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Non-blue *Haslea* species (Bacillariophyceae: Naviculaceae) in the benthic marine flora of Guam (Mariana Islands, Western Pacific Ocean).

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In recent years the taxonomy of *Haslea* has been in flux. We describe several *Haslea* taxa from Guam, including four new species, in light of these recent findings. Four delicate, fusiform *Haslea* species are described from a variety of benthic habitats on coral reefs. These species, *H. fusiformis* comb. nov. and *H. alexanderi, H. apoloniae* and *H. guahanensis* spp. nov. are distinguished by stria density, foramen ('areola') shape, and cell size from each other and from similar taxa. A key to all these taxa is provided. In addition, an arcuate species is described as *Haslea arculata* sp. nov. The presence of a bilayered wall is shown for all these taxa. We compare the morphology of specimens reported as *H. howeana* from Guam with *Navicula tsukamotoi*, recently described from Okinawa, and with '*Navicula* cf. *howeana*' from Puerto Rico. We provide the first account of the complex cingulum of *N. tsukamotoi*. We conclude that our specimens match *N. tsukamotoi* and that *H. howeana* should be returned to *Navicula*, but we leave open the question of conspecificity.

Keywords: *biodiversity, copulae, coral reefs, diatoms, Mariana Islands, Naviculales*

Introduction

The diatom genus *Haslea* Simonsen (1974) (formerly *Navicula* section *Fusiformes*) is widespread in benthic and planktonic habitats. It was recently shown to be more diverse than previously thought and to be structurally related to *Pleurosigma* W. Smith and *Gyrosigma* A. Hassall (Sterrenburg et al. 2015). Morphologically the genus was redefined by Sterrenburg et al. (2015) on the basis of a double-layered wall structure, comprising an inner (basal, ontogenetically earlier), grid-like layer separated from the outer (tegumental) layer of longitudinal strips by parallel, perforated walls they termed saepes. However, Li et al. (2017) showed that genetically some species placed in *Haslea*, including two of those by Sterrenburg et al. (2015), belong in *Navicula* Bory, and that '[the] presence of continuous longitudinal strips on the external valve surface is not a synapomorphy, but rather a homoplastic character for *Haslea*.' Unfortunately, saepes are difficult to observe, e.g. where suitable breaks in the valves expose them, and indeed for the species transferred by Li et al. (2017), Sterrenburg et al. (2015) noted that they had not observed them.

Several other characters differ between *Haslea* and *Navicula*, though none is synapomorphic for *Haslea*. For example, in *Haslea* the raphe is overhung by an axial costa, which can be seen twisting over the raphe slit near the apex (Reid 2012: 52 and pl. 7, Fig. 3) (superb cross-sectional views in Poulin et al. 2004) and the central area in *Haslea* is flanked by short accessory ribs or flaps (Schrader 1973, Sterrenburg et al. 2015, Li et al. 2017), similar to those in *Pleurosigma* and *Gyrosigma*, but absent in *Navicula*.

Haslea includes a subset of very similar taxa that are weakly silicified and lack a pseudostauros; Hustedt (1961) distinguished these on the basis of size and stria densities (when discernable) plus the rather dubious criterion of geographic provenance. The best known is the type species, *Haslea ostrearia* (Gaillon) Simonsen (recently typified by Poulin et al. 2019), the 'blue diatom' coloured by marennine in its vacuoles (Nassiri et al. 1998, Pouvreau et al. 2006), which is famous for producing a desirable colour in oysters. Blue pigment is a very distinctive feature of *H. ostrearia*, but more recently three new species, *H. karadagensis* Davidovich, Gastineau & Mouget, *H. provincialis* Gastineau, Hansen & Mouget and *H. nusantara* Mouget, Gastineau & Syakti, morphologically very similar but molecularly distinct, have been reported to contain a different blue pigment, as were further, morphologically distinct blue *Haslea* species (Gastineau et al. 2012, 2014a, 2014b, 2016, Prasetiya et al. 2019).

Also in this group is one of the least-known taxa, *Navicula fusiformis* Grunow [non *Navicula fusiformis* Ehrenberg; see VanLandingham 1975]. Grunow (1877) included

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Navicula ostrearia (Gaillon) Turpin in Bory as a variety of *N. fusiformis*, but Sauvageau (1907) treated *N. fusiformis* as a variety of *N. ostrearia* (the latter being the older name), and Hustedt (1961) maintained each at species rank. In erecting *Haslea*, Simonsen (1974) reduced *N. fusiformis* to a synonym of *H. ostrearia*. Apart from Grunow's record from Honduras, Hustedt (1961) noted only a few observations by himself from Dubrovnik. The hyaline appearance of the valves in LM makes it difficult to distinguish *N. fusiformis* from *H. ostrearia* except by its larger size. Hustedt's (1961) systematic remarks on the two forms, to which Simonsen (1974) referred in making the synonymy, also note that it is not known whether *N. fusiformis* has blue pigment.

We found several species of this group in Guam, two of which were similar to *N. fusiformis*, and in trying to resolve their identities, we began by seeking unmounted type material. In this paper, we describe our findings from these samples and propose four new species of lanceolate *Haslea* and a new combination for *N. fusiformis*. In addition, we investigated a commonly observed arcuate cell with biarcuate raphe branches, which resembled *Haslea* in the outer covering of longitudinal strips. The variety of valve shapes within *Haslea*, as depicted by Sterrenburg et al. (2015) could encompass this form, if it had the appropriate valve structure. For this species we were able to get gene sequences, but most of our attempts to culture Guam *Haslea* species have not been successful. Finally, a species previously identified as *Haslea howeana* (Hagelstein) Giffen is common in Guam (Lobban et al. 2012) but appeared very similar to *Haslea tsukamotoi* Sterrenburg, which Li et al. (2017) transferred to *Navicula* and also needs reconsidering in the light of these recent studies.

Methods

Raw samples were collected from intertidal and subtidal reefs around Guam (Table 1) and processed for LM and SEM using standard laboratory protocols (Lobban 2015). In brief, samples preserved in formalin were rinsed, boiled with nitric acid and rinsed to neutrality. Drops of the resultant suspension were dried onto cover slips for LM and cellulose nitrate filters for SEM. LM observations were made with a Nikon 80*i* microscope with differential interference contrast and phase contrast illumination, SEM observations were made with a desktop Phenom G2 Pro (PhenomWorld US, Hillsboro, OR, USA).

In searching for type material that could be examined in SEM, we were fortunate that David Mann had visited the Grunow collection in Vienna and written that for *Navicula*

> "the present Curator of Cryptogams, Dr. Anton Igersheim, has been through all the folders, opened the envelopes to find out which sample is present, and stamped the envelope with the sample number. Furthermore, an Excel spreadsheet is available that lists the *Navicula* envelopes and gives their contents, and this is an invaluable resource. (Mann 2009)"

Dr. Igersheim was able to loan us several packets containing residual material from the Honduras *Sargassum* samples, though none of it was marked as containing *N. fusiformis*. The SEM stubs are stored in the UGUAM Diatom Herbarium.

Type slides were deposited at the Academy of Natural Sciences of Drexel University, Philadelphia (ANSP).

DNA extraction, sequencing and molecular phylogenetic analysis

Strains cultured in this study were isolated from wild material, outlined in Table S1. Strains from Guam were isolated into f/2 media and grown in a Percival model I-36LL incubator at 27°C in a 12:12 h light: dark photoperiod. Harvested material from cultures was stored in pellets made after centrifugation in an Eppendorf 5415C centrifuge (Eppendorf North America, Hauppauge, NY, USA) for 5 min at 10,000 rpm. For DNA extraction, the QIAGEN DNeasy Plant Mini Kit (QIAGEN Sciences, Valencia, California, USA) was used, with initial cell disruption by 1.0 mm glass beads in a Mini-Beadbeater (Biospec Products, Inc, Bartlesville, OK, USA) for 45 sec. PCR-based DNA amplification and di-deoxy Sanger sequencing of small-subunit nuclear rRNA and the chloroplast-encoded rbcL and psbC markers followed Theriot et al. (2010).

Phylogenetic analysis of the three-gene dataset nuclear-encoded small subunit (SSU) rRNA, and plastid-encoded *rbc*L and *psb*C followed the molecular phylogenetic methods outlined in Lobban et al. (2019). The dataset was constructed from the pennate dataset used in Sabir et al. (2018), with additional representatives of the Naviculaceae added from GenBank and the strains isolated as above. The final dataset contained 411 taxa of araphid and raphid pennate diatoms, using '*Asterionellopsis socialis* HK181' as an outgroup. SSU sequences were aligned using the program SSUalign (Nawrocki et al. 2009), with the covariance model based on the diatoms included in the download, plus secondary structure models from an additional 23 diatoms found on the CRW website (Cannone et al. 2002). SSU and plastid sequences were concatenated into a single matrix, which is available in the Supplementary Data as Appendix S1. We created eight partitions for the data (SSU paired sites, SSU unpaired sites, and the first, second and third codon positions of each of *rbcL* and *psbC*). PartitionFinder results (Lanfear et al. 2014) suggested combining first and second codon positions with SSU paired and unpaired sites, and combining the third positions as separate partitions (BIC criterion). This dataset and partitioning scheme was run using the GTR + G model under maximum likelihood using RAxML ver. 8.2.7 (Stamatakis 2014) compiled as the pthread-AVX version on an Intel i7 based processor under Xubuntu 14.4. We ran 20 replicates, each with 500 rapid BS replicates, with ML optimizations. Bootstrap

Table 1. List of raw materials in which *Haslea* species were observed.

Sample	Locality	Sample notes[a]	*Haslea* spp.
GU7AA-4	UOG Marine Lab	Floating algal mat on seawater flow-through tank, coll. C. Lobban, 23 Feb 2015	*H. arculata*
GU43C	Tagachang reef flat, Yona	Epiphytes on green algal filaments in a very shallow pool, inner edge of reef flat, coll. 22 Sep. 2012	*H. alexanderi*
GU44BF-1A	Gab Gab reef, Apra Harbor	Sparse algal turf in farmer-fish[b] territory ca. 5 m deep, coll. 12 Oct. 2014	*H. apoloniae*
GU44BK-6	Gab Gab reef	Dense algal turf in farmer-fish[c] territory 1 m deep, coll. 30 Mar. 2015	*H. apoloniae, H. arculata*
GU44BV-1	Gab Gab reef	*Halimeda* with fringe of diatoms, ca. 10 m deep, coll. 28 Oct. 2017	*H. fusiformis*
GU52K-7	Outhouse Beach, Apra Harbor	Dense red algal turf in farmer-fish[c] territory 1 m deep, coll. 3 May 2009	*H. apoloniae, H. arculata*
GU52P-9	Outhouse Beach	Dense red algal turf in farmer-fish[c] territory ca. 3 m deep, coll. 25 Apr. 2011	*H. guahanensis, H. alexanderi*
GU52X-1	Outhouse Beach	Long, dense red algal turf in farmer-fish[c] territory ca. 3 m deep, coll. 10 May 2015	*H. apoloniae, H. arculata, H. alexanderi*
GU66F-8	Gab Gab II reef, Apra Harbor	Red algal mat from base of blue sponge in farmer-fish[b] territory 16 m deep, coll. 6 Apr. 2014	*H. guahanensis*
GU66G-2	Gab Gab II reef	Sparse algal turf on lettuce coral in farmer-fish[b] territory 16 m deep, coll. 9 Nov. 2014	*H. apoloniae, H. arculata*
GU66H-1	Gab Gab II reef	Sparse algal turf on brown sponge in farmer-fish[b] territory at 15 m deep, coll. 14 Dec. 2014	*H. fusiformis, H. apoloniae, H. alexanderi*
GU83A-1	Family Beach, Apra Harbor	Loosely associated with *Galaxaura* (red seaweed), coll. R.W. Jordan, 11 Mar. 2019	*H. apoloniae, H. guahanensis*

[a] All collections by C.S. Lobban and M. Schefter, except as noted.
[b] *Plectroglyphidodon lacrymatus.*
[c] *Stegastes nigricans.*

support was assessed utilizing the BS replicates from the run producing the optimal ML score.

Terminology. We follow Sterrenburg et al. (2015) and Li et al. (2017). As noted in the Introduction, the structure of *Haslea* comprises an inner basal layer in the form of a grid, and an outer layer of tegumentary strips, the layers held apart by perforated longitudinal walls called saepes. Since the walls are only longitudinal, the structure is not loculate, and what appear to be areolae in LM are in fact the spaces in the grid, i.e. foramina. In some *Haslea* spp. a few central virgae are thickened to form a pseudostauros – the correct term for this rather than stauros (as used by Hustedt 1961) (Massé et al. 2001, Cox 2012).

Morphological criteria for determining generic allocation

In addition to the bi-layered wall with saepes (Sterrenburg et al. 2015), a combination of other characters (none individually synapomorphic) also distinguish *Haslea* and *Navicula*, and are more easily seen:

(1) internal aspect of the central area with asymmetrical flanking bars present in *Haslea* (more prominent on the side with the axial costa), absent in *Navicula*.
(2) raphe partially overhung by an axial costa, and showing a characteristic twist where the overhang ends near the helictoglossa, (as noted by Reid 2012: 52, pl. 7, Fig. 3).
(3) the helictoglossa is markedly elongated where it emerges beyond the axial costa, whereas, in the species that Li et al. (2017) transferred to *Navicula* the helictoglossa is very short and supported by lateral thickenings.

Results

Sequence data

The 3-gene DNA sequence phylogeny resolved a monophyletic Naviculaceae (1; see Supplemental Fig. S1 for complete tree), though without particularly strong support (bootstrap support [bs] = 70%). The Naviculaceae was sister (bs = 98%) to a clade containing the Plagiotropidaceae and Pleurosigmataceae. Within the Naviculaceae, *Haslea* sensu stricto was monophyletic (bs = 100%) and sister to the other Naviculaceae (bs = 70%). One of the taxa described here, *Haslea arculata*, was sister to a strain of *H. pseudostrearia* Massé, Rincé & E.J. Cox (bs = 82%).

The 'hasleoid' *Navicula* species, *N. avium* (Tiffany, Herwig & Sterrenburg) Yuhang Li & Kuidong Xu and

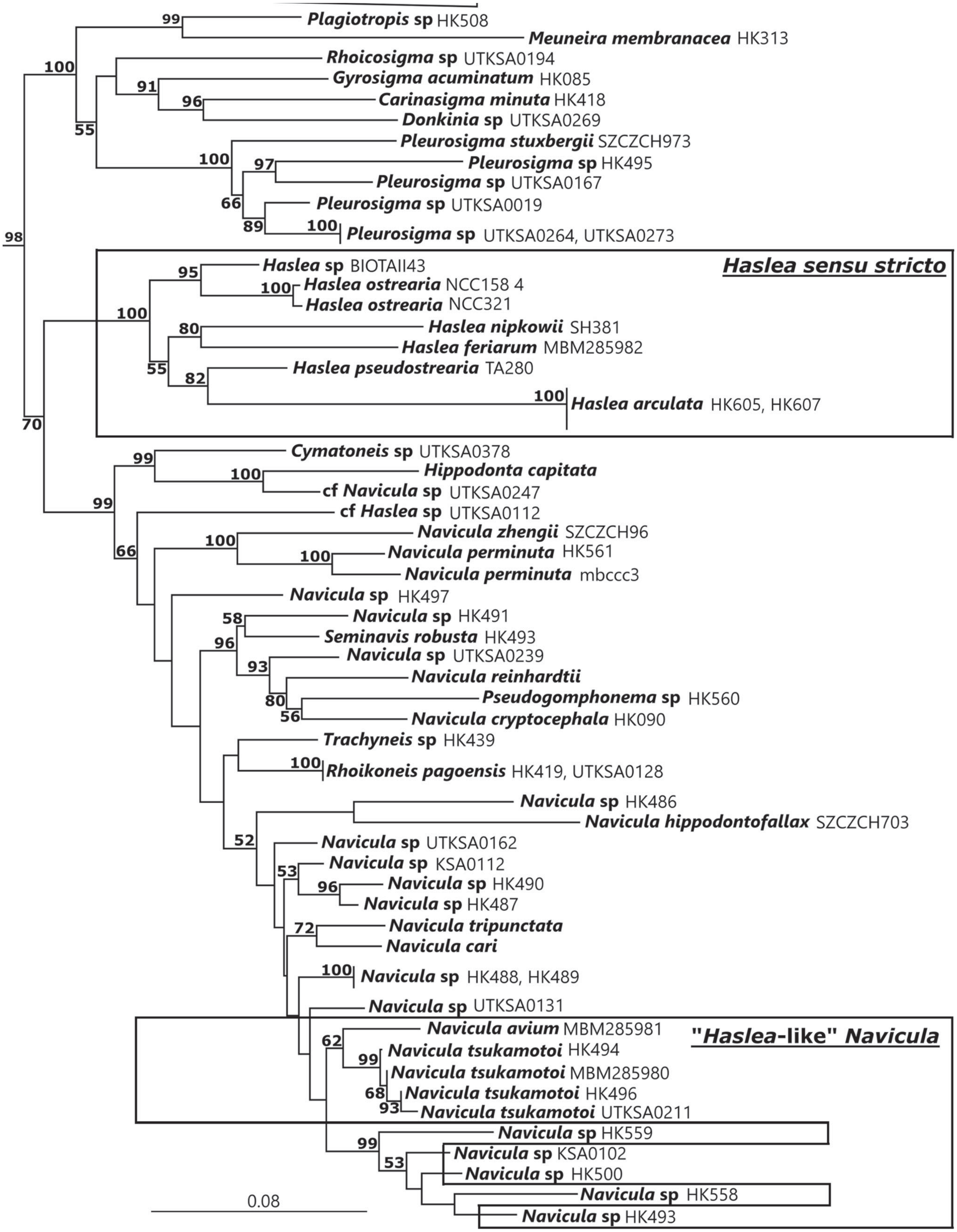


Fig. 1. Phylogenetic tree from the RAxML analysis of the 3-gene dataset created for this manuscript. Only the clade representing the Naviculaceae (and the immediate sister clade) is presented here; the complete tree is displayed in Figure S1. Bootstrap support values > 50% shown at nodes. The *Haslea sensu stricto* clade is boxed for clarity, as are the *Navicula* strains which show a '*Haslea*-like' valve structure.

Table 2. Comparison of characters of new *Haslea* and related delicate, fusiform species with perpendicular striation and lacking a pseudostauros.[a]

Character	Length (μm)	Width (μm)	Transapical striae in 10 μm	Longitudinal striae in 10 μm (approx.)	Apparent foramen shape
H. fusiformis Grunow (data from Hustedt)	115–150	8.5–10	33	36	square
H. fusiformis from Grunow packet 839–4611	c. 112–125	c. 9–11	33–34	32	square
H. fusiformis from Guam	103–132	8–10	31	35–36	± square
H. alexanderi n. sp., subtidal	228–366	27–40	24–26	29–30	± square
H. alexanderi n. sp., tidal pool	145–200	21–32	24–26	30–33	± square
H. apoloniae n. sp.	40–83	6–12	33–35	40–45	Apically rectangular ± square
H. guahanensis n. sp.	107–145	9–13	28–31	42–49	Apically rectangular
H. clevei Hinz, Hargraves & Sterrenburg	85–110	10–12	23	30	± square
H. crystallina (Hustedt) Simonsen	106–155	12–15	26	30	± square
H. frauenfeldii (Hustedt) Simonsen	100–190	17–25	29	29	Exactly square
H. gigantea var. *gigantea* (Hustedt) Simonsen	300–410	32–48	16–17	20–22	± square
H. gigantea var. *tenuis* von Stosch	180–315	18–23	16.5–18.5	23.5–27.0	Apically rectangular
H. gretharum Simonsen	150–200	19.5–22	18–19	36–40	Strongly apically rectangular
H. hyalinissima Simonsen	45–60	5–7	27–28	Not resolved but > 30	Unknown, but probably apically elongate
H. ostrearia (Gaillon) Simonsen	68–69	6.5–7.5	36	53	Strongly apically rectangular
H. pseudostrearia Massé, Rincé and Cox	37–43	11.0–12	17	20	square
H. trompii (Cleve) Simonsen	70–160	10.0–14	28–30	17–20	Transapically rectangular

[a] Arrangement: Following the organization of the text, the various sources of information for *H. fusiformis* are given first, followed by the new Guam taxa in alphabetical order, then similar species from the literature in alphabetical order, with the omission of *H. kjellmannii* and *H. vitrea* (Arctic species).

[b] Data for *H. gigantea* are the corrected data from Simonsen 1974.

N. tsukamotoi, are sister to a clade of unnamed *Navicula* species, some of which have 'hasleoid' valve morphology (HK493, HK500, KSA0102), others 'normal' naviculoid morphology (HK558, HK559).

Morphology and taxonomy

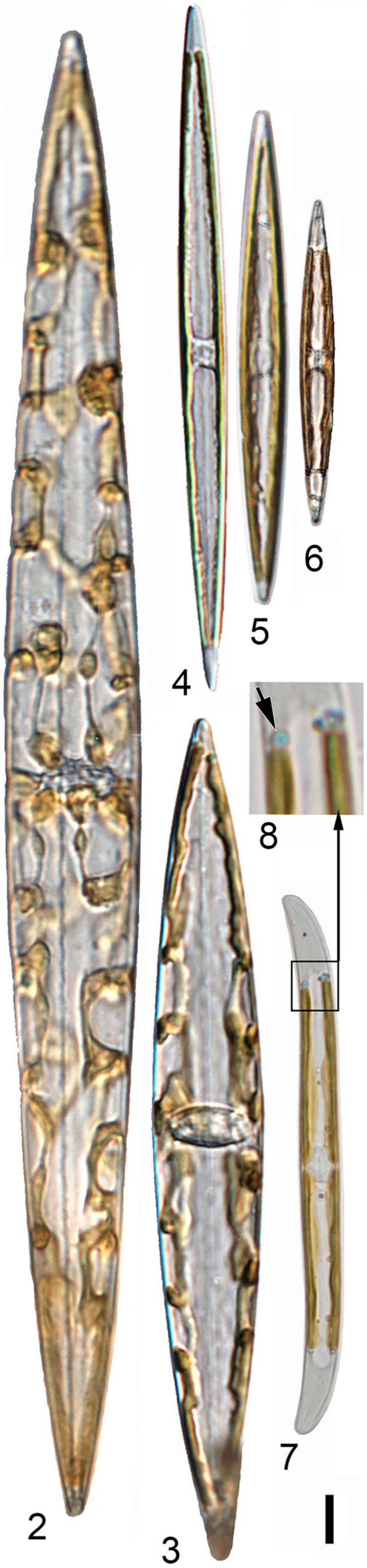
Species displayed a wide range in size and two plastid arrangements (Figs 2–8; Table 2). Some specimens from Guam conformed to *N. fusiformis* based on Grunow's (1877), and Hustedt's (1961) descriptions and drawings, and the few fragments we observed in SEM from Grunow's *Sargassum* material, being aware that he also reported *N. ostrearia* from this material. We confirmed the bilayered wall structure and propose the transfer of Grunow's taxon to *Haslea*.

Four new fusiform species were distinguished in the Guam samples. A comparison of these species and similar ones is given in Table 2, and we also provide a key to this subset of the genus. The curved species had several characters that indicated it belonged in *Haslea*. Guam specimens previously identified as *H. howeana* did not have a bilayered wall and most closely resembled *N. tsukamotoi*; they are described under that name.

***Haslea fusiformis* (Grunow) Lobban & C.O.Perez stat. nov., comb. nov.** Fig 5, Figs 9–17

References: Grunow 1877, p. 178, pl. 195: Fig. 11 (a-b). Hustedt 1961, p. 39, Fig. 1192b (as *N. fusiformis*).

Description: Based on Grunow packet 839–4611 (Fig. 13) and Guam samples GU66H-1, GU44BV-1 and GU44BV-3, valves narrowly lanceolate with acute apices, 102–125 µm long, 9–11 µm wide (Figs 9–10; Table 2), two plate-like plastids on the girdle sides, no evidence of blue pigment (Fig. 5). Foramina ('areolae') square and arranged in perpendicular rows (Figs 13–17), transapical striae 33–34 in 10 µm, longitudinal striae 32 in 10 µm. Dimensions as given by Hustedt (1961), 115–150 µm long by 8.5–10 µm wide, transapical and longitudinal striae 33 and 36 in 10 µm, respectively (Table 2). Externally, the tegumentary strips cover the entire surface, the marginal strips tapering and ending as the apex narrows (Figs 11–12); the



Figs 2–8. Live specimens. Figs 2–3. *H. alexanderi* larger cell from subtidal habitat, smaller cell from shallow reef-flat pool; lobed ribbon-like plastids. Figs 4–6. *H. guahanensis*, *H. fusiformis* and *H. apoloniae,* straight cells with a plate-like plastid along each girdle face. Figs 7–8. *H. arculata*, curved valve with plate-like plastids and small vacuoles of blue pigment at the tips of the plastids (Fig. 8, arrow). Scale bar = 10 µm.

external central raphe endings slightly deflected and ending in a large pore (Fig. 12). Internally, the raphe was overhung by a rib along the sternum, which had the characteristic twist near the apices, before the long terminal helictoglossa, which was not supported by lateral thickenings (Figs 13–16). Elevated small central bars on both sides of the internal central nodule were observed in both the Grunow and Guam material (Figs 13–15). Evidence of the saepes is suggested by the rows of peg-like structures seen where the integumentary strips were torn off or bent aside (Fig. 17 arrows).

Haslea alexanderi **Lobban & Perez sp. nov.** Figs 3, 18–28

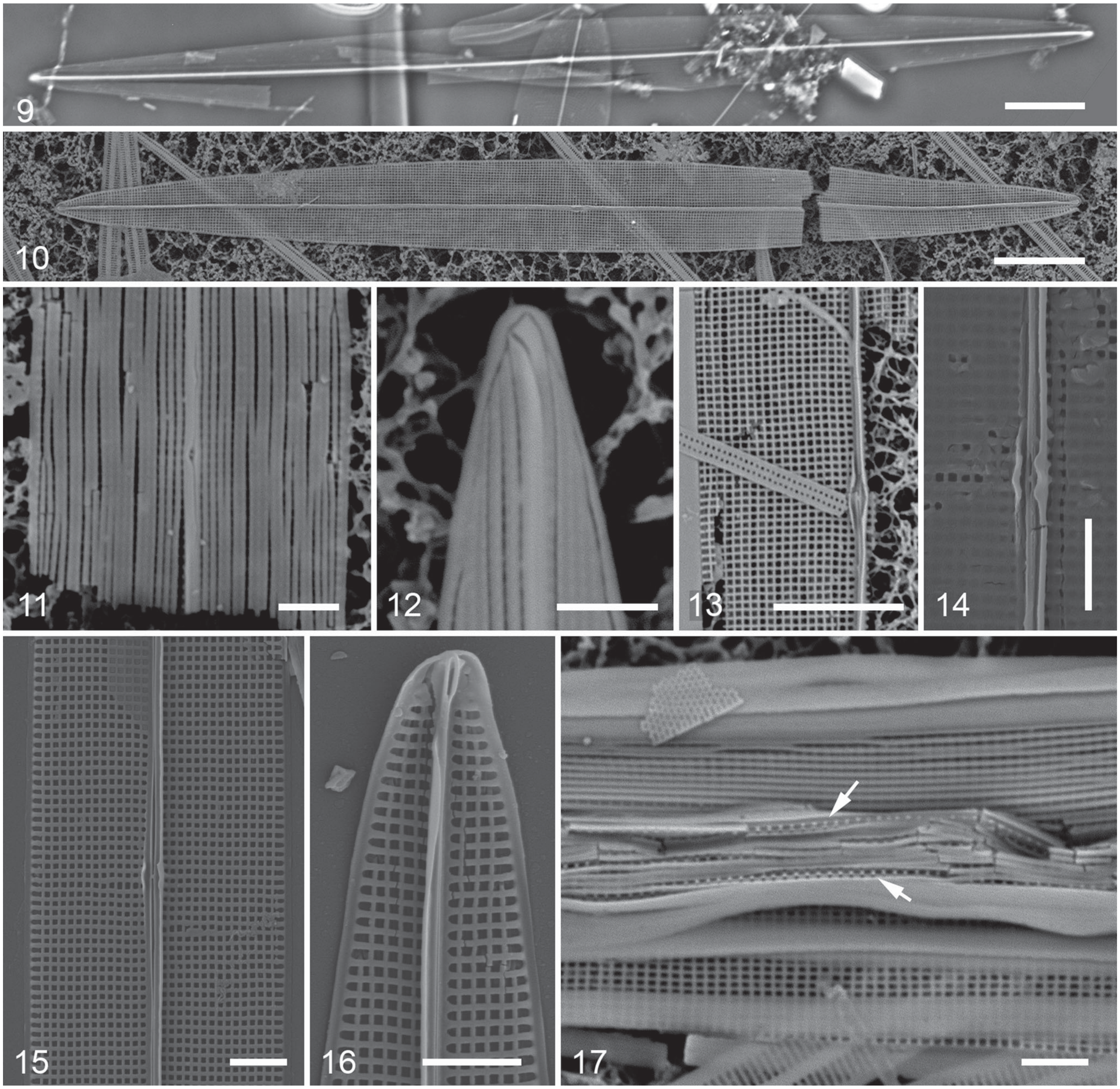
Diagnosis: Differing from *H. gigantea* Hustedt in its smaller size (ranges overlap), plastid type, finer transverse striae, and benthic habitat, and from *H. frauenfeldii* Grunow in its greater width and unequal transverse and longitudinal stria densities, i.e. foramina slightly rectangular.

Holotype: GU66F-8, slide 1417, specimen at 20.0 mm E, 13.0 mm S of the mark on the slide (Fig. 18); deposited at ANSP, accession number ANSPGC36368.

Type locality: GabGab II reef, Apra Harbor, Guam, 13.444 N, 144.644 E. Sample details in Table 1.

Other materials examined: GU52P-9, GU52X-1, GU66H-1, GU52AD-1, TK28

Description: Very large, delicate cells, lacking a pseudostauros. Valves broadly lanceolate with acute apices, subtidal populations 228–366 µm long (mean 316; $n = 19$), 27–40 µm wide (mean 31, $n = 19$) (Figs 2, 18–24). A population from a shallow sandy pool on the inner reef flat at Taga'chang was substantially smaller (Figs 3,29), 145–200 µm long (mean 182, $n = 32$), 21–32 µm wide (mean 27, $n = 31$) but otherwise indistinguishable (Figs 25–28, Table 2). Two (?) lobed, ribbon-like plastids in valve view (Figs 2, 3). longitudinal and transapical striation visible with light microscopy (Fig. 19). Transapical striation parallel throughout, 24–26 in 10 µm (mean 25; $n = 9$), longitudinal striation 29–30 µm (mean 29; $n = 14$). Saepes observed externally in broken section with SEM (Fig. 21, arrow), central raphe endings slightly deflected, very close, and slightly expanded (Fig. 20), terminal raphe ending straight (Fig. 22). Internally, the basal grid nearly quadrate, foramina smaller near the central nodule (Figs 23–24, 27). Raphe straight; the raphe ridge was partially tilted, except near the centre and at the apices (Figs 23–24; 27, 28), central area flanked by bilobed flaps of silica (Figs 23, 27). A representative specimen of the pool population (GU43C) can be found on slide 2765 at 20.2 mm E and 11.7 mm S of the mark; deposited at ANSP, accession number ANSPGC36365 (Fig. 25).



Figs 9–17. *Haslea fusiformis*; Guam specimens, except Fig. 13. Figs 9–10. whole valves in LM (phase contrast) and SEM. Figs 11–12. External views of apex and central area. Fig. 13. Fragment of valve and cingulum from Grunow 839-4611, internal view showing basal layer with square foramina and central area. Figs 14–16. High-resolution images from cultured clone showing internal central areas and apex. Fig. 17. Broken frustule showing saepes (arrows). Scale bars: Figs 9–10 = 10 µm, Fig. 13 = 5 µm, Figs 11–12, 14–17 = 2 µm.

Etymology: Named in remembrance of Lobban's grandfather, Alexander Lobban (1877–1949), son of Rev. Alexander Lobban (1847–1903), and three other Alexanders in his fatherline, back to 1702.

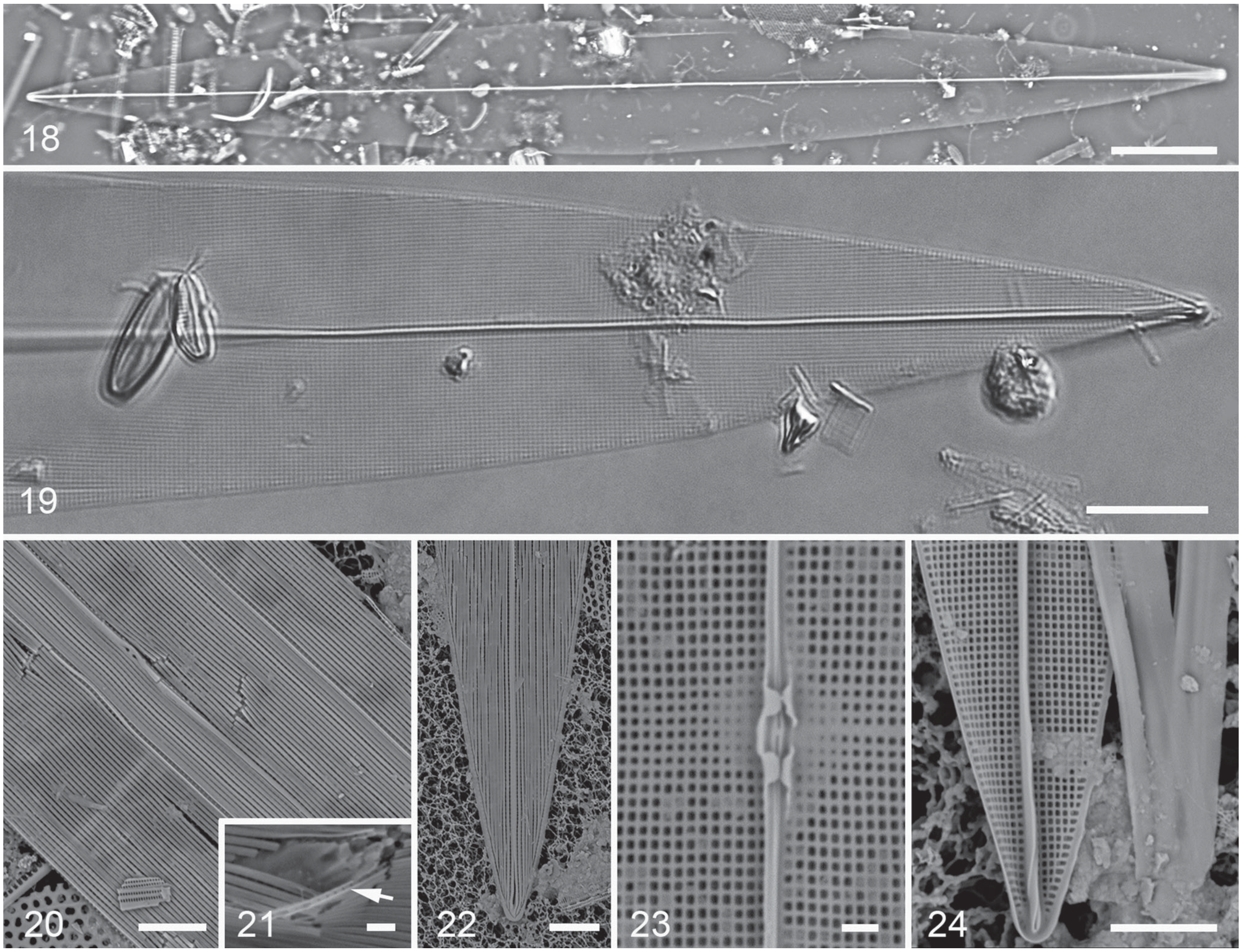
Haslea apoloniae **Lobban & C.O.Perez sp. nov.** Figs 6, 30–37

Diagnosis: Differing from *H. hyalinissima* Simonsen in the broader valve and higher transverse stria density (longitudinal stria density of *H. hyalinissima* not resolved), and from *H. guahanensis* sp. nov. (see below) in the shorter valves and higher transverse stria density.

Holotype: GU44BK-6, slide 1828 specimen at 18.8 E and 11.1 S of the mark; deposited at ANSP, accession number ANSPGC36366 (Fig. 30).

Type locality: GabGab Reef, Apra Harbor, Guam, 13.443 N, 144.643 E. Sample details in Table 1.

Etymology: Named in memory of Perez' grandmother, Apolonia Orca (1941–2006).



Figs 18–24. *Haslea alexanderi*, n. sp., subtidal population, SEM except Figs 18–19. Fig. 18. Holotype, phase contrast. Fig. 19. Apex of a valve, showing striae; DIC. Figs 20, 22. External views of central portion of valve and apex in SEM, showing tegumentary strips. Fig. 21. Detail of broken valve showing bilayered wall structure (arrow). Figs 23–24. Internal view of central area showing central bars and of apex. Scale bars: Fig. 18 = 25 µm, Fig. 19 = 10 µm, Figs 20, 22, 24 = 5 µm, Fig. 21, 23 = 1 µm.

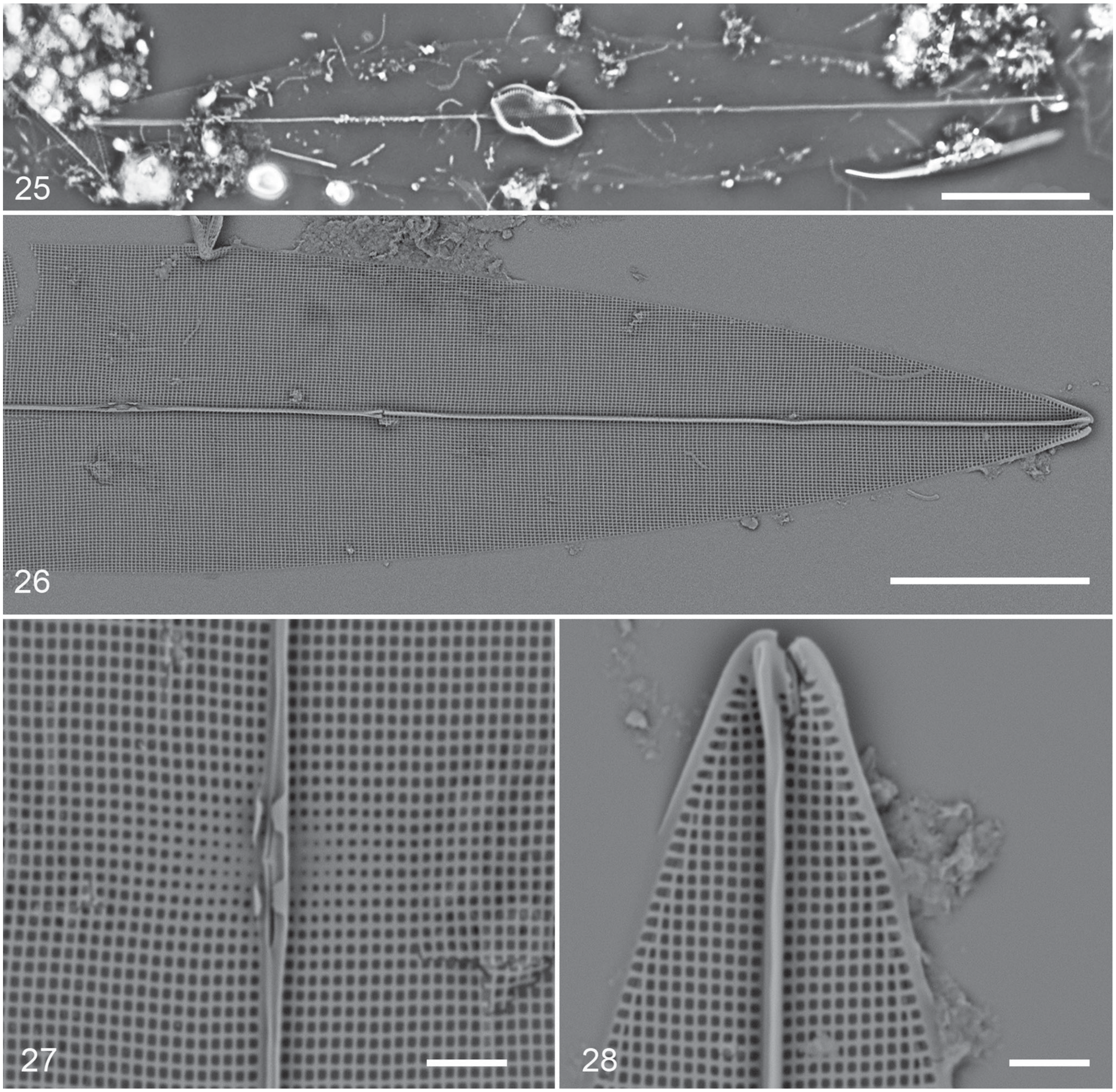
Other material examined: GU52X-1, GU66H-1, GU44BK-6, GU52K-7; GU44BF-1A

Description. Delicate fusiform cells, lacking a pseudostauros. Valves are narrowly lanceolate with acute apices (Figs 6, 30, 31), 40–83 µm long 27–40 µm wide. Two band-like plastids that do not extend to the apex, lie appressed to the girdle faces (Fig. 6). Striae perpendicular, not resolved in LM (Figs 30–31), transapical striae 34–35 in 10 µm, longitudinal striae 40–44 µm. Wall of two layers, separated by apical lines of saepes (observed in a torn specimen, Figs 32–33, and in external view, Fig. 34). Tegumental (outer) layer of delicate longitudinal silica strips parallel to the raphe that merge with peripheral slits at times (Figs 34–35). Basal layer a grating of apically elongated rectangular foramina (Figs 36–37). Raphe straight. Raphe fissures in external view slightly undulating towards the centre, raphe endings simple, slightly deflected to one side (Fig. 35). Terminal raphe fissures apparently long and curved (Fig. 34). Raphe ridge deflected except at the centre and at the apices; central nodule flanked by small silica bars (Fig. 36). An accessory ridge parallels the raphe (Figs 36–37); lateral rib curled near apex and long helictoglossa (Fig. 37).

Haslea guahanensis **Lobban & C.O.Perez, n. sp.** Figs 4, 38–44

Diagnosis: Differing from *H. apoloniae* in the longer valves and lower transverse stria density, and from *H. gretharum* Simonsen in the much shorter and narrower valves and finer transverse striae.

Holotype: GU52P-9, slide 826, specimen at 18.8 mm E and 10.6 mm S of the mark (Fig. 38); deposited at ANSP, accession number ANSPGC36367.



Figs 25–28. *Haslea alexanderi*, n. sp., pool population, SEM except Fig. 25. Fig. 25. Valve, LM phase contrast. Fig. 26. Half of a valve in internal view. Figs 27–28. Internal views, details of apex and central area. Scale bars: Fig. 25 = 25 µm, Fig. 26 = 10 µm, Figs 27–28 = 2 µm.

Type locality: 'Outhouse Beach,' Apra Harbor, Guam, 13.464 N, 144.656 E. Sample details in Table 1.

Etymology: Named for the island of Guam, Guahan in the indigenous language.

Other materials examined: **GU66F-8,** GU83A-1

Description: Delicate fusiform cells, lacking a pseudostauros. Valves narrowly lanceolate with acute apices, 107–145 µm, long, 9–13 µm wide (Figs 4, 38), two ribbon-shaped plastids (Fig. 4). Foramina apically rectangular, transapical striation 28–31 in 10 µm, longitudinal striation 42–49 in 10 µm, not resolved in LM (Figs 38–41, 43). External raphe straight, undulating toward the centre, raphe endings deflected to one side (Fig. 42, arrow). Internal raphe endings straight, with a small elevated flap on each side (Figs 40, 41), terminal endings in a twisted helictoglossa (Fig. 39). Evidence for the saepes can be seen where a section of tegumental strip has been torn away (Fig. 43–44 arrow): the saepes appear as a series of pegs along the longitudinal line of the basal grid (cf. Sterrenburg et al. 2015, Fig. 56).

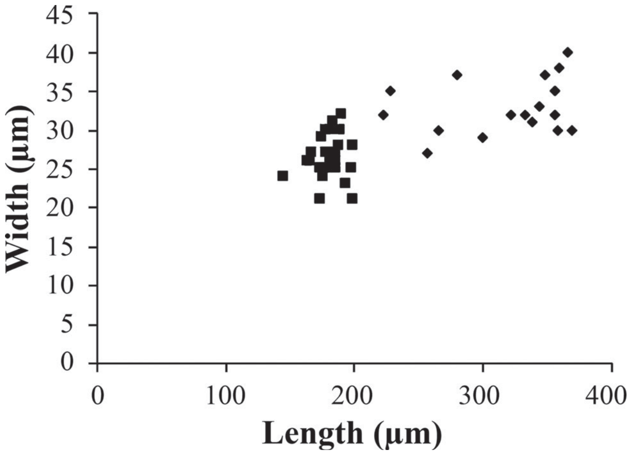


Fig 29. Size ranges of *Haslea alexanderi* populations from Apra Harbor subtidal (diamonds) and Taga'chang shallow reef flat pool (squares).

***Haslea arculata* Lobban & Ashworth, n. sp.** Figs 7–8, Figs 45–49

Diagnosis: Distinguished from all other *Haslea* spp. by the arcuate valve outline and bi-arcuate raphe path.

Holotype: Specimen at 18.6 E and 13.6 S of the mark on slide 1828, deposited at ANSP, accession number ANSPGC36366 (Fig. 45).

Type locality: GabGab Reef, Apra Harbor, Guam, 13.443 N, 144.643 E, sample GU44BK-6. Dense filamentous algal turf in farmer fish (*Stegastes nigricans*) territory, 2 m depth. Coll. C.S. Lobban and M. Schefter, 30 Mar. 2015.

Etymology: From *arculus*, a small bow, + *-atus*, likeness.

Other materials examined: GU7AA-4, GU52X-1, GU66G-2, GU66H-1, GU52K-7.

Description: Cells highly mobile, two plate-like plastids along the girdle faces, a blue pigment in tiny vacuoles at the polar ends of the plastids (Figs 7–8). Valves, lacking a pseudostauros, slightly arcuate with the apices deflected in the same direction, 86–109 µm long, 7–7.7 µm wide (Figs 45–46); striae not resolved in LM, transverse striae 32 in 10 µm, longitudinal striae 73 in 10 µm (Figs 47–48). Raphe-sternum bi-arcuate, curving from near the 'dorsal' side at the apices to the 'ventral' side at the central area (Figs 45–46); the raphe slit overhung by the axial costa (Figs 47–48) but there was no twist in the costa where it ended (contrast Fig. 48 with Fig. 16). Central bars with irregular edges but apparently with a single peak (Fig. 47). External central raphe endings slightly deflected toward the 'dorsal' side (Fig. 49), terminal raphe endings often hard to follow because of the tegumental strips, but curving slightly toward the 'dorsal' side, apparently not hooked (Fig. 50).

Saepes not observed, but gene sequences (Fig. 1) show that this species, despite some morphological differences from the fusiform *Haslea* spp., is in this genus rather than *Navicula*.

***Navicula tsukamotoi* (Sterrenburg et al. 2015) Yuhang Li et Kuidong Xu** Figs 51–62

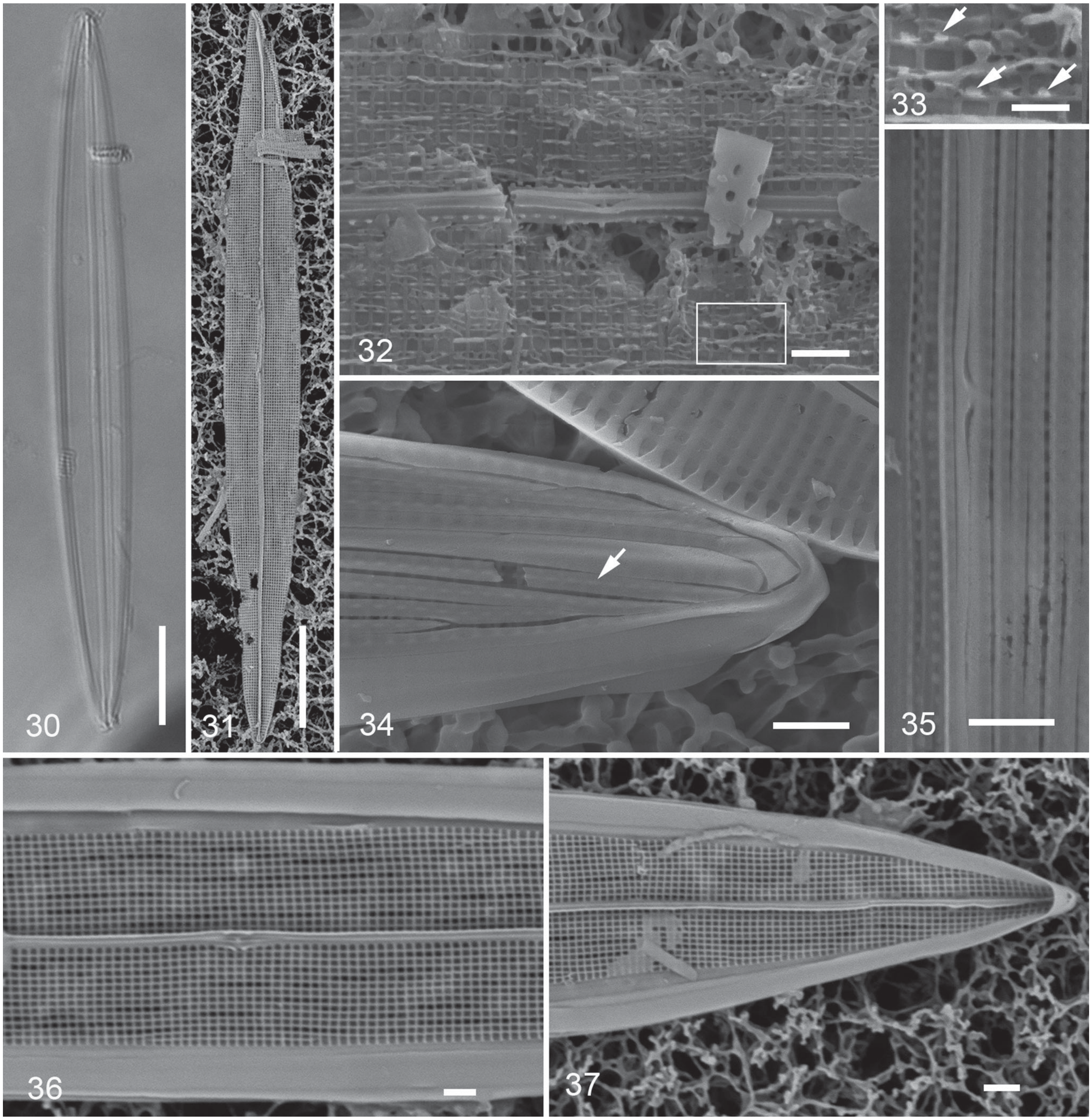
Published illustrations: Sterrenburg et al. 2015, Figs 33–38; Li et al. 2017, Figs 5–21; Lobban et al. 2012, pl. 47, figs 6, 7 as *Haslea howeana* (Hagelstein) Giffen

Materials examined: GU7Y-4, 3; GU21AM-2; GU44AR-2, GU44BK-6, GU44BV-1; GU52Q-10a, GU52W-1.

This species is common in Guam but, in the light of recent publications cited above and sequence data shown in Fig. 1, is correctly identified as *N. tsukamotoi*.

Dimensions: Length 30–68 µm, width 7–12 µm, transapical striae 17–19 in 10 µm, weakly radiating in the middle, and longitudinal striae 20–23 in 10 µm (Figs 51–53; Table 3). Even though the longitudinal stria density is the same across the cell, the external ridges and internal foramina are narrower closer to the raphe-sternum (Figs 54–58). The longitudinal ridges were supported along their distal margin but overhung most of the foramina, leaving the appearance of a small slit straight through to the interior (Figs 54–55, 58); in Figs 54 and 54 the internal foramina are seen through the outer ridges (Fig. 55 inset), and in Fig. 58 the edge of the overhanging ridge was seen as slits below the medial margins of the foramina. Unlike the closely-spaced integumentary strips in *Haslea*, the ridges in these *Navicula* spp. were widely separated. There was a single apical pore (Fig. 55), and internal thickening supporting the apical helictoglossae (Fig. 57), as noted by Sterrenburg et al. (2015). The cingulum did not consist of a single copula, but comprised first a wide, segmented valvocopula, interpreted as having six components (Figs 59–62): two asymmetrical girdle pieces were segmented, each with two parts (V_1, V_2; V_3, V_4) and there were two different apical caps (V_5, V_6). Second, there were several narrow pleurae, at least one of which had an integral apical cap (Figs 59–60, 62).

Our culture collections included a sample of *N.* cf. *howeana* from Condado Lagoon, Puerto Rico and we provide images of its ultrastructure here briefly for comparison with Pacific *N. tsukamotoi* (Figs 63–66) and *Navicula howeana* (Table 3). Stria densities: transverse 15–19 in 10 µm, longitudinal 18–20 in 10 µm ($n = 10$). Certain features are shared with *N. tsukamotoi*: the apical pore (Fig. 63), buttressed helictoglossa (Fig. 65), crozier-hook shape of external central raphe endings (Fig. 64), and narrowing of the foramina toward the raphe-sternum (Figs 64, 66). The most obvious difference is the evident remains of ricae in the foramina (Fig. 66), which are not evident in Guam specimens (Figs 56, 58, 61), nor in images in Sterrenburg et al. (2015).



Figs 30–37. *Haslea apoloniae*, n. sp. SEM except Fig. 30. Fig. 30. Holotype, frustule in LM, DIC. Fig. 31. Valve, internal view. Figs 32–33. Valve with almost all tegumentary strips torn away, enlargement of area in rectangle in Fig. 33 showing saepes (arrows). Figs 34–35. External views of apex and central area; arrow in Fig. 34 indicates the saepes showing through the tegumentary strips. Fig. 36. Internal view of central area. Fig. 37. Internal apex showing curled end of the axial rib and the long helictoglossa. Scale bars: Fig. 30, 31 = 10 µm, Figs. 32, 34–37 = 1 µm, Fig. 33 = 500 nm.

Discussion

Following the study by Li et al. (2017) there were 35 *Haslea* taxa, of which most appear robust and/or have a distinct pseudostauros. The four new fusiform taxa and the similar ones in the literature, 15 species in all, can be separated on the basis of size and/or stria densities (transapical and longitudinal) and consequent foramen proportions, as shown in Table 2 and discriminated in the key below. Authorities for taxa not previously mentioned, but included in the key, are: *H. trompii* (Cleve) Simonsen, *H. gigantea* var. *tenuis* von Stosch, *H. clevei* F.Hinz, P.E.Hargraves & Sterrenburg, and *H. crystallina* (Hustedt) Simonsen.

Key To Warm/Temperate, Delicate, Fusiform Species Of *Haslea* Lacking A Pseudostauros

1. Foramina (‘areolae’) transapically rectangular . . . . *H. trompii*
1. Foramina (‘areolae’) square or nearly so, or apically rectangular 2

2. Foramina apically rectangular 3
2. Foramina square or nearly so, i.e. longitudinal and transverse stria densities similar 8

3. Foramina strongly apically elongated, longitudinal stria density > 50 in 10 µm, cells producing blue pigment . . . . *H. ostrearia* / *H. karadagensis* / *H. nusantara* / *H. provincialis**
3. Foramina apically elongated, longitudinal stria density < 50 in 10 µm, lacking blue pigment 5

4. Longitudinal stria density 23–27 in 10 µm . . . . *H. gigantea* var. *tenuis*
4. Longitudinal stria density 35–49 in 10 µm 5

5. Length < 100 µm 6
5. Length > 100 µm 7

6. Valves 40–83 × 6–12 µm; transverse striae 34–35/10 µm, longitudinal striae 40–45/10 µm . . . . *H. apoloniae*
6. Valves 45–60 × 5–7 µm; 27–28/10 µm, longitudinal striae unknown but > 30/10 µm . . . . *H. hyalinissima*

7. Valves 107–145 × 8–9 µm, transverse striae 28–31/10 µm, longitudinal striae 42–49/10 µm . . . . *H. guahanensis*
7. Valves 150–200 × 19.5–22 µm; transverse striae 18–19/10 µm, longitudinal striae 36-40/10 µm . . . . *H. gretharum*

8. Transverse striae < 20 in 10 µm 9
8. Transverse striae > 20 in 10 µm 10

9. Cells small, < 50 µm long . . . . *H. pseudostrearia*
9. Cells very large, > 300 µm long . . . . *H. gigantea*

10. Cells narrow, ≤ 12 µm wide 11
10. Cells broader, > 12 µm wide 12

11. Cells 85–110 µm long, transverse striae 23 in 10 µm . . . . *H. clevei*
11. Cells 110–150 µm long, transverse striae 31–34 in 10 µm . . . . *H. fusiformis*

12. Cells 12–15 µm wide, 106–155 µm long . . . . *H. crystallina*
12. Cells 17–40 µm wide 13

13. Cells 17–25 µm wide; foramina exactly square, 29 in 10 µm . . . . *H. frauenfeldii*
13. Cells 21–40 µm wide; transverse striae 24–25, longitudinal striae 30–33 in 10 µm, foramina slightly rectangular . . . . *H. alexanderi*

*The first three species cannot be distinguished morphologically, but Gastineau et al. (2012) and Prasetiya et al. (2019) established that they do not interbreed, have considerable genetic distance, and produce different blue pigments. *H. provincialis* can be distinguished morphologically but is ‘semicryptic’ (Gastineau et al. 2016).

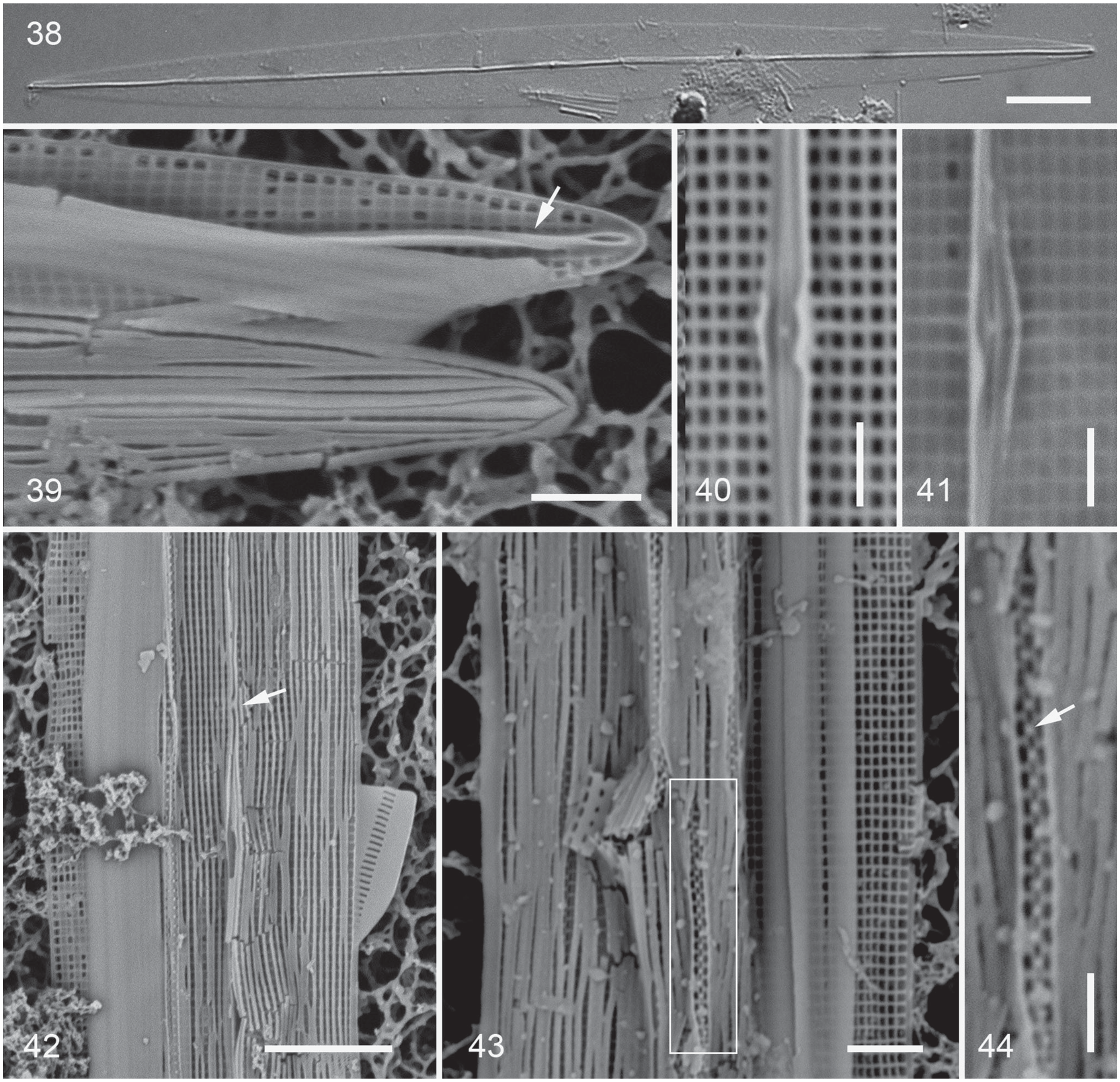
Table 3. Comparison of characters of *Navicula howeana* and *Haslea* (*Navicula*) *tsukamotoi*.

Species and reference	Length (μm)	Width (μm)	Transapical striae in 10 μm	Longitudinal striae in 10 μm (approx.)	Shape of apex
N. howeana (data from Hagelstein 1939)	75–85	11–12	15	15	‘Slightly produced, subacute ends’[a]
H. tsukamotoi (data from Sterrenburg et al. 2015)	40–45	8–9.5	16–19	18–21	‘Acute’
N. tsukamotoi (data from Li et al. 2017)	25.0–45.6	6.9–8.6	18–20	23–25	‘Acute or subacute’
N. tsukamotoi (Red Sea strain in database of Sabir et al. 2018)	38–55	9–10	20–22	21–22	Acute
N. tsukamotoi (this study)	30–68	7–12	17–19	20–23	Acute
N. tsukamotoi (Puerto Rico; this study)	30–62.5	10–10.7	15–19	18–20	Acute

[a] Also drawn as such by Giffen (1980).

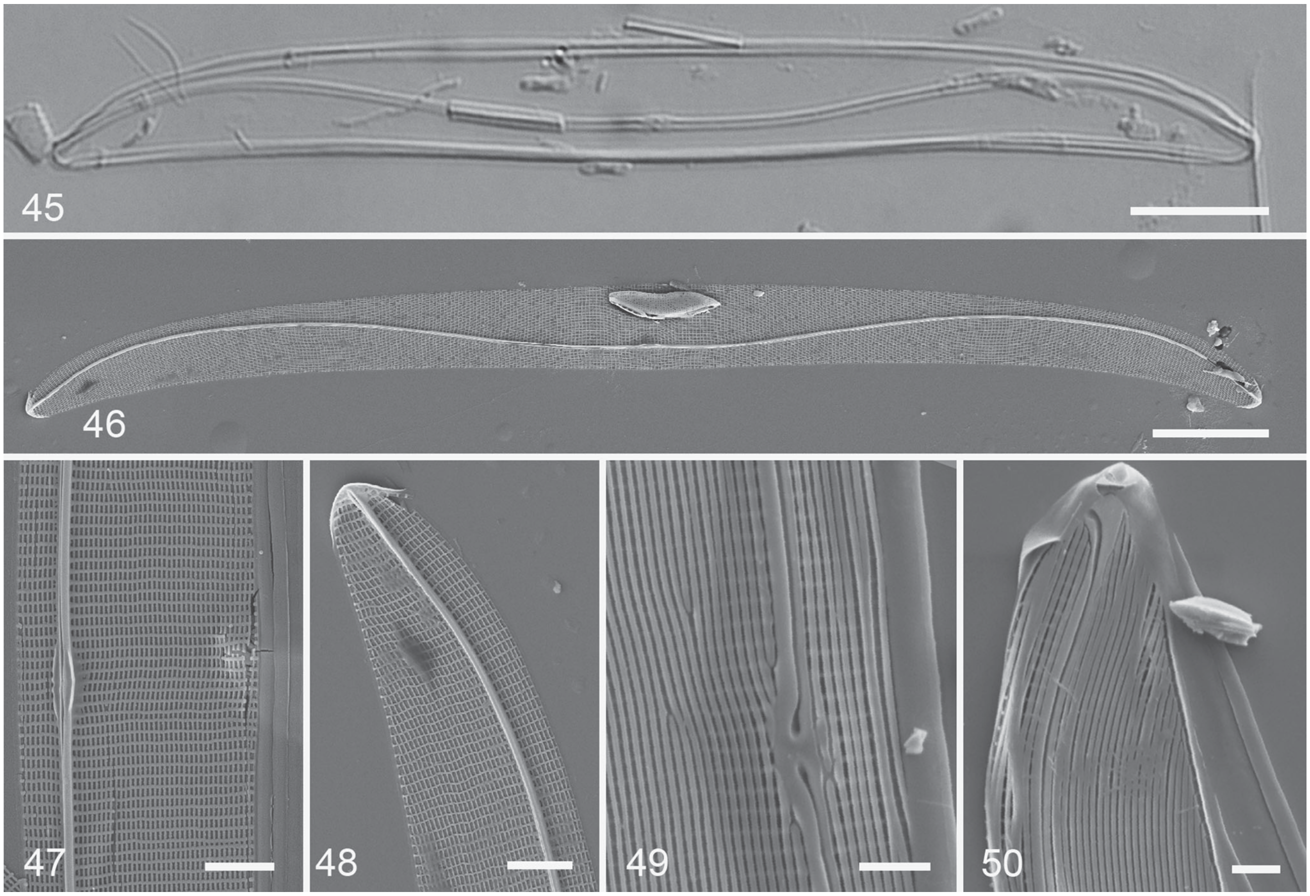
Status of *Navicula fusiformis* Grunow 1877 (non Ehrenberg 1832)

Simonsen (1974) pointed out that *N. fusiformis* Grunow (1877) was an illegitimate name because *N. fusiformis* Ehrenberg (1832) had priority, but solved the problem by considering Grunow’s species synonymous with *N. ostrearia*, which he then transferred to his new genus, *Haslea*. *Navicula fusiformis* Ehrenberg may in fact be a *nomen nudum* (Guiry & Guiry 2019), as it was mentioned as a new species in Ehrenberg (1832, pp. 8, 17,



Figs 38–44. *Haslea guahanensis*, n.sp. SEM except Fig. 38. Fig. 38. Holotype in LM, DIC. Fig. 39. External view of apex, also showing part of internal apex with long helictoglossa (arrow). Figs 40–41. Central region showing central bars; Fig. 41 also shows rotae still intact covering most of the foramina. Fig. 42. External view of central portion with central raphe endings (arrow), also showing the hyaline cingulum. Figs 43–44. Broken frustule with saepes visible where tegumentary strip missing (Fig. 44, arrow). Scale bars: Fig. 38 = 10 µm, Fig. 42 = 5 µm, Figs 39, 43 = 2 µm, Figs 40–41, 44 = 1 µm.

20), but without any description, and apparently no illustration. Specimens from the Grunow sample have 33–34 transapical striae and 32 longitudinal striae in 10 µm, lower densities than Hustedt's data. However, even with this difference, the specimens we found cannot be *H. ostrearia.* Therefore, we conclude that the specimens were indeed *N. fusiformis*, just with a slightly different stria count, since we only had two specimens from the three samples given by the museum. We are also confident that the Guam material described above matches Grunow's material from Honduras. We still do not know whether *N. fusiformis* has blue pigment. *Navicula fusiformis* is clearly different from *H. ostrearia,* with the latter having higher stria densities, resulting in rectangular foramina (see Table 2). Thus *N. fusiformis* cannot be maintained in synonymy with *H. ostrearia*, and we therefore propose it as a separate species. On the basis of the central bars, internal central and terminal raphe endings, and evidence for saepes, we conclude that is indeed a *Haslea.* The formal transfer is as follows:



Figs 45–50. *Haslea arculata*, n. sp. Fig. 45. Holotype frustule in LM, DIC. Fig. 46. SEM internal view of entire valve. Figs 47–48. Internal details of central area and apex. Figs 49–50. External details of central area and apex showing deflected raphe endings. Scale bars: Figs 45–46 = 10 μm, Figs 47–48 = 2 μm, Figs 49–50 = 1 μm.

***Haslea fusiformis* (Grunow) Lobban & C.O.Perez comb. nov**.

Basionym: *Navicula fusiformis* Grunow (1877) *Monthly Microscopical Journal, London* 18: 178, pl. 195: Fig. 11 a-b (nom. illegit.); non *N. fusiformis* Ehrenberg (1832): 8, 17, 20, nom. inval.

Synonyms: *Haslea ostrearia* (Gaillon) var. *fusiformis* (Grunow) Sauvageau (1907)

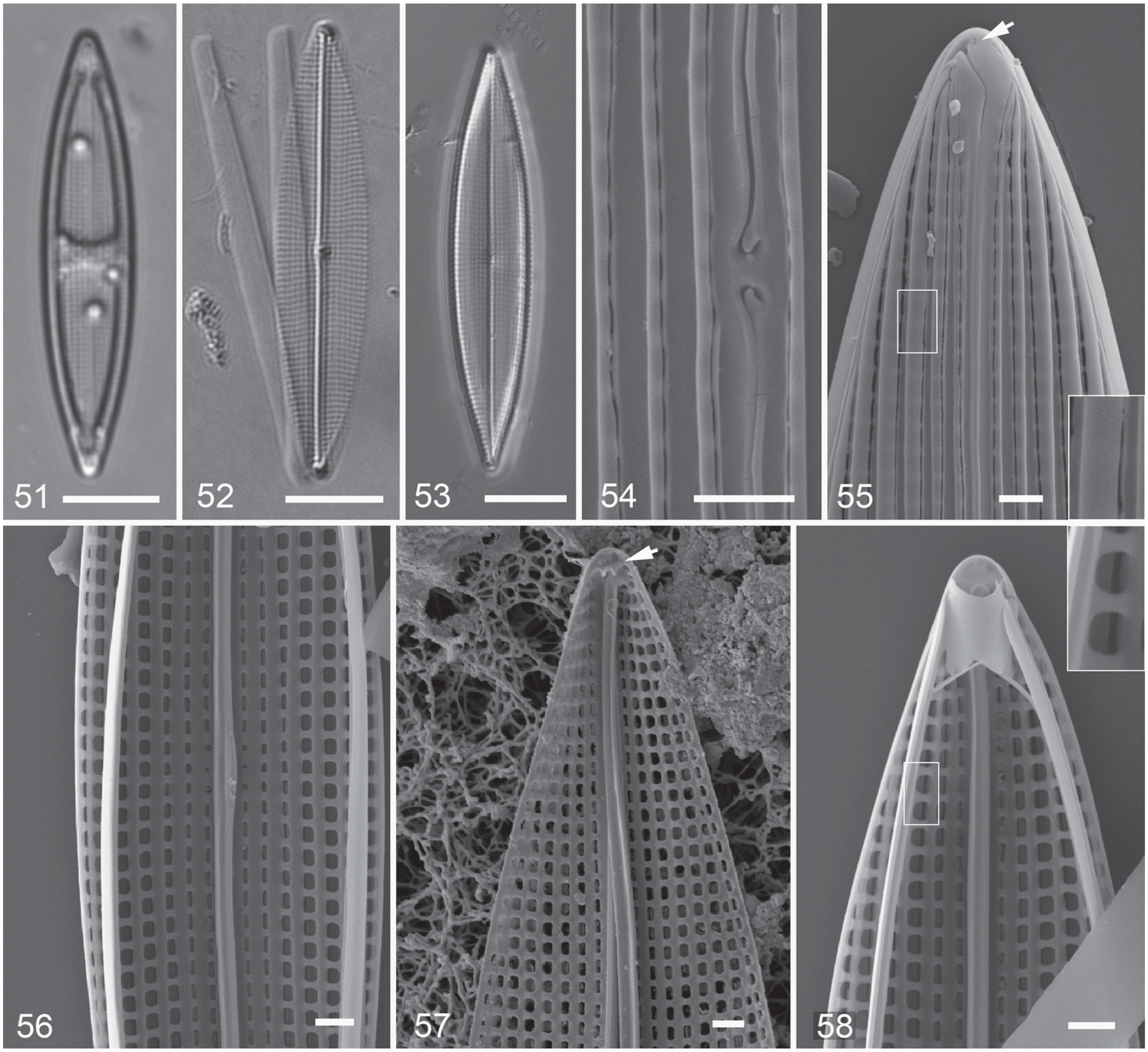
Haslea ostrearia (Gaillon) Simonsen (1974), pro parte '*Meteor' Forschungsergebnisse, Reihe D: Biologie* 19: 46.

The new Guam species

Haslea species generally occurred frequently but in low numbers in the samples, often with more than one species present (Table 1), but a few samples had significant populations (*H. alexanderi* in GU43C; *H. arculata* in GU7AA-4; *H. guahanensis* and *H. alexanderi* in GU52P-9).

Plastid shape differed between smaller versus large cells (Figs 2–8) but it is unclear whether this has any taxonomic implications; the images shown here are all from freshly collected living cells. Plastids have rarely been recorded in this group of *Haslea*; Hustedt (1961) makes no mention of them except for *H. ostrearia*, which has a plate-like plastid along each girdle face, as in our smaller ones and *H. ferarium* (observed by Li et al. 2017). Simonsen (1974) makes no mention of them. However, Al-Yamani & Subarova (2019, pl. 121) have excellent images of *H. gigantea*, showing numerous relatively small, elongate plastids scattered in the cytoplasm or clustered around the nucleus. Plastids in our two larger species, in contrast are massive, more convoluted than in our smaller species but still lying along each girdle side. Although blue species of *Haslea* have recently been shown to be widespread and diverse (Gastineau et al. 2014b, 2016), none of our species showed blue pigment except for the tiny vacuoles in *H. arculata*, which are quite unlike the *H. ostrearia* group.

We sought finer ultrastructure details that might be helpful in discriminating species in this group. Sterrenburg et al. (2015) considered the central bars but dismissed them as not useful ('Central internal raphe endings present no special features in the specimens we have seen, central bars may be vestigial or absent'), but that comment must be interpreted in the light of their inclusion of several species that are now considered to be *Navicula* and lack bars.

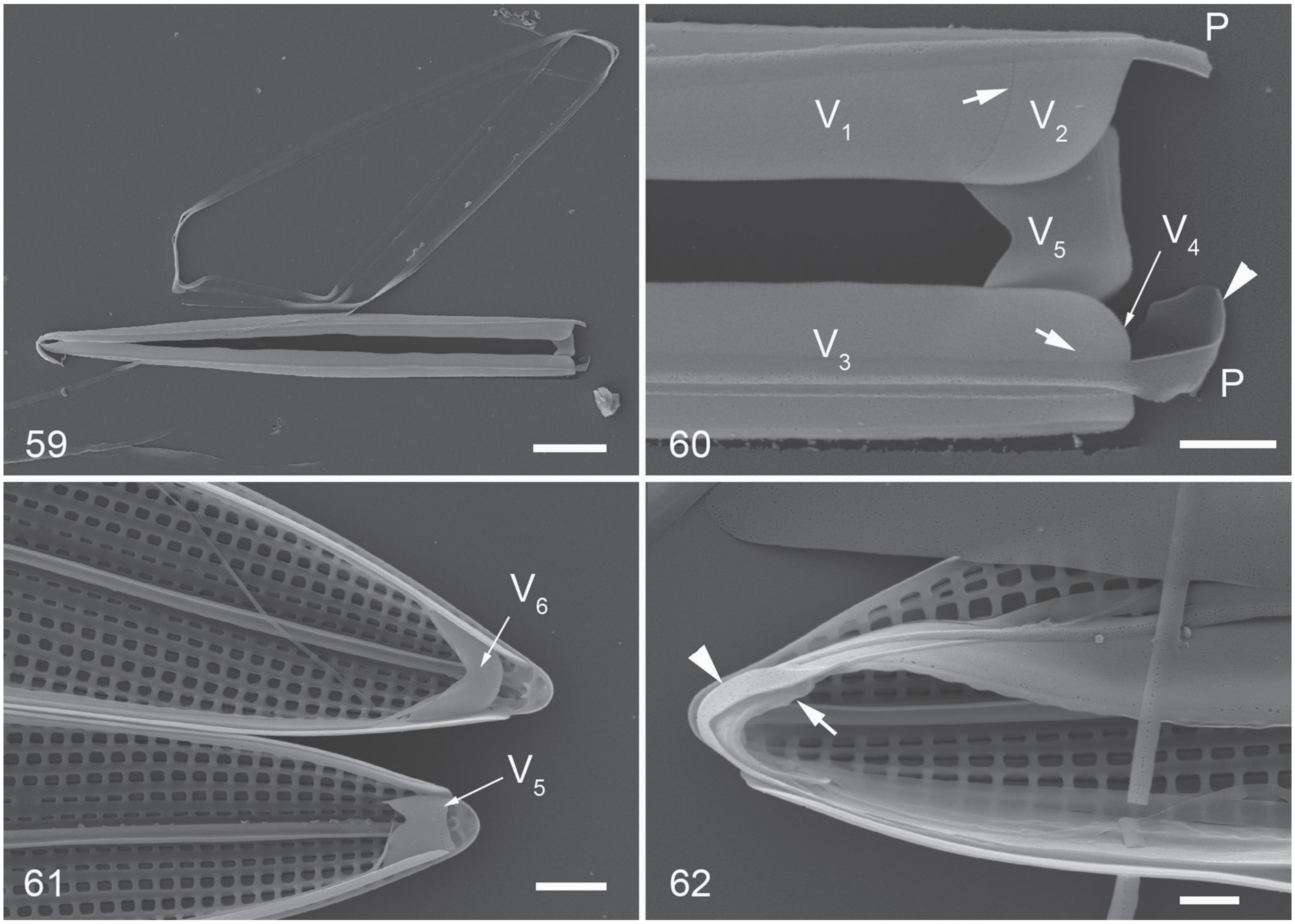


Figs 51–58. *Navicula tsukamotoi*. Fig. 51. Live cell showing girdle-appressed plastids. Figs 52–53. Acid cleaned valves in LM, Fig 52 also showing the girdle pieces of the cingulum. Fig. 54. External detail of central area. Figs 55, 58. External and internal views of the apex, showing relationship of foramina to external grooves and ridges. The rectangles and inset enlargements show the location of two underlying foramina and the gap between the edge of a ridge and the edge of the next groove. Figs 56–57 Internal views of central and apical portions showing decreasing width of foramina towards the raphe-sternum and short, buttressed helictoglossa (arrow on 57). Scale bars: Figs 51–53 = 10 µm, 55–58 = 1 µm.

Nevertheless, the central bars or flaps in our *Haslea* spp. seemed weakly developed, sometimes thin flaps rather than solid bars, and we are inclined to agree that they are not useful for discriminating species within *Haslea*, although they are useful in distinguishing *Haslea* from *Navicula*. Prasetiya et al. (2019) used the presence of a central bar to help distinguish *H. nusantara* from *H. ostrearia* and *H. karadagensis*. Sterrenburg et al. (2015) also noted that the striae were offset in *H. clevei* and cited some other examples. We also saw offset striae in our specimens (e.g. Figs 14,16), but it did not appear to be consistent either within or between species, and does not seem to be taxonomically useful.

Two populations of H. alexanderi or two species?

In the absence of molecular data, we have struggled to decide whether the populations of large cells with convoluted plastids (Figs 2,3) are the same species or not. Size alone is notoriously unreliable as a taxonomic criterion,



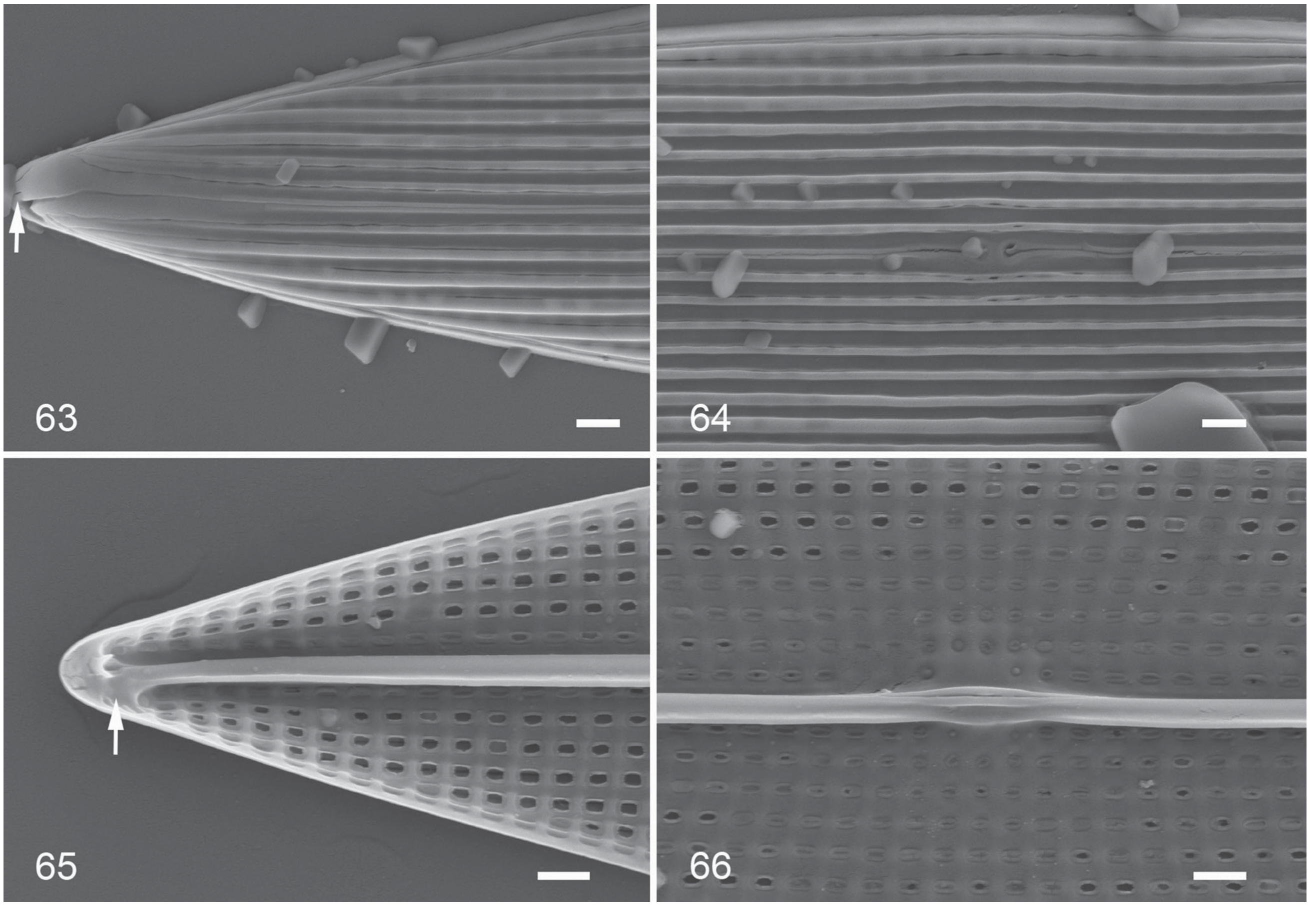
Figs 59–62. *Navicula tsukamotoi* cingulum structure. Figs 59–60. Valvocopula and several pleurae, general view, and detail of one end of the cingulum. Small arrows indicate the joints between the girdle components of the valvocopula (V_1, V_2; V_3, V_4), large arrowhead the apical cap of the pleura (P). V_5 is the wider of the two apical caps. Fig. 61. Apices of two valves with valvocopulae, showing the two different apical caps, V_5 and V_6. Fig. 62. Apex of valve with attached valvocopula and pleurae. Part of a pleura runs from top to bottom of the image. Arrowhead points to the apical cap on one of the pleurae, arrow to the apical cap piece (V_6) of the valvocopula. Scale bars: Fig. 59 = 5 µm, Fig. 61 = 2 µm, Figs 60, 62 = 1 µm.

and while the graph of size range shows no overlap between the populations, the ranges are adjacent, and the graph can equally well be interpreted as showing a single population. The cells in the low range came from a different habitat, a very shallow intertidal pool, than the rest, which are subtidal from two locations in Apra Harbor. Again, the difference cannot be used to discriminate them, as the smaller size could be the result of the environmental conditions on a single species, or equally two species with different habitats. We cannot tell which is the cause and which the effect. Moreover, two specimens discovered in a sample of epiphytic seaweeds from a shallow reef site in Chuuk Lagoon (TK28) were also in the smaller range. Apart from size, differences are slight and unconvincing as evidence for either hypothesis. We therefore take a conservative approach and identify all these specimens are belonging to one species until more genetic data and/or ecological diversification data for tropical benthic raphid diatoms become available.

Comparison with published species

Here we add a few comments to elaborate on the summaries in the Diagnoses, Table 2 and Key.

Morphological discrimination between some species in this group depends on very fine distinctions in morphometrics. For instance, Hustedt (1961), in describing *N. crystallina* as a new species, prepared a table comparing it with *N. frauenfeldii* and *N. vitrea* [*Haslea vitrea* (Cleve) Simonsen] (data reproduced here in Table 2, except for geographic provenance), which clearly distinguished it from *N. vitrea* on stria densities and size, but less clearly from *N. frauenfeldii*, which was broader but with overlapping lengths. However, he stated that the transverse/longitudinal striae, both 29 in 10 µm in *N. frauenfeldii* vs. 26/30 in 10 µm in *N. crystallina,* had the same density ('gleicher Entfernung'). He also noted that *N. frauenfeldii* was subtropical/tropical vs temperate. Hustedt (1961: 42) clearly recognized the potential for similar species to exist and the difficulty in distinguishing them: 'Ob damit alle



Figs 63–66. *Navicula tsukamotoi* cells from a culture isolated from Puerto Rico, showing internal and external views of apical and central regions. Arrows indicate apical pore (Fig. 63), buttressed helictoglossa (Fig. 65). Scale bars = 1 µm.

Arten dieser Gruppe erfaßt sind, is natürlich fraglich. Jedenfalls ist bei den Untersuchungen größte Genauigkeit hinsichtlich der Struktur geboten, die wegen der Zartheit meistens nur an genauen Abbildungen, am besten an Mikrophotographien, ausgezählt warden kann.' [Whether all kinds of this group are covered is, of course, questionable. In any case, the investigations require the greatest possible accuracy in terms of structure, which, because of its delicacy, can usually only be counted on precise images, preferably on photomicrographs.]

Specifically within the *Haslea gigantea-frauenfeldii-vitrea-crystallina* group, Simonsen (1974) introduced *Navicula gretharum* Simonsen*,* quickly corrected to *Haslea gretharum* (Simonsen 1975) and *H. hyalinissima.* He distinguished the former from the others by its much finer longitudinal striae (36–40 in 10 µm), i.e. apically elongate foramina. *Haslea hyalinissima* was incompletely described because he could not resolve the longitudinal striae: while they were finer than the transverse striae (27–28 in 10 µm), it is important to know if they are 30 or 40 or more in 10 µm, i.e. if the foramina are ± square or apically rectangular.

More recently Sterrenburg et al. (2015) added *H. clevei*, which differs from *H. crystallina* in having somewhat coarser transverse striae (23 vs 26 in 10 µm) and being shorter and narrower. Gastineau et al. (2012) struggled to distinguish *H. karadagensis* from *H. ostrearia* on morphological grounds. Clearly genetic sequence data are needed for this group, but we could not succeed in culturing any of this group, only *H. arculata*. Although the morphological differences are small among some of our species, and between them and some similar species from other places, we are confident that they are distinct, based on the criteria presently used in the genus.

Simonsen (1974) concluded from his own and others' records that *H. gigantea* is planktonic, and this seems to be true (Al-Yamani & Subarova 2019). However, other large, delicate taxa have been assumed to be planktonic but found not to be, such as *Stictocyclus stictodiscus* (Grunow) Ross, one of our common benthic taxa, whose benthic habit was first observed by Round (1978), whereas our samples of large *Haslea*, from several samples at two sites in Apra Harbor, Guam, were living in a benthic environment. Recently Sabir et al. (2018) explored the possibility that some benthic diatoms might have adaptations that would allow them to move between benthic and planktonic habitats (not just swept up stochastically as tychoplankton). That might apply to several taxa in our flora with large thin-walled cells, such as these *Haslea* spp., *S. stictodiscus*, *Biddulphiopsis membranacea* (Cleve) H.A. von Stosch & R. Simonsen and *Chrysanthemodiscus floriatus* A. Mann, or that form loosely attached, necklace chains, such as *Bleakeleya notata* (Grunow) F.E. Round and *Perideraion* Lobban & R.W.Jordan spp., that have colonies very similar to the planktonic *Asterionella glacialis* (Castracane) F.E. Round.

Haslea arculata

As seen in LM, both alive and acid cleaned, *H. arculata* somewhat resembles a small, curved species of *Climaconeis* Grunow, of which three are known in Guam, all with biarcuate raphe paths (Lobban et al. 2010, Lobban 2018). However, while we dubbed this *Haslea* 'little bow', its outline is nearly straight with deflected apices, rather than a true arc like the *Climaconeis* spp., and the plastids are two flat plates, in contrast to two pairs of plates joined by pyrenoids. Of course, the *Haslea* valve structure is also entirely different. *Haslea arculata* also differs in shape from the non-fusiform species shown by Sterrenburg et al. (2015), which included sigmoid [*H. nipkowii* (Meister) Poulin & Massé, *H. sigma* Talgatti, Sar & Torgan, and *H. staurosigmoidea* Sterrenburg & Tiffany] and amphoroid (*H. feriarum* Tiffany & Sterrenburg) (the last three all have a pseudostauros, in contrast to all the Guam species).

Haslea tsukamotoi vs. H. howeana

Having concluded that our specimens originally identified as *H. howeana* match *N. tsukamotoi*, two points need consideration here: (1) Is *H. howeana* really a *Navicula*? (2) Are *N. tsukamotoi* and *N. howeana* conspecific?

Li et al. (2017) published gene sequences for *H. howeana* (from GenBank) but noted that there was no voucher specimen, so the determination was not certain. For this reason, they did not make the transfer even though the sequences were in the *Navicula* clade. On basis of our molecular data and observations of a Puerto Rico population, we can confidently move *H. howeana* back to *Navicula*, as *N. howeana* Hagelstein. Even were the Puerto Rico and St. Croix populations eventually shown to be different species, it is extremely unlikely that this would alter the generic conclusion, and all similar taxa, including *H. nautica* (Cholnoky) Giffen and *H. britannica* (Hustedt) Witkowski, Lange-Bertalot & Metzeltin should also revert to *Navicula*.

The question of whether or not *N. tsukamotoi* and *N. howeana* are conspecific remains an open question pending further study of Hagelstein's (1939) type material (if any) or collections from the type locality (Harbor of Christiansted, Saint Croix, Virgin Islands). Giffen (1980) reported material identical to Hagelstein's from the Seychelles, with a drawing but no stria densities, and transferred it to *Haslea*, based on gross similarities to *H. kjellmannii* Cleve, which Simonsen (1974) had already transferred to *Haslea.* Sterrenburg et al. (2015) described *H.* (*N.*) *tsukamotoi* as being 40–55 µm long, 8–9.5 µm wide, with 16–19 transverse striae in 10 µm, 18–21 longitudinal striae in 10 µm (they appear apically elongate in the images, even though the difference in dimensions is small). Sterrenburg et al. (2015) distinguished it on the grounds that it 'has much finer striae than *H. howeana.*' We also note that Sterrenburg et al. described the valve apices of *N. tsukamotoi* as 'acute,' whereas Hagelstein (1939) described *N. howeana* apices as having 'slightly produced, subacute ends' (Table 3). Li et al. (2017) did not disagree that the two species are distinct; for *N. tsukamotoi* they gave dimensions very similar to those in Sterrenburg et al. (2015). As Table 3 shows, the striae are somewhat coarser in *N. howeana*, as defined by Hagelstein (1939).

As for the cingulum of *N. tsukamotoi*, Li et al. (2017) included two images, interpreted as showing it is composed of 'two bands, one being a long band and the other a short ligula.' It is clear from our images that the structure is more complex; the presence of two different, separate apical caps ('ligules') suggests that the valvocopula is not an open band but two separate side pieces. Each side piece also shows a crack, that based on Medlin's (1985) images of the *Rhoikoneis sponsalia* (Giffen) Medlin valvocopula, we interpret as two joined pieces. In addition, there are clearly several delicate pleurae present in the cingulum.

Reconsideration of the Pleurosigmataceae

Given the bilayered wall structure and the central bars around the internal raphe endings, along with the diversity of shape noted in recent papers and here, we reconsider the Pleurosigmataceae and the place of *Haslea*. This family has a very convoluted history, reviewed by Reid (2012: Fig. 1). There have been two recent taxonomic considerations, Reid (2012) and Cox (2015). Using morphological data, Reid (2012: 85–86) showed that *Haslea* was sister to the 'sigmoid' clade, i.e. the pairs of genera that are more or less sigmoid with transverse vs. decussate striae, the list presently comprising *Gyrosigma* / *Pleurosigma*, *Rhoicosigma* Grunow / *Cochlearisigma* G.Reid; *Carinasigma* G.Reid / *Donkinia* J.Ralfs, and *Arcuatasigma* G.Reid / *Toxonidisigma* Lobban & G.Reid (Lobban & Reid 2018) along with *Plagiotropis, Costasigma* G.Reid and *Toxonidea* Donkin (Reid 2012). Cox (2015) maintained *Haslea* in the Naviculaceae but included *Plagiotropis* Pfitzer in Mann's (Round et al. 1990) family Plagiotropidaceae, to which she added *Meuneira* Deeke and *Pachyneis* Simonsen. Molecular data on *Haslea* in Li et al. (2017) showed two clades, one of which they restored to *Navicula*, but they did not comment of the place of the other clade. Our results show *Haslea* (*sensu stricto*) sister to *Navicula*, but *Plagiotropis* and *Meuneira* sister to the

Pleurosigmataceae, so that *Haslea* cannot be included in Pleurosigmataceae, in spite of the morphological parallels.

It is not surprising that the *Haslea* species reported from these samples are new to science, given the richness of new species in several other genera that we have investigated on Guam [*Climaconeis* (Lobban et al. 2010, Lobban 2018); *Licmophora* (Lobban et al. 2018, Macatugal et al. 2019, inter alia), conopeate *Nitzschia* (Lobban et al. 2019), *Cyclophora* (Ashworth et al. 2012), etc.]. This study does not complete the *Haslea* flora of Guam. The majority of our sampling has been done in farmer-fish territories in Apra Harbor, but *H. alexanderi* also occurred in a much different habitat. We have indications of other species from other habitats, for which we have insufficient evidence yet to proceed. As we continue to explore the benthic marine diatom flora of Guam, it is becoming increasingly clear that it represents part of a largely unexplored community, i.e. tropical Western Pacific, with many new taxa. It is also clear from the molecular and biochemical work on blue *Haslea* spp. that still more biodiversity may be hidden beneath the morphotaxa.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

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CORRECTION

Lobban, C.S., C.O. Perez & M.P. Ashworth. 2020. Non-blue *Haslea* species (Bacillariophyceae: Naviculaceae) in the Guam benthic marine flora. *Diatom Research* 35: 163–183.

The basionym given for the new combination *Haslea fusiformis* (Grunow) Lobban & Perez is wrongly given as *N. fusiformis* Grunow 1877, since Grunow (1877) equated this with *Berkeleya fusidium* Grunow 1867, referring to the same Honduras sample, which we examined. He gives his reasons for changing his mind about the generic assignment, and could not keep the epithet *fusidium* as it was already taken by *N. fusidium* Ehr. Ironically so was *N. fusiformis*. The following correction is therefore needed to legitimize our change:

***Haslea fusidium* (Grunow) Lobban & C.O.Perez, comb. nov.**

Basionym: *Berkeleya fusidium* Grunow 1867, *Hedwigia* 6, p. 17

Synonyms: *Navicula fusiformis* Grunow 1877 (nom. illegit.)

Haslea ostrearia (Gaillon) var. *fusiformis* (Grunow) Sauvageau

Haslea ostrearia (Gaillon) Simonsen (in part) (and in Poulin et al. 2019)

We note in Craig's dictionary (1849) that *fusidium* is a noun, used for a genus name in Fungi, and also that Grunow (1867) in his paper in German wrote the word with a capital, indicating that he took it to be a noun. As a noun in apposition rather than an adjective, it is correctly spelled *fusidium*.

Thanks to Richard L. Moe for bringing this to our attention and to Luc Ector and Bart van der Vijver for dialogue on the epithet spelling.

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