obiektach hydrologicznych umożliwiły zbadanie autekologii zbiorowisk okrzemek w nich występujących. Z 381 taksonów odnotowanych w wytypowanych do badań obiektach hydrologicznych wskazano 38 gatunków dla nich charakterystycznych. Wykorzystując wielowątkowe podejście w celu weryfikacji autekologii gatunków charakterystycznych obejmujące analizę warunków środowiskowych panujących w badanym ekosystemie, analizę ekologii gatunków w oparciu o piśmiennictwo oraz określenie warunków środowiskowych na podstawie znanej już autekologii gatunków współwystępujących, dla 16 taksonów okrzemek charakterystycznych ustanowiono lub zmieniono 36 ekologicznych wartości wskaźnikowych w klasyfikacji Van Dama i współautorów.

X. Abstract

Since the beginning of 20th century diatoms (Bacillariophyta) have been widely used as bioindicators of the quality of surface waters. The progress in advanced microscopic techniques and molecular studies resulted in increasing number of new taxa descriptions, especially in recent years. However, taxonomic studies very often miss ecological data and does not bring any insight into the species autecology. Such data are supplemented over time during diversity studies in various ecosystems of the world. Biomonitoring procedures rely on autecology of indicator species; thus, for the proper and reliable assessment it is crucial to include ecological data while describing new taxa and to update the range of tolerance to environmental factors if applicable.

The basic evaluation tool in surface water quality assessment is the OMNIDIA software. The software contains large database of over 24,000 diatom species described with ecological indicator values allowing to calculate diatomaceous indices and also includes ecological classification systems. The main classification system is the Van Dam and co-authors system proposed in 1994. This system classifies diatom taxa to seven environmental features of aquatic ecosystems according to their ecological preferences. Despite that in recent years, new publications on diatoms provide further autecological data, the database of OMNIDIA software is not constantly updated; therefore, the assessment of the ecological state of ecosystems using this tool may be fraught with an error. To avoid possible mistakes it is necessary to carry out multi-stranded analysis on diatoms' autecology and to provide complete ecological data establishing new or updating already described indicator values for these bioindicators.

Aquatic ecosystems of anthropogenic origin that were created after the exploitation of minerals are characterized by specific and unique environmental conditions. The hydrogeochemical conditions of these ecosystems shape diatom assemblages making them unique among other surface water ecosystems of natural origin. Therefore, these sites are excellent for taxonomical and ecological studies of this group of algae. Diatom assemblages in post-mining reservoirs have been studied so far to trace climate changes over time and to determine possible species diversity. However, diatom research in such ecosystems provide crucial data for biomonitoring procedures, because they give a scientific foundation for verification or description of new ecological indicator values for species being characteristic for these type of ecosystems.

Three hydrological sites of anthropogenic origin were selected for comprehensive studies on the diversity, intraspecific and interspecies variability and autecology of diatoms. The sites were distinguished by different environmental conditions: the hydrological complex of Pełczyska was characterized by high concentration of chloride ions and high electrolytic conductivity of water; the complex of reservoirs in Łęczyca by a high water pH reaction and the lowest concentration of bicarbonate ions; while the Bogdałów reservoir by an alkaline waters and the lowest concentration of K^+, Cl^- and NH_4^+ ions.

The essential part of the research was (1) to examine intraspecific and interspecific variability of diatoms, and (2) to examine the autecology of diatoms in post-mining water ecosystems. For this purpose, 62 diatomaceous samples from studied hydrological sites were examined and 31 water samples were analysed to investigate physical and chemical properties of water. The Pełczyska hydrological complex was subjected to additional analysis aimed at determining changes in the structure of diatom communities over a period of 50 years using archival biological samples. The structure of diatom assemblages in relation to environmental data was investigated using MDS, nMDS, HCA, Shade Plot, SIMPER and PCA analyses in the PRIMER 7.0.13 and STATISTICA 13 software. The conclusions on autecology of diatoms were compared and combined with databases of the OMNIDIA 6.0.6 software.

A detailed hydrobiologial and hydrochemical analysis of the Pełczyska complex showed that over the past 50 years, along with the decreasing chloride ion concentration, the structure of the diatom assemblages has changed. Some brackish and marine diatom species were still noted. According to the research findings, the extending of salinity scale in the OMNIDIA software, from 4 to 7 degrees was proposed along with description of new indicator values for water salinity for selected diatom species: *Halamphora tenerrima, Parlibellus crucicula, Staurophora salina, Halamphora dominici, H. subsalina, Achnanthes brevipes, Navicula perminuta* and *Opephora mutabilis.*

Analysis of interspecies variability in the studied ecosystems, using light microscope and scanning electron microscope, showed diversity in the morphology of *Discostella woltereckii* and *D. stelligera* cell walls and confirmed the occurrence of both species in the same environment. These results enriched the knowledge on autecology of these species and proven that the type of environment cannot be a distinguishing feature, since they may co-occur in one ecosystem.

The wide interspecies variability of diatoms in the studied ecosystems enabled a detailed investigation on the morphological variability of selected species.

Based on comparative analyses of specimens from type material and environmental samples, two new species for science were described: *Chamaepinnularia plinskii* Żelazna-Wieczorek & Olszyński in the hydrological complex Pełczyska and *Aulacoseira pseudomuzzanensis* Olszyński & Żelazna-Wieczorek in the complex of reservoirs in Łęczyca.

Specific environmental conditions prevailing in the studied hydrological sites enabled the study on autecology of diatoms. From the 381 taxa recorded at the study sites, 38 were designated as type-specific or characteristic for those type of ecosystems. Using a multi-stranded approach to verify the autecology of characteristic species, including the analysis of environmental conditions of studied ecosystems, analysis of species autecology based on literature and determination of environmental conditions based on the autecology of co-occurring species, 36 ecological indicator values have been established or changed for 16 type-specific diatoms in Van Dam and co-authors classification system.

XI. Literatura uzupełniająca

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