Taylor & Francis
Taylor & Francis Group

Craspedostauros alatus sp. nov., a new diatom (Bacillariophyta) species found on museum sea turtle specimens

Roksana Majewska, Matt P. Ashworth, Eric Lazo-Wasem, Nathan J. Robinson, Lourdes Rojas, Bart Van de Vijver & Theodora Pinou

View supplementary material

Published online: 16 Oct 2018.

Submit your article to this journal

Article views: 170

View related articles

View Crossmark data

Citing articles: 7 View citing articles

Taylor & Francis
Taylor & Francis Group

Check for updates

Craspedostauros alatus sp. nov., a new diatom (Bacillariophyta) species found on museum sea turtle specimens

ROKSANA MAJEWSKA [1,2]*, MATT P. ASHWORTH[3], ERIC LAZO-WASEM[4], NATHAN J. ROBINSON[5], LOURDES ROJAS[4], BART VAN DE VIJVER[6,7] & THEODORA PINOU[8]

[1]*Unit for Environmental Sciences and Management, School of Biological Sciences, North-West University, Potchefstroom, South Africa*
[2]*South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa*
[3]*Department of Molecular Biosciences, University of Texas, Austin, TX, USA*
[4]*Division of Invertebrate Zoology, Peabody Museum of Natural History, Yale University, New Haven, CT, USA*
[5]*Cape Eleuthera Institute, The Cape Eleuthera Island School, Eleuthera, The Bahamas*
[6]*Botanic Garden Meise, Research Department, Meise, Belgium*
[7]*Department of Biology, ECOBE, University of Antwerp, Antwerpen, Belgium*
[8]*Department of Biological and Environmental Sciences, Western Connecticut State University, Danbury, CT, USA*

Several populations of a new *Craspedostauros* species were observed on museum specimens of juvenile green turtle and Kemp's ridleys collected from Long Island Beach, New York, USA. The new taxon, *Craspedostauros alatus* Majewska & Ashworth sp. nov., exhibits a distinctive set of morphological features typical of the genus, including cribrate areolae, a stauros narrower than the fascia, multiple doubly perforated girdle bands, and two fore and aft chloroplasts, but clearly differs from all known *Craspedostauros* species in possessing partially fused proximal helictoglossae forming an internal thickening with a shallow central cavity, distinctive external wing-like silica flaps near the apices, and a combination of valve dimensions and stria density not observed in other taxa. The same taxon was identified in the NCMA culture collection (strain CCMP120), isolated in 1967 from a sample collected from the equatorial upwelling zone in the Pacific Ocean. Nine samples from three sea turtle species were collected from the same location (Long Island Beach). Statistical analyses suggested that the epizoic diatom flora composition was not affected by the collection season but differed among sea turtle species. The most significant difference was observed between the samples with and without *C. alatus*. Samples with *C. alatus* were always dominated by *Achnanthes elongata* and *Berkeleya* cf. *rutilans*, while those lacking the new taxon were characterized by remarkably higher contributions of epizoic *Poulinea* species. This observation suggests that the microhabitat provided by each sea turtle differs among specimens, which may be related to the different stages of biofilm development on the host sea turtle. Additionally, the value of zoological museum collections for epizoic diatom surveys is briefly discussed.

Keywords: Craspedostauros*, marine diatom, epizoic, museum specimens, Kemp's ridley, sea turtle*

Introduction

Aquatic animals, both marine and freshwater, provide unique habitats for various macro- and microorganisms. Although growth on a living, and especially mobile, substratum may prove impossible for many plant and animal taxa, some species have developed adaptations that allow them to thrive in these extreme environments and thus utilize a niche inaccessible to many other organisms. Among them, various diatom species seem to cope especially well with challenges posed by rapidly changing conditions related to the host's biology and behaviour, and are therefore found on a diversity of host organisms. For instance, different species of diatoms have been found growing on ciliates (Dute et al. 2000), barnacles (Round & Alexander 2002), unionid bivalves (Francoeur et al. 2002), hydrozoans (Di Camillo et al. 2005), copepods, isopods, and euphausiids (Russell & Norris 1971, McClatchie et al. 1990, Winemiller & Winsborough 1990, Cook et al. 1998, Fernandes & Calixto-Feres 2012), mayflies (Wujek 2013), crabs (Madkour et al. 2012), horseshoe crabs (Patil & Anil 2000), and gastropods (Gillan & Cadée 2000, Radea et al. 2008). Moreover, diatoms have long been known to grow on marine vertebrates such as sea-birds, whales, and dolphins (Holmes & Croll 1984, Denys 1997, Denys & Van Bonn 2001 and references therein). In past decades, several new vertebrate-associated diatom genera, never observed on non-animal substrata, and thus presumably exclusively epizoic, such as *Bennetella* R.W. Holmes (1985), *Epiphalaina* R.W. Holmes, Nagasawa et Takano (Holmes et al. 1993), and *Plumosigma* T. Nemoto (1956), have been described. Although these reports and discoveries suggest that epizoic diatoms are not uncommon in aquatic habitats,

---

*Corresponding author. E-mail: roksana.majewska@nwu.ac.za

their biodiversity, ecological function, and contribution to global primary production remain unknown, and it is conceivable that the small number of currently known epizoic diatom species is directly related to the generally small and geographically uneven sampling effort for marine diatoms.

Only recently, have the first studies exploring sea turtle-associated diatoms been conducted and several new diatom taxa, including three new genera, have been described (Majewska et al. 2015a, 2017a, 2018, Frankovich et al. 2016, Riaux-Gobin et al. 2017a, b). Interestingly, diatoms on sea turtles seem to attract growing attention, partly due to their potential use in exploring sea turtle ecology and as health indicators (Robinson et al. 2016).

Robinson et al. (2016) examined museum sea turtle samples of various ages, fixed and stored in different ways, showing that even relatively poorly preserved specimens may still contain a high number of unbroken diatom valves, retaining their suitability for diatom analysis. During a careful examination of sea turtle samples donated to the Yale Peabody Museum of Natural History (New Haven, CT, USA), an interesting diatom species bearing evident morphological similarities to the genus *Craspedostauros* E.J. Cox was found.

Craspedostauros is currently a relatively species-poor genus comprising nine validated species, eight of which are presumably typically marine (Cox 1999, Ashworth et al. 2017) and one brackish (Sabbe et al. 2003, Van de Vijver et al. 2012). *Craspedostauros* taxa have been recorded and described from a wide variety of substrata and locations, including the English Channel, eastern and western coasts of Great Britain, southern coasts of Australia, Red Sea (water sample), western coasts of Guam (water sample), western coasts of South Africa, Philippines, and Antarctic saline lakes (Cox 1999, Sabbe et al. 2003, Ashworth et al. 2017). Morphological features of the genus include cribrate areolae, a stauros narrower than the fascia, multiple doubly perforated girdle bands, and two fore and aft chloroplasts (Cox 1999). The DNA phylogeny, as well as morphological similarities, indicate that the genus is closely related to two other marine genera, *Achnanthes* Bory and *Staurotropis* T.B.B. Paddock, and most likely to the recently described *Druehlago* Lobban et Ashworth, for which no DNA sequence data have yet been obtained (Ashworth et al. 2017).

The present paper describes the newly discovered diatom as a new species: *Craspedostauros alatus* Majewska & Ashworth sp. nov., based on its frustule morphology, which displays several features typical for the genus, such as a stauros narrower than the fascia, cribrate areolae, and internal central thickening between the proximal raphe endings.

Material and methods

Samples were taken from frozen sea turtle carcasses donated to the Yale Peabody Museum of Natural History.

Table 1. A list of the Yale Peabody Museum of Natural History sea turtle specimens used in this study.

Species	Original specimen number	Diatom slide number	Specimen collection date
Lepidochelys kempii	NY4942-13[a]	TPD-04-16	24/11/2013
	NY4722-12[a]	TPD-05-16	20/12/2012
Kemp's ridley	NY4954-13[a]	TPD-06-16	29/07/2014
Caretta caretta	NY5307-15	TPD-08-16	30/07/2015
	NY5308-15	TPD-09-16	02/08/2015
Loggerhead	NY5310-15	TPD-10-16	04/08/2015
Chelonia mydas	NY4569-12	TPD-20-16	26/01/2012
	NY4961-13[a]	TPD-21-16	29/07/2014
Green turtle	NY5311-15	TPD-22-16	07/08/2015

[a]Samples that contained *Craspedostauros alatus*.

These were three juvenile green turtles (*Chelonia mydas* L.), three loggerheads (*Caretta caretta* L.), and three Kemp's ridleys (*Lepidochelys kempii* Garman) found stranded (cold-stunned and still alive, though beyond rehabilitation) along Long Island Beach (New York State, USA) between 2012 and 2015 (Table 1). In addition, morphologically similar specimens were found in the diatom culture collection of the University of Texas at Austin (USA) under the name '*Stauroneis constricta*' Ehrenberg, strain ID CCMP1120, and a sample was taken for further analyses.

For diatom analysis, the sea turtle carcasses were partly thawed and 5–10 cm^2 sections were cut from several arbitrarily chosen locations on the carapace. The carapace pieces were placed in separate 100 mL beakers filled with ca. 25 mL distilled water and sonicated (Branson B-22-4 Ultrasonic Cleaner, Branson Ultrasonics Corporation, Danbury, USA) for 30 min to detach the epizoic diatoms. Subsequently, water samples were left under the ventilation hood for ca. 24 h, which allowed the sample volume to be reduced to about 25% of the initial volume prior to standard diatom cleaning. To remove all organic debris, a mixture of boiling concentrated acids (1:3 volume ratio of 64% nitric acid and 97% sulphuric acid) was used following von Stosch's method (Hasle & Syvertsen 1997). Culture collection material was cleaned using a mixture of equal volumes of sample, 30% hydrogen peroxide, and 70% nitric acid. After the reaction was completed, the cleaned material was centrifuged using Premiere XC-2000 Bench-Top Centrifuge (C & A Scientific, Manassas, VA, USA) and Sorvall RC-5B Refrigerated Superspeed Centrifuge (DuPont Company, Newton, CT, USA) at 2500 rpm for 10 min and rinsed several times with distilled water until the pH of the sample became neutral, which was determined using litmus paper. For light microscopy (LM) examination, cleaned diatom material was pipetted onto

glass cover slips (22 × 22 mm) and air-dried. Permanent slides were prepared using Naphrax® and observed under a Nikon 80i microscope equipped with differential interference contrast optics and 100× 1.4 N.A. oil immersion objective and a Zeiss Axioskop (Carl Zeiss Microscopy, Thornwood, NY, USA). LM images were obtained using a Nikon DS-Fi1 digital microscope camera.

Due to the high percentage of broken *C. alatus* and *Chelonicola* spp. frustules in the digested material, another set of permanent slides was prepared using uncleaned water samples. For scanning electron microscopy (SEM), both cleaned and uncleaned diatom material was filtered through 1-μm Isopore™ polycarbonate membrane filters (Merck Millipore), after which the filters were attached to aluminium stubs with carbon tabs, air-dried, and sputter-coated (Cressington 108Auto, Cressington Scientific Instruments, Watford, UK) with gold. A field emission SEM (XL-30 ESEM FEG) operating at 5–15 kV and 10 mm working distance was used for the analysis. Cleaned cultured material was dried onto 12 mm round coverslips, coated with iridium using a Cressington 208 Bench Top Sputter Coater, and observed with a Zeiss SUPRA 40 VP SEM (Carl Zeiss Microscopy, Thornwood, NY, USA). Samples, SEM stubs, and permanent slides are stored at the Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa and the Yale Peabody Museum of Natural History, New Haven, USA (sea turtle samples), and UTEX Culture Collection of Algae, University of Texas at Austin, Austin, USA (cultured material).

The terminology used largely follows Ross et al. (1979), Round et al. (1990), Cox (1999), and Van de Vijver et al. (2010). The morphology of the new taxon has been compared with descriptions and images of all known *Craspedostauros* species (Cox 1999, Van de Vijver et al. 2012, Ashworth et al. 2017).

In every sample, at least 400 diatom valves were counted along random transects using SEM. To study variation patterns in diatom flora composition among different sea turtle specimens, species, and seasons, standard statistical analyses such as Analysis of Similarities (ANOSIM), Similarity Percentages (SIMPER), and non-metric Multidimensional Scaling (nMDS) were performed on square root-transformed diatom abundance data using the PRIMER v6 computer software (Clarke & Gorley 2006).

Results

Craspedostauros alatus Majewska & Ashworth, sp. nov.

Type

United States, New York: Riverhead, samples taken from carapaces of three Kemp's ridleys (*Lepidochelys kempii*) and one green turtle (*Chelonia mydas*) found alive but beyond rehabilitation on the beach by an anonymous collector, 20 December 2012, 24 November 2013, and 29 July 2014. The holotype (SADC-TPD-05-16) is deposited in the South African Diatom Collection housed by North-West University, Potchefstroom, South Africa; isotype (TPD-05-16) deposited at the Yale Peabody Museum of Natural History, New Haven, USA; paratypes TPD-04-16, TPD-06-16, TPD-21-16.

The holotype and isotype slides contain diatom specimens isolated from the same individual sea turtle (Kemp's ridley), but the paratype slides contain diatom specimens isolated from different sea turtle individuals (Kemp's ridley and green turtle) (Table 1).

Etymology

The specific epithet refers to the presence of short elevated silica flaps near the apices that resemble small wings (Latin *alatus* = winged).

Light microscopy (Figs 1–6):

Due to their deep girdles, intact frustules almost always lie in girdle view, in which position they appear clearly constricted at the centre (Figs 1–6). Girdle view broad with several perforated girdle bands (Figs 3–6). Valves lightly silicified and fragile, often broken along the raphe and/or valve face–mantle junction. In valve view (not shown), valves are narrow, linear to linear-lanceolate, slightly constricted at the stauros, becoming narrower towards the bluntly rounded apices. Valve dimensions ($n = 40$): 20–37 μm long, 3–5 μm wide, length/width ratio: 5.3–7.8. Valve face–mantle junction visible as a faint line running on each side of the raphe (Fig. 2, arrows), from the apices throughout the entire valve length, crossing the stauros at about the mid-point of each half. Transapical striae parallel throughout the entire valve, 26–28 in 10 μm. Areolae not discernible in LM. Narrow, rectangular fascia clearly visible, widening slightly towards the slightly biarcuate valve margins (Figs 5, 6). Unthickened hyaline area extending from the valve margins, wider at the stauros (Figs 5, 6). Sternum clearly visible (Figs 1, 2, 4), slightly elevated near the apices on one side of the raphe, corresponding to external wing-like thickenings (Fig. 1, arrows). Raphe straight, biarcuate in girdle view (Figs 1–5).

Scanning electron microscopy (Figs 7–20):

Valves linear-lanceolate, slightly centrally constricted, with blunt, sometimes protracted apices (Figs 7–9). Striae uniseriate, parallel throughout the whole valve, alternate or opposite, composed of 1–3 (very rarely 4) areolae on the valve face and 1–6 (very rarely 7) on the mantle (Figs 7–9). Copulae open, perforated by two rows of typically elongated areolae (Figs 10–12). Valvocopula curved and hyaline at the stauros with smaller, irregular areolae (Figs 10, 12).
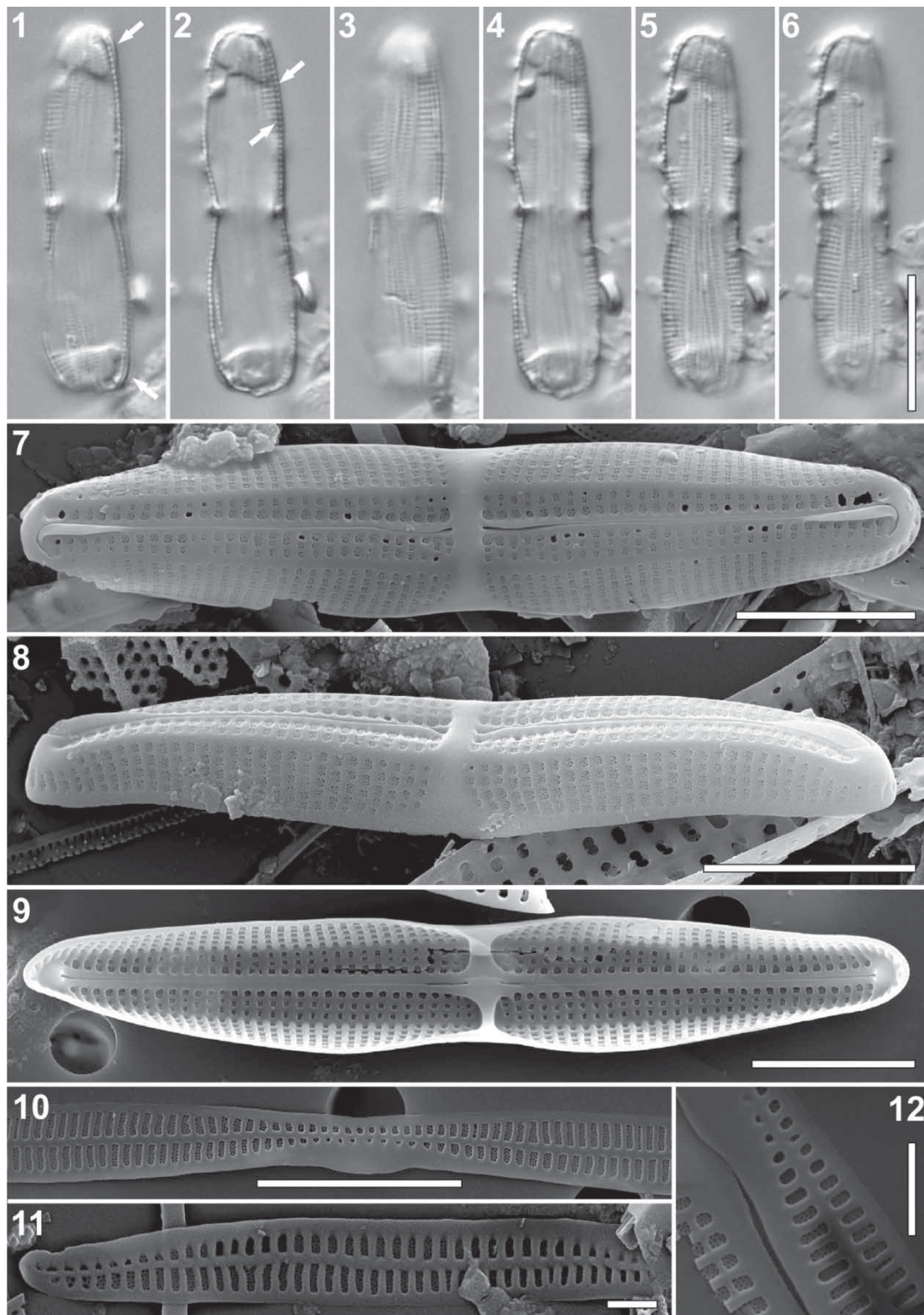
Figs 1–12. LM (Figs 1–6) and SEM (Figs 7–12) images showing frustule of *Craspedostauros alatus* in girdle view in different focal planes. Arrows indicate apical wing-like thickenings (Fig. 1) and valve face–mantle junction (Fig. 2). Figs 7–12. SEM images. Figs 7, 8. *C. alatus* valve in external view. Fig. 9. *C. alatus* valve in internal view. Figs 10–12. Open valvocopula of *C. alatus*. Fig. 10. Central part with smaller and irregular areolae. Fig. 11. Detail of an open end. Fig. 12. Central part with hyaline area at the stauros. All images were taken from the holotype population. Scale bars: 10 μm (Figs 1–6), 5 μm (Figs 7–10), 1 μm (Figs 11, 12).

External valve view (Figs 7, 8, 13–16): Valve face narrow and flat, with a slightly raised raphe sternum (Fig. 8). Almost rectangular fascia gradually widening near the valve face–mantle junction and towards the valve margin on the mantle (Figs 7, 8, 13, 14). Weakly raised hyaline ridge marking the valve face–mantle junction (Figs 7, 8) running from apex to apex. Mantle very deep (Fig. 8). Near the apices, valve face connecting to the mantle almost at a right angle, becoming sharp closer to the stauros (Figs 7, 8). Raphe sternum narrow, merging with the fascia at the centre (Figs 7, 8, 13, 14). Raphe branches almost straight. Proximal raphe endings weakly expanded, unilaterally deflected due to very narrow silica flaps extending from one side of the raphe sternum, partially covering the raphe branches (Figs 7, 13, 14). Distal raphe fissures unilaterally curved (Figs 7, 16). At the apices, thickened pore-free area continuing beyond the terminal raphe fissures (Figs 7, 8, 16). Prominent wing-like silica flaps partially covering the first row of areolae bordering the raphe sternum near the valve apices (Fig. 16). Areolae cribrate, irregular, squarish to roundish, variable in size (Figs 7–9, 13–16). Areolae bordering the raphe sternum clearly larger than on the rest of the valve face (Figs 7, 13, 14). Transapically elongated or confluent areolae often present close to the valve margin (Figs 8, 15). One row of narrow, elongated mantle areolae running around the apices (Figs 8, 16). Cribrum pores (up to 15 per cribrum in larger areolae) markedly elongated and curved at the areola margins (Figs 13–16).

Internal valve view (Figs 9, 17–20): Narrow pore-free longitudinal lines running from apex to apex clearly marking the valve face–mantle junction (Fig. 9). Raised stauros distinctly narrower than the fascia (Figs 9, 17, 18), broadening and decreasing in thickness close to the valve margins (Figs 9, 18). Proximal raphe fissures terminating on an elongated, weakly constricted rectelevatum (Figs 17, 18, arrows). Cribrate areolae closer to the external valve surface (Figs 14, 17–20). Distal raphe endings straight, elongated, terminating in prominent helictoglossae (Figs 9, 20). Pore-free area continuing beyond the terminal helictoglossae (Figs 9–20).

Culture collection specimens

Morphologically similar specimens were found in the diatom culture collection of the University of Texas at Austin (USA) (Figs 21–28). The diatom strain in question had been acquired from the National Center for Marine Algae and Microbiota (NCMA), at Bigelow Laboratory for Ocean Science (USA) under the name '*Stauroneis constricta*' Ehrenberg, strain ID CCMP1120. According to the collection data on NCMA's website (https://ncma.bigelow.org), this strain was isolated in 1967 from a sample collected from the equatorial upwelling zone in the Pacific Ocean and maintained at a temperature of ca. 24°C (see

Supplementary Table S1 for more information about the strain).

Most of the observed features agreed well with the taxon described from the sea turtle carcasses. Numerous abnormally developed cells were present in the cultured strain, most likely due to its long-term maintenance (Estes & Dute 1994). Cells with regular morphologies were slightly wider (5–7 μm; *n* = 30), with a larger length range (16–38 μm; length/width ratio: 3–5.9, *n* = 30) than those found on the sea turtles, whereas the number of striae in 10 μm was lower (22–25/10 μm vs. 26–28/10 μm in sea turtle samples). Both valve malformations and changes in morphometrics are not uncommon in clones maintained over long periods and we believe that both taxa probably belong to the same species. Access to the cultures allowed us to observe the two fore and aft H-shaped chloroplasts (Fig. 21, arrows), a feature of other *Craspedostauros* species (Cox 1999, Ashworth et al. 2017). All observed frustules possessed the typical external wing-like structures near the apices, a mantle wider than the valve face (Figs 22–26), open girdle bands with two rows of cribrate perforations (Fig. 27), and internal proximal raphe fissures terminating in elongated and partially fused helictoglossae (Fig. 28). Some valves exhibited slight asymmetry, both apically and transapically (e.g. Figs 24, 28), and the lack of a central constriction (Fig. 26).

DNA sequence data

DNA sequence data for the CCMP1120 strain have been included in at least three published datasets: Rampen et al. (2009, nuclear ribosomal SSU), MacGillivary & Kaczmarska (2011, partial *rbc*L), and Ashworth et al. (2017, nuclear ribosomal SSU, *rbc*L and *psb*C). Only the last dataset focused specifically on the molecular phylogeny of *Craspedostauros*, including three additional *Craspedostauros* species, one of which was another NCMA strain, CCMP797, which had also been previously identified as a *Stauroneis* spp. In that study, CCMP1120 was identified as '*Craspedostauros* cf. *neoconstrictans*' [*sic*] and was sister to a clade containing *C. alyoubii* J. Sabir et Ashworth and *C. paradoxa* Ashworth et Lobban with high bootstrap support (98% under Maximum Likelihood). Together with CCMP797 (labelled '*Craspedostauros amphoroides*'), the genus was monophyletic (80% bootstrap support under Maximum Likelihood) and sister to a clade containing *Achnanthes*, *Staurotropis*, *Undatella* Paddock et P.A. Sims and the Bacillariales (80% bootstrap support under Maximum Likelihood).

Ecology and associated diatom taxa

Frustules of *C. alatus* were found on all (three) Kemp's ridley carapaces examined, regardless of the year and season of collection, and on one (out of three examined) green turtle carapaces collected in July 2014 (Table 1). No valves of *C. alatus* were found on loggerhead carapaces collected
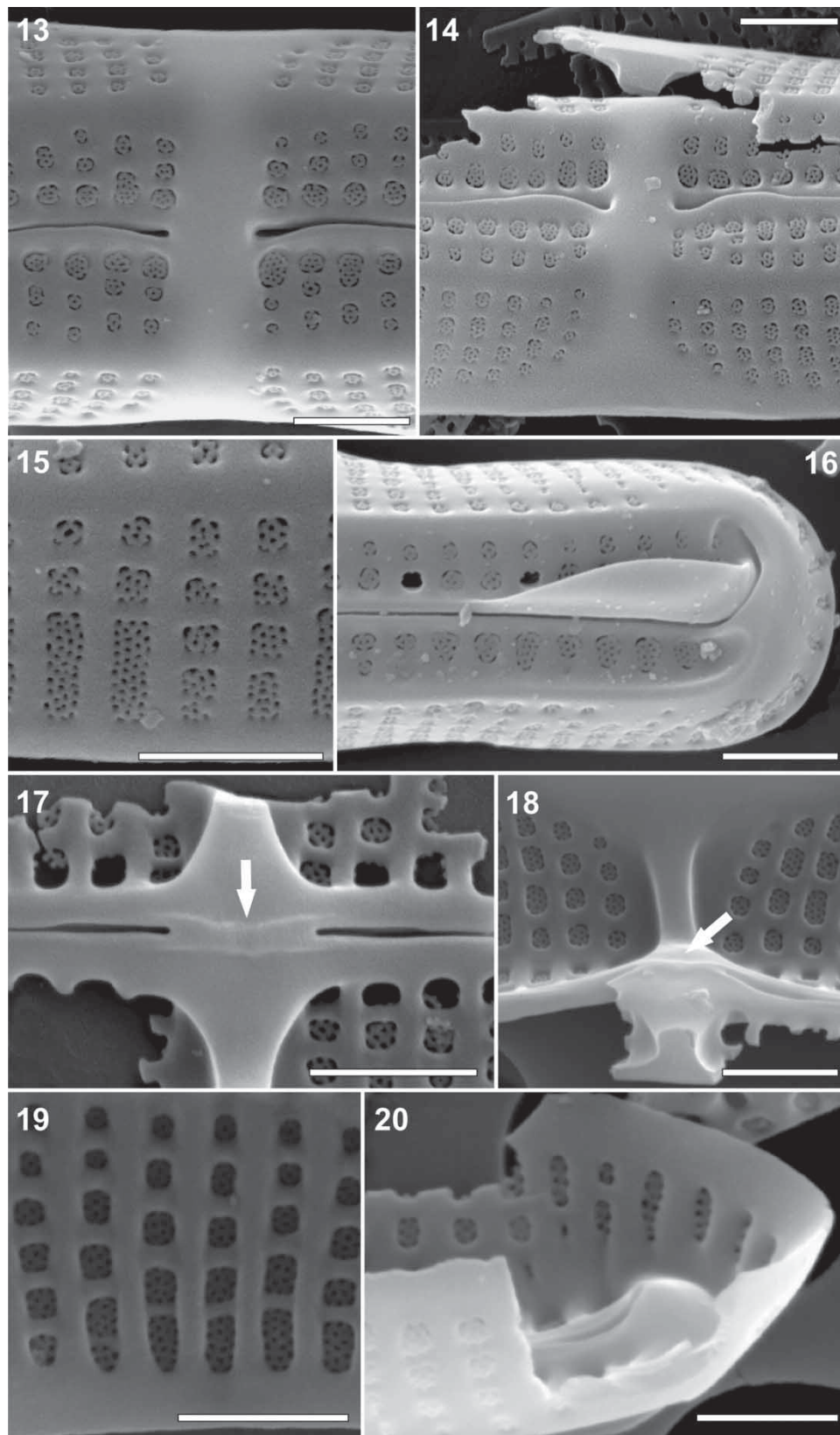
Figure 13–20. SEM images of *Craspedostauros alatus*. Figs 13–16. External details. Figs 13, 14. Central area. Fig. 15. Mantle areolae. Fig. 16. Apex with wing-like thickening. Figs 17–20. Internal details. Figs 17, 18. Central area with proximal raphe fissures terminating in elongated and partially fused helictoglossae creating a small and shallow cavity in the middle of the stauros (arrows). Fig. 19. Mantle areolae. Fig. 20. Apical part with prominent helictoglossa. All images were taken from the holotype population. Scale bars: 1 μm.
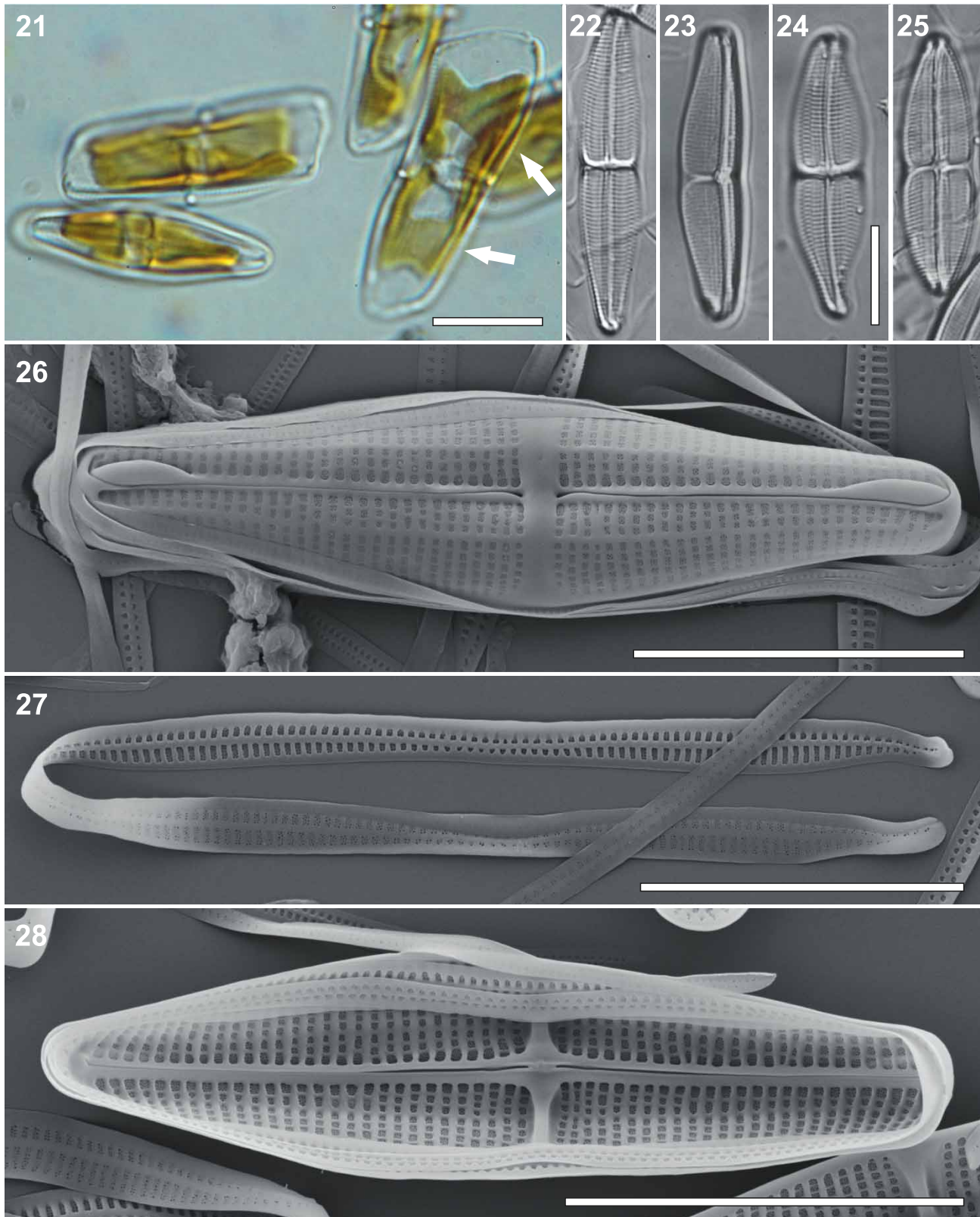
Figs 21–28. LM images of *Craspedostauros alatus*. Fig. 21. Living cells with two fore and aft H-shaped chloroplasts (arrows). Figs 22–25. Cleaned valves. Figs 26–28. SEM images of *C. alatus*. Fig. 26. Valve in external view. Fig. 27. Open valvocopula. Fig. 28. Valve in internal view. All images show specimens from the CCMP1120 strain. Scale bars: 10 μm.
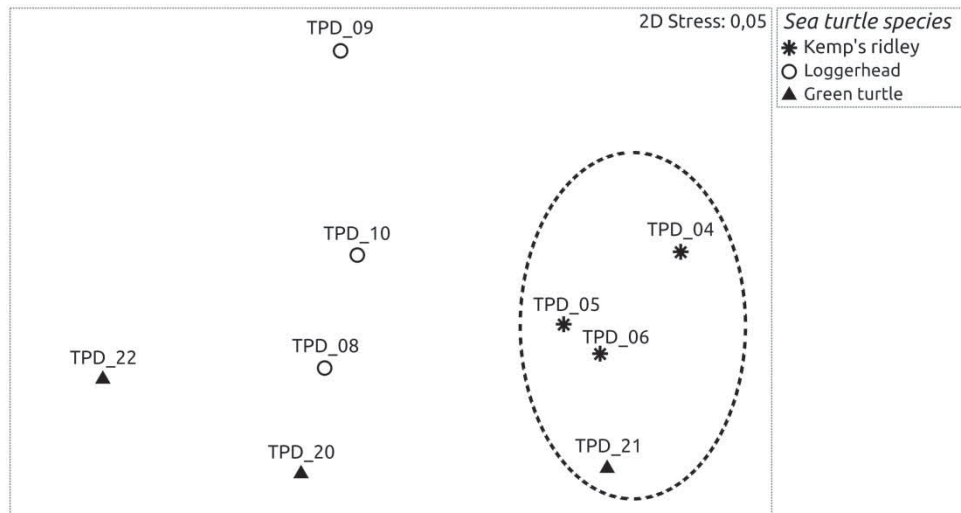
Fig. 29. Non-metric multidimensional scaling graph showing distances between the diatom samples (points) as compositional dissimilarity of those samples. The cluster containing samples with *Craspedostauros alatus* is indicated by a dashed line.

from the same location (Table 1). Although the relative abundance of *C. alatus* did not exceed 5.4% in any of the samples, the taxon was not rare and altogether more than 50 valves have been analysed.

The diatom flora of the four samples in which *C. alatus* occurred was always dominated by *Achnanthes elongata* Majewska et Van de Vijver (18–41%), *Berkeleya* cf. *rutilans* (15–25%), and *Poulinea* Majewska, De Stefano et Van de Vijver (6–13%), with *Amphora* Ehrenberg ex Kützing *sensu lato*, *Navicula* Bory, *Nitzschia* Hassall, and *Proschkinia* N.I. Karajeva also present (Supplementary Figure S1), and was significantly different from that in the five samples in which *C. alatus* was not found (Fig. 29, Supplementary Figure S1; ANOSIM: global $R = 0.769$, $p < .01$). The average dissimilarity between the two sample groups was 72.9%, with 63.7% and 56% average similarity among samples with and without *C. alatus*, respectively (SIMPER). In general, samples with *C. alatus* contained fewer *Poulinea* spp. (30% of the average dissimilarity) and more *A. elongata* (15% of the average dissimilarity) and *B.* cf. *rutilans* (14% of the average dissimilarity) than those where *C. alatus* was absent (SIMPER). According to the ANOSIM results, there was a relatively weak correlation between the epizoic diatom flora composition and sea turtle species (global $R = 0.473$, $p < .05$). The highest level of dissimilarity among the diatom flora growing on different sea turtle species was revealed for Kemp's ridleys and loggerheads (69.7%, SIMPER), followed by Kemp's ridleys and green turtles (65%, SIMPER), and green turtles and loggerheads (53.7%, SIMPER). No correlation between the diatom flora composition and the season (winter vs. summer) was found (ANOSIM: global $R = -0.185$; $p \gg .05$).

Discussion

The new taxon clearly belongs to *Craspedostauros* based on the cribrate structure of both valve and girdle areolae, the stauros being narrower than the fascia, the multiple doubly perforated girdle bands, and the two fore and aft chloroplasts (Cox 1999). Nonetheless, it shows some notable differences from all known *Craspedostauros* species. *Craspedostauros alatus* is distinctly smaller, both in valve length (20–37 µm in *C. alatus* vs. > 80 µm) and width (3–5 µm vs. > 6 µm), and exhibits much lower stria density (26–28 in 10 µm vs. > 36 in 10 µm) than two recently described species, *C. alyoubii* and *C. paradoxa* (Ashworth et al. 2017). It is also smaller than *C. neoconstrictus* E.J. Cox (valve length 40–110 µm, valve width 5–7 µm) and *C. australis* E.J. Cox (valve length 35–78 µm, valve width 4–6 µm), has a higher stria density than *C. capensis* E.J. Cox (19 in 10 µm), *C. decipiens* (Hustedt) E.J. Cox (20–22 in 10 µm), and *C. britannicus* E.J. Cox (ca. 24 in 10 µm), and a lower stria density than *C. australis* (35 in 10 µm) and *C. amphoroides* (Grunow) E.J. Cox (30–32 µm) (Table 2). Furthermore, it differs from all other *Craspedostauros* species in possessing a typical rectelevatum, with a small, shallow cavity in the middle of the stauros. Its most distinctive feature, however, is the presence of the prominent wing-like silica flaps near the apices.

Ecological characteristics

Craspedostauros alatus was found in samples of four out of nine sea turtle carcasses found on Long Island Beach. Its presence on the sea turtle carapaces did not seem to depend on the season as it was found in samples collected in summer and in winter. This may suggest

Table 2. Comparison of *Craspedostauros alatus* with several morphologically similar *Craspedostauros* taxa (after Cox 1999).

Character	*C. neoconstrictus*	*C. decipiens*	*C. capensis*	*C. britannicus*	*C. australis*	*C. amphoroides*	*C. alatus*[a]
Valve outline	± Linear, constricted	Lanceolate	Lanceolate, constricted	Linear to narrow lanceolate	Linear	Lanceolate to slightly constricted	Linear to linear-lanceolate, slightly constricted
Valve length (µm)	40–110	20–38	25–35	14–60	35–78	28–45	20–37 (16–38)
Valve width (µm)	5–7	3–5	4.5–5.5	5–6	4–6	3.5–7	3–5 (5–7)
Stria density (in 10 µm)	∼25	20–22	19	∼24	35	30–32	26–28 (22–25)
Areola size	Similar	Variable	Variable	Similar	Similar	Variable	Variable
Internal central raphe endings	Slight helictoglossae	Unknown	Knob	Helictoglossae	Knob	Slight helictoglossae	Rectelevatum
Valve face–mantle junction	Slight	Distinct	None	None	None	Strong	Strong (distinct)
Valve margin at centre	Expanded	Straight	Straight	Slightly expanded	Straight	Straight	Very slightly expanded
Apical wing-like silica flaps	Absent	Absent	Absent	Absent	Absent	Absent	Always present

[a]Values and descriptions given in brackets refer to the cultured strain CCMP1120.

that sea turtle-associated diatoms depend less on external environmental factors (such as water temperature or salinity) and cope well with the changing abiotic conditions experienced by their hosts.

There was a highly significant difference (ANOSIM: global $R = 0.769$, $p < .01$) in the diatom flora composition between the samples with *C. alatus* and those without, suggesting that the habitats provided by the nine sea turtle individuals were not the same. Samples containing *C. alatus* were always dominated by *A. elongata* (recently described from olive ridleys nesting on the Pacific coast of Costa Rica, Majewska et al. 2015b) and *B.* cf. *rutilans*, with a lower contribution of *Poulinea* spp. This last genus was first described from olive ridleys in Costa Rica (Majewska et al. 2015a) but has since been observed on several other sea turtle populations living in different parts of the world (Majewska et al. 2017b, R. Majewska, unpub., T.A. Frankovich, pers. comm., S. Bosak, pers. comm.). As was previously suggested (Majewska et al. 2017b), two recently described genera, *Poulinea* and *Chelonicola* (and possibly several other taxa), seem to be truly epizoic and conceivably sea turtle-specific taxa that are able to colonize the carapace in the early stages of sea turtle life history. This likely occurs through physical contact with other aquatic megafauna, including other sea turtles and sea mammals (Wetzel et al. 2012), whereas other non-epizoic taxa are being recruited as the biofilm develops and the host sea turtle visits different foraging habitats. In the present study, *Poulinea* spp. and *Chelonicola* spp. were

found in nine and six samples, respectively, supporting this hypothesis. Accordingly, larger and less specialized taxa would attach only if the biofilm thickness and other parameters prove suitable for their growth. Currently it is still unclear whether taxa such as *A. elongata* or *C. alatus* are truly epizoic or less specialized and more opportunistic species. Interestingly, the *C. alatus* strain acquired from the National Center for Marine Algae and Microbiota (USA) was isolated from a sample collected in the equatorial Pacific Ocean, far from the continents. Limited by the information provided by NCMA, we can only speculate on the geographical range and ecological preferences of *C. alatus*. However, the fact that *C. alatus* was found in two different oceans and at different latitudes does not contradict the theory that diatoms found on sea turtles are sea turtle-borne and thus may live within the certain sea turtle population range, but are occasionally transferred to other sea turtle populations and species when single individuals forage in the same area, or mate (Caine 1986, Robinson et al. 2017, Majewska et al. 2017b).

Statistical analysis (ANOSIM) performed on diatom abundance data indicated that the epizoic diatom flora composition might be related to the host sea turtle species. However, due to the relatively low number of analysed sea turtles (three of each of the three sea turtle species found in Riverhead, NY) any general conclusions must be drawn with caution. Further studies are needed to shed more light on various aspects of the probably common, but still unexplored, diatom epibiosis on aquatic animals, including the

sea turtle-associated diatoms, their taxonomy, biogeography, and ecological preferences.

Museum specimens

This study shows how museum specimens of aquatic vertebrates can be used for epizoic diatom analysis, and thus contributes to our knowledge of epibiotic diatom biodiversity and biogeography. A similar approach was adopted by Robinson et al. (2016), who used museum specimens to show that diatoms are universally present on all existing sea turtle species, and Wu & Bergey (2017), who investigated freshwater and aerophilic diatoms on North American snapping turtles.

Although zoological museum collections may constitute an excellent source of unique and often very rich epizoic diatom material, there are several important limitations to such study. It must be highlighted that storage, handling, and preservation method of museum specimens will have a profound impact on surface-associated diatoms. Specimens in various zoological collections were not handled or preserved according to standard protocols used in diatom studies, and thus it should not be assumed that diatoms still present on those specimens' surface (if any) reflect the original diatom community structure, as many diatoms are likely to have been detached and lost. Therefore, in most cases, museum specimens cannot be used for quantitative assessments, contrary to the proposal of Wu & Bergey (2017), and detailed comparisons of diatom flora composition should only be made between samples preserved and stored in the same way. Moreover, the extraction method used to remove diatom frustules from the animal tissue surface may greatly affect not only absolute diatom abundances, but also their species composition. Thus, we propose tissue sonication as a highly efficient, non-destructive diatom extraction method that can be applied to many valuable specimens when museum policy precludes digestion with concentrated acids, a standard protocol for diatom studies.

Over the last few years, there has been a surge in interest in epizoic diatom studies, and specifically in sea turtle-associated diatoms, with several sea turtle diatom taxa being described. It is likely that more diatoms will be discovered in future surveys exploring various epizoic habitats based on both fresh, and museum, zoological specimens.

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed at https://doi.org/10.1080/0269249X.2018.1491426.

ORCID

Roksana Majewska ⬤ http://orcid.org/0000-0003-2681-4304

References

ASHWORTH M.P., LOBBAN C.S., WITKOWSKI A., THERIOT E.C., SABIR M.J., BAESHEN M.N., HAJARAH N.H., BAESHEN N.A., SABIR J.S. & JANSEN R.K. 2017. Molecular and morphological investigations of the stauros-bearing, raphid pennate diatoms (Bacillariophyceae): *Craspedostauros* E.J. Cox, and *Staurotropis* T.B.B. Paddock, and their relationship to the rest of the Mastogloiales. *Protist* 168: 48–70.

CAINE A.C. 1986. Carapace epibionts of nesting loggerhead sea turtles: Atlantic coast of U.S.A. *Journal of Experimental Marine Biology and Ecology* 95: 15–26.

CLARKE K.R. & GORLEY R.N. 2006. *PRIMER v6: user manual/tutorial*. PRIMER-E Ltd, Plymouth. 192 pp.

COOK J.A., CHUBB J.C. & VELTKAMP C.J. 1998. Epibionts of *Asellus aquaticus* (L.) (Crustacea, Isopoda): an SEM study. *Freshwater Biology* 39: 423–438.

COX E.J. 1999. *Craspedostauros* gen. nov., a new diatom genus for some unusual marine raphid species previously placed in *Stauroneis* Ehrenberg and *Stauronella* Mereschkowsky. *European Journal of Phycology* 34: 131–147.

DENYS L. 1997. Morphology and taxonomy of epizoic diatoms (*Epiphalaina* and *Tursiocola*) on a sperm whale (*Physeter macrocephalus*) stranded on the coast of Belgium. *Diatom Research* 12: 1–18.

DENYS L. & VAN BONN W. 2001. A second species in the epizoic diatom genus *Epipellis*: *E. heptunei* sp. nov. In: *Lange-Bertalot Festschrift. Studies on diatoms. Dedicated to Prof. Dr. Dr. h.c. Horst Lange-Bertalot on the occasion of his 65th birthday* (Ed. by R. JAHN, J.P. KOCIOLEK, A. WITKOWSKI & P. COMPÈRE), pp. 167–176. A.R.G. Gantner Verlag, Ruggell.

DI CAMILLO C., PUCE S., ROMAGNOLI T., TAZIOLI S., TOTTI C. & BAVESTRELLO G. 2005. Relationships between benthic diatoms and hydrozoans (Cnidaria). *Journal of the Marine Biological Association of the UK* 85: 1373–1380.

DUTE R.R., SULLIVAN M.J. & SHUNNARAH L.E. 2000. The diatom assemblages of *Ophrydium* colonies from South Alabama. *Diatom Research* 15: 31–42.

ESTES A. & DUTE R.R. 1994. Valve abnormalities in diatom clones maintained in long-term culture. *Diatom Research* 9: 249–258.

FERNANDES L.F. & CALIXTO-FERES M. 2012. Morphology and distribution of two epizoic diatoms (Bacillariophyta) in Brazil. *Acta Botanica Brasilica* 26: 836–841.

FRANCOEUR S.N., PINOWSKA A., CLASON T.A., MAKOSKY S. & LOWE R.L. 2002. Unionid bivalve influence on benthic algal community composition in a Michigan lake. *Journal of Freshwater Ecology* 17: 489–500.

FRANKOVICH T.A., ASHWORTH M.P., SULLIVAN M.J., VESELÁ J. & STACY N.I. 2016. *Medlinella amphoroidea gen. et sp. nov.* (Bacillariophyta) from the neck skin of loggerhead sea turtles (*Caretta caretta*). *Phytotaxa* 272: 101–114.

GILLAN D.C. & CADÉE G.C. 2000. Iron-encrusted diatoms and bacteria epibiotic on *Hydrobia ulvae* (Gastropoda: Prosobranchia). *Journal of Sea Research* 43: 83–91.

HASLE G.R. & SYVERTSEN E.E. 1997. Marine diatoms. In: *Identifying marine phytoplankton* (Ed. by C.R. TOMAS), pp. 5–385. Academic Press, San Diego.

HOLMES R.W. 1985. The morphology of diatoms epizoic on Cetaceans and their transfer from *Cocconeis* to two new genera, *Bennettella* and *Epipellis*. *British Phycological Journal* 20: 43–57.

HOLMES R.W. & CROLL D.A. 1984. Initial observations on the composition of dense diatom growths on the body feathers of three species of diving seabirds. In: *Proceedings of the Seventh International Diatom Symposium, Philadelphia, August 22–27, 1982* (Ed. by D.G. MANN), pp. 265–278. Koeltz Science Publishers, Königstein.

HOLMES R.W., NAGASAWA S. & TAKANO H. 1993. The morphology and geographic distribution of epidermal diatoms of the Dall's porpoise (*Phocoenoides dalli* True) in the Northern Pacific Ocean. *Bulletin of the National Science Museum Tokyo, Series B, Botany* 19: 1–18.

MACGILLIVARY M.L. & KACZMARSKA I. 2011. Survey of the efficacy of a short fragment of the *rbc*L gene as a supplemental DNA barcode for diatoms. *Journal of Eukaryotic Microbiology* 58: 529–536.

MADKOUR F.F., SALLAM W.S. & WICKSTEN M.K. 2012. Epibiota of the spider crab *Schizophrys dahlak* (Brachyura: Majidae) from the Suez Canal with special reference to epizoic diatoms. *Marine Biodiversity Records* 5: E64. doi:10.1017/S1755267212000437.

MAJEWSKA R., KOCIOLEK J.P., THOMAS E.W., DE STEFANO M., SANTORO M., BOLAÑOS F. & VAN DE VIJVER B. 2015a. *Chelonicola* and *Poulinea*, two new gomphonemoid diatom genera (Bacillariophyta) living on marine turtles from Costa Rica. *Phytotaxa* 233: 236–250.

MAJEWSKA R., SANTORO M., BOLAÑOS F., CHAVES G. & DE STEFANO M. 2015b. Diatoms and other epibionts associated with olive ridley (*Lepidochelys olivacea*) sea turtles from the Pacific coast of Costa Rica. *PLoS ONE* 10: e0130351. doi:10.1371/journal.pone.0130351.

MAJEWSKA R., DE STEFANO M., ECTOR L., BOLAÑOS F., FRANKOVICH T.A., SULLIVAN M.J., ASHWORTH M.P. & VAN DE VIJVER B. 2017a. Two new epizoic *Achnanthes* species (Bacillariophyta) living on marine turtles from Costa Rica. *Botanica Marina* 60: 303–318.

MAJEWSKA R., VAN DE VIJVER B., NASROLAHI A., EHSANPOUR M., AFKHAMI M., BOLAÑOS F., IAMUNNO F., SANTORO M. & DE STEFANO M. 2017b. Shared epizoic taxa and differences in diatom community structure between green turtles (*Chelonia mydas*) from distant habitats. *Microbial Ecology* 74: 969–978.

MAJEWSKA R., DE STEFANO M. & VAN DE VIJVER B. 2018. *Labellicula lecohuiana*, a new epizoic diatom species living on green turtles in Costa Rica. *Nova Hedwigia, Beihefte* 146: 23–31.

MCCLATCHIE S., KAWACHI R. & DALLEY D.E. 1990. Epizoic diatoms on the euphausiid *Nyctiphanes australis*: consequences for gut-pigment analyses of whole krill. *Marine Biology* 104: 227–232.

NCMA. https://ncma.bigelow.org [Accessed 11 February 2018].

NEMOTO T. 1956. On the diatoms of the skin film of whales in the Northern Pacific. *Scientific Reports of the Whales Research Institute, Tokyo* 11: 99–132.

PATIL S.J. & ANIL A.C. 2000. Epibiotic community of the horseshoe crab *Tachypleus gigas*. *Marine Biology* 136: 699–713.

RADEA C., LOUVROU I. & ECONOMOU-AMILLI A. 2008. First record of the New Zealand mud snail *Potamopyrgus antipodarum* J.E. Gray 1843 (Mollusca: Hydrobiidae) in Greece – notes on its population structure and the associated microalgae. *Aquatic Invasions* 3: 341–344.

RAMPEN S.W., SCHOUTEN S., PANOTO F.E., BRINK M., ANDERSEN R.A., MUYZER G, ABBAS B. & SINNINGHEDAMSTÉ J.S. 2009. Phylogenetic position of *Attheya longicornis* and *Attheya septentrionalis* (Bacillariophyta). *Journal of Phycology* 45: 444–453.

RIAUX-GOBIN C., WITKOWSKI A., CHEVALLIER D. & DANISZEWSKA-KOWALCZYK G. 2017a. Two new *Tursiocola* species (Bacillariophyta) epizoic on green turtles (*Chelonia mydas*) in French Guiana and Eastern Caribbean. *Fottea* 17: 150–163.

RIAUX-GOBIN C., WITKOWSKI A., KOCIOLEK J.P., ECTOR L., CHEVALLIER D. & COMPÈRE P. 2017b. New epizoic diatom (Bacillariophyta) species from sea turtles in the Eastern Caribbean and South Pacific. *Diatom Research* 32: 109–125.

ROBINSON N.J., MAJEWSKA R., LAZO-WASEM E., NEL R., PALADINO F.V., ROJAS L., ZARDUS J.D. & PINOU T. 2016. Epibiotic diatoms are universally present on all sea turtle species. *PLoS ONE* 11: e0157011. doi:10.1371/journal.pone.0157011.

ROBINSON N.J., LAZO-WASEM E.A., PALADINO F.V., ZARDUS J.D. & PINOU T. 2017. Assortative epibiosis of leatherback, olive ridley and green sea turtles in the Eastern Tropical Pacific. *Journal of the Marine Biological Association of the United Kingdom* 97: 1233–1240.

ROSS R., COX E.J., KARAYEVA N.I., MANN D.G., PADDOCK T.B.B., SIMONSEN R. & SIMS PA. 1979. An amended terminology for the siliceous components of the diatom cell. *Nova Hedwigia, Beiheft* 64: 513–533.

ROUND F.E. & ALEXANDER C.G. 2002. *Licmosoma* - a new diatom genus growing on barnacle cirri. *Diatom Research* 17: 319–326.

ROUND F.E., CRAWFORD R.M. & MANN D.G. 1990. *The diatoms: biology & morphology of the genera*. Cambridge University Press, Cambridge. 747 pp.

RUSSELL D.J. & NORRIS R.E. 1971. Ecology and taxonomy of an epizoic diatom. *Pacific Science* 25: 357–367.

SABBE K., VERLEYEN E., HODGSON D.A., VANHOUTTE K. & VYVERMAN W. 2003. Benthic diatom flora of freshwater and saline lakes in the Larsemann Hills and Rauer Islands, East Antarctica. *Antarctic Science* 15: 227–248.

VAN DE VIJVER B., MATALONI G., STANISH L. & SPAULDING S.A. 2010. New and interesting species of the genus *Muelleria* (Bacillariophyta) from the Antarctic region and South Africa. *Phycologia* 49: 22–41.

VAN DE VIJVER B., TAVERNIER I., KELLOGG T.B., GIBSON J.A., VERLEYEN E., VYVERMAN W. & SABBE K. 2012. Revision of type materials of Antarctic diatom species (Bacillariophyta) described by West & West (1911), with the description of two new species. *Fottea* 12: 149–169.

WETZEL C.E., VAN DE VIJVER B., COX E.J., BICUDO D.C. & ECTOR L. 2012. *Tursiocola podocnemicola* sp. nov.,

a new epizoic freshwater diatom species from the Rio Negro in the Brazilian Amazon Basin. *Diatom Research* 27: 1–8.

WINEMILLER K.O. & WINSBOROUGH B.M. 1990. Occurrence of epizoic communities on the parasitic copepod *Lernaea carassii* (lernaeidae). *The Southwestern Naturalist* 35: 206–210.

WU S.C. & BERGEY E.A. 2017. Diatoms on the carapace of common snapping turtles: *Luticola* spp. dominate despite spatial variation in assemblages. *PLoS ONE* 12: e0171910. doi:10.1371/journal.pone.0171910.

WUJEK D.E. 2013. Epizooic diatoms on the cerci of Ephemeroptera (Caenidae) naiads. *The Great Lakes Entomologist* 46: 116–119.