

Nagumoea serrata, a new diatom species (Bacillariophyceae) found on seagrass from the south–eastern coast of Africa (Indian Ocean)

Roksana MAJEWSKA^{1,2*} & Bart VAN DE VIJVER^{3,4}

¹Unit for Environmental Sciences and Management, School of Biological Sciences, North–West University, Potchefstroom 2520, South Africa; *Corresponding author e-mail: roksana.majewska@nwu.ac.za

²South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown 6140, South Africa

³Meise Botanic Garden, Research Department, Nieuwelaan 38, B–1860 Meise, Belgium

⁴University of Antwerp, Department of Biology, ECOBE, Universiteitsplein 1, B–2610 Wilrijk, Antwerpen, Belgium

Abstract: A novel diatom species belonging to the genus *Nagumoea* is described based on detailed light and scanning electron microscopy observations. The new taxon was found abundantly in several samples collected from seagrass on the southernmost coasts of Mozambique, Southern Africa. While the new species shows characters (e.g. scalariform valvocopula, fibulae of complex shape and structure) consistent with the genus description, it differs distinctly from currently known members of *Nagumoea*. Differences include generally larger (7–29 µm) and more slender (1.0–1.5 µm) valves with acutely rounded apices and 4–5 areolae per stria, a doubly-perforated valvocopula, and the last-formed copula with a highly regular serrated margin. Based on the current knowledge and understanding of the diatom morphology and phylogeny, *Anaulus vallus* Nikolaev, previously included within the genus *Denticula*, is transferred to the genus *Nagumoea*. This brings the current number of the genus members to five. Ecological preferences of *Nagumoea* are briefly discussed.

Key words: *Anaulus*, *Cymodocea*, *Denticula vallus*, epiphytic diatom, morphology, Mozambique, *Nagumoea*, new species, seagrass, taxonomy

INTRODUCTION

The small marine diatom genus *Nagumoea* Kociolek et Witkowski was described in 2011 based on *Denticula neritica* R.W.Holmes et D.A.Croll (HOLMES & CROLL 1984; WITKOWSKI et al. 2011). The genus is characterized by small-celled species possessing typical scalariform valvocopulae (previously called septa, see VAN DE VIJVER et al. 2012), uniseriate striae with areolae covered externally by individual hymenes, a canal raphe with wide portules and an external filiform raphe not contained in a keel (WITKOWSKI et al. 2011). The most prominent feature of the genus is the structure of the fibulae, the latter being very complex, composed of two wide arches with narrow siliceous elevations connecting the lower arches (see WITKOWSKI et al. 2011, figs 62–66). At present, only three species comprise the genus: *N. neritica* Witkowski et Kociolek, the genotype, *N. africana* Kociolek et al. and *N. livingstonensis* Van de Vijver et Kociolek, described recently from the Maritime Antarctic Region (WITKOWSKI et al. 2011; VAN DE VIJVER & KOCIOLEK 2018).

VAN DE VIJVER & KOCIOLEK (2018) discuss the

placement of the genus within either the Bacillariales, Rhopalodiales or Surirellales. They concluded that the absence of a keel most likely indicated that *Nagumoea* forms part of a Bacillariales branch that evolved before a keel was developed, a feature nowadays present in almost all members of the Bacillariales.

In the present paper, a new diatom species is described found epiphytically on seagrass (*Cymodocea* sp.) based on detailed light and scanning electron microscopy. Diatoms living on marine angiosperms have been documented for the last 40 years from many places around the world. TIFFANY (2011) presents an overview of the epizoic and epiphytic diatom forms and discusses in detail many records of diatoms found on different seagrass and surfgrass species (*Ruppia*, *Zostera*, *Posidonia*, *Thalassia* and *Phyllospadix*). Most studies deal with the ecology and phenology of diatom communities associated with this specific habitat (e.g. STERRENBURG et al. 1995; FRANKOVICH et al. 2006; HASEGAWA et al. 2007; CHUNG & LEE 2008; MAJEWSKA et al. 2014; KANJER et al. 2019). A few studies report on epiphytic diatoms on various *Cymodocea* species (SULLIVAN 1979; MABROUK et al. 2014). Despite the relatively high number of studies on seagrass diatoms, new species have rarely been

reported from this substratum.

The present paper describes the frustule ultrastructure of *Nagumoea serrata* Majewska et Van de Vijver sp. nov. based on detailed light and scanning electron microscopy observations. The study is the first to analyse and document cells of *Nagumoea* still attached to its original substratum adding information about the genus ecology and life form. The new species is compared with all currently known *Nagumoea* species. Furthermore, in line with the current understanding of the genus morphology, *Anaulus vallus* Nikolaev is transferred to *Nagumoea* (NIKOLAEV 1969; SULLIVAN 2010).

MATERIALS AND METHODS

Study site and sampling. Samples of *Cymodocea* sp. were collected from the intertidal zone of the sandy/rocky beach in the vicinity of Ponta do Ouro, Mozambique, during a low tide in June 2017. Ponta do Ouro (eng. 'tip of gold') is a small coastal town in the far south of Mozambique, situated on the Mozambique Channel. The local climate is strongly influenced by the warm Agulhas Current (Chemane et al. 1997). Although locally known and visited for its beaches, flora and fauna, and diving and fishing, Ponta do Ouro is rather isolated and can only be reached by a 4×4 vehicle.

The collected plants were placed in separate plastic bags and transported to the laboratory on ice (in a cooler box). Several 2–cm² sections of the blades were placed in a 4% formaldehyde solution in seawater for further analyses of the attached epiphytes. After ca. 24h, the preserved fragments were dehydrated in an alcohol series, critical-point-dried, and mounted on aluminium stubs following the method described in MAJEWSKA et al. (2015).

The remaining portion of the seagrass material was prepared for light (LM) and scanning electron microscopy (SEM) observations. The samples were cleaned by adding a mixture of boiling concentrated acids (2:1 volume ratio of 55% HNO₃ and 98% of H₂SO₄) according to the protocol proposed by von Stosch (HASLE & SYVERTSEN 1997). Following digestion and centrifugation (3 × 10 minutes at 1400 g), the resulting cleaned material was diluted with distilled water to avoid excessive concentrations of diatom valves on the slides. Cleaned diatom material was mounted in Naphrax. The slides were analysed using an Olympus BX53 microscope, equipped with Differential Interference Contrast (Nomarski) and the UC30 Imaging System. For SEM, portions of the oxidized suspension were filtered through polycarbonate membrane filters with a pore diameter of 1 µm, pieces of which were fixed on aluminum stubs after air-drying. The stubs with both cleaned material and dehydrated seagrass pieces were sputter-coated with Gold-Palladium and studied in a ZEISS ULTRA SEM microscope at 3 kV (Natural History Museum (BM), London, UK) and a FEI Quanta Feg 250 at 5–10 kV (North-West University, Potchefstroom, South Africa). Samples, stubs and slides are stored at the BR-collection (Meise Botanic Garden, Belgium) and South African National Diatom Collection (North-West University, Potchefstroom, South Africa). Morphological terminology follows ROUND et al. (1990) and WITKOWSKI et al. (2011). The morphology of the new species has been compared to the ultrastructure of all currently known taxa.

OBSERVATIONS

Nagumoea serrata Majewska et Van de Vijver sp. nov. (Figs 1–30)

Description

Light Microscopy (Figs 1–23): Frustules in girdle view rectangular, isopolar, symmetrical along apical and transapical axis (Figs 1–11). Valves linear in larger specimens to linear-lanceolate in smaller cells, with parallel to weakly convex margins and non-protracted, acutely rounded apices (Figs 12–23). Valve dimensions (n=50): length 7–29 µm, width 1.0–1.5 µm. Raphe in LM barely detectable (Figs 21, 22, 23, arrows). Fibulae thick, very distinct, visible in both valve and girdle view, 8–12 in 10 µm. Maximum number of fibulae per valve = 24. Structural details of striae and areolae indiscernible.

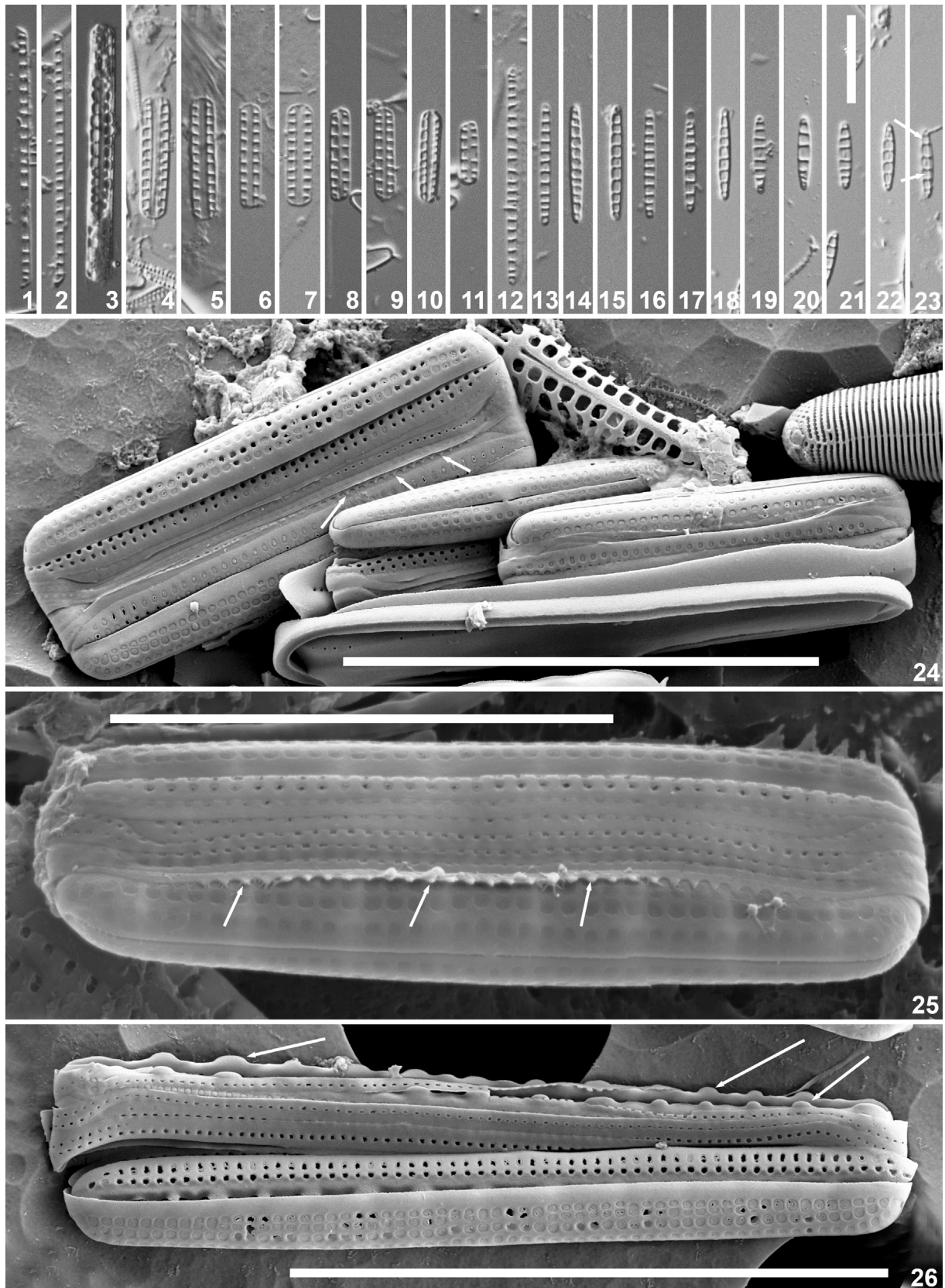
Scanning electron microscopy (Figs 24–32): Mantle deep (Figs 24–26). Mantle striae composed of 2–3 large, rounded to quadratic areolae, leaving a small hyaline zone at mantle edge (Figs 24, 26). Transapical striae on valve face composed of two areolae. Areolae bordering axial area apically elongated (Figs 27, 28). Second row of areolae at valve margin large and rounded, externally occluded by individual hymenes (Fig. 28), separated from mantle striae by a narrow, but distinct hyaline zone. Central area small, rectangular, slightly asymmetrical (Figs 27, 28). Raphe branches almost straight, very slightly curving (Figs 26, 27). Central raphe endings straight, weakly expanded, and close together (Figs 26, 27). Terminal raphe fissures continuing onto valve mantle, unilaterally hooked (Fig. 26). Internally, raphe simple with central endings terminating on a raised, narrow central nodule (Figs 29, 30, 31). Terminal raphe endings with small helictoglossae (Figs 29, 30, 32). Fibulae robust, forming a complex shape typical for *Nagumoea* (Figs 31, 32), plate-like, widening from middle to valve margins (Fig. 30). Siliceous elevations connecting lower parts of fibulae and fusing with upper thickened edge present at valve face-mantle junction (Figs 30, 31, 32, arrows). Cingulum with open copulae (~8+) perforated by one or two (valvocopula) rows of hymenate, roundish or transapically elongated areolae, ca. 55 in 10 µm (Figs 24–26). Shortened row of small rounded pores present in apical parts of abvalvar copulae (Figs 24–26). Valvocopula with digitate margin (Fig. 26, arrows). Last-formed copula of epitheca with regular, serrated edge (Figs 24 & 25, arrows).

Holotype: BR-4573 (Meise Botanic Garden, Belgium)

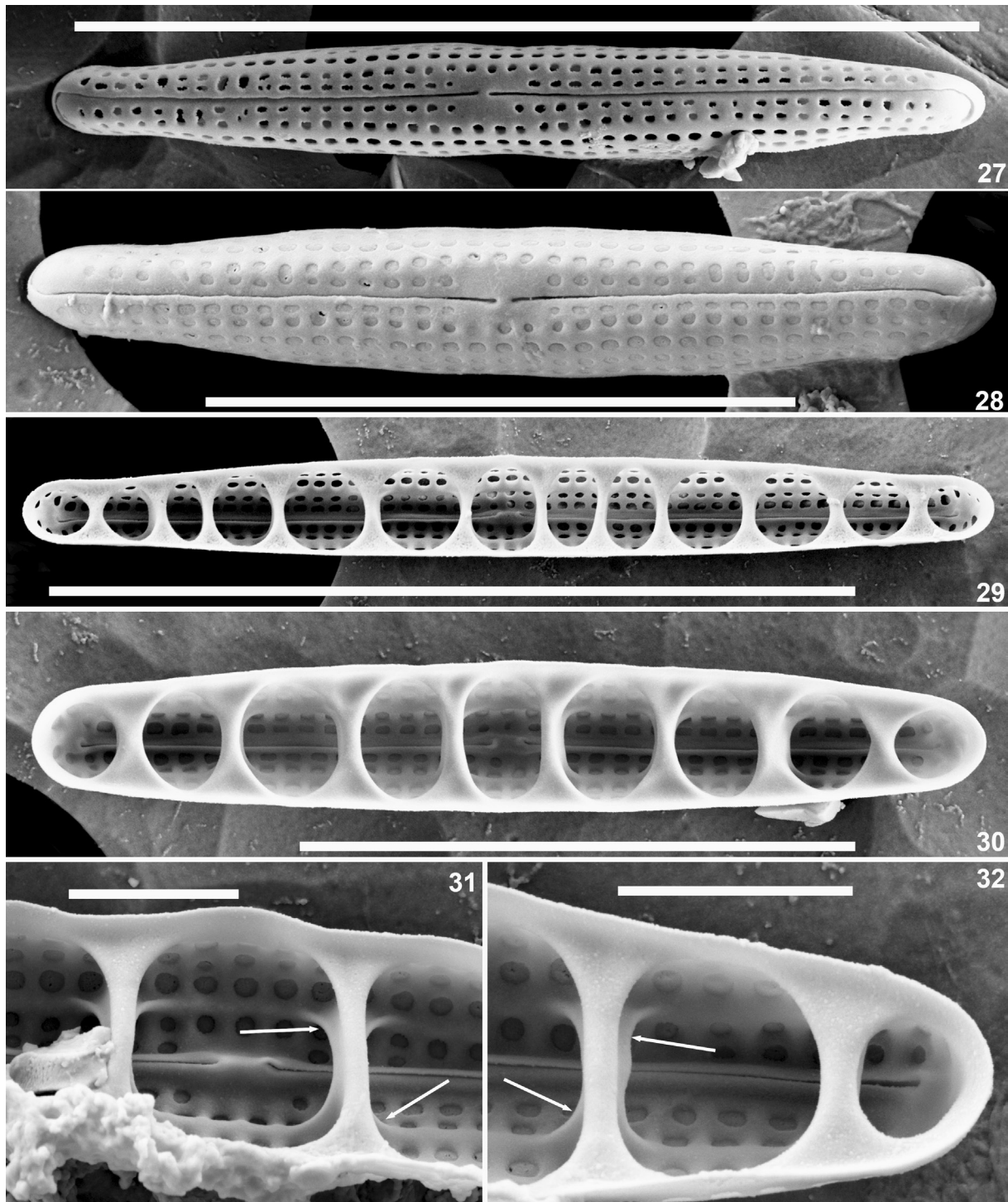
Isotypes: PLP-366 (University of Antwerp, Belgium) and SANDC-SG103 (South African National Diatom Collection, North-West University, South Africa)

Type locality: Ponta do Ouro, Maputo Province, Mozambique, sample SANDC-SG103 collected from the blades of seagrass *Cymodocea* sp. (leg. R. Majewska), coll. date 02/06/2017 (S 26°51' 2.166"; E 32°53'39.114").

Etymology: The specific epithet is based on the Latin word *serratus* ('serrated') with reference to the serrated



Figs 1–26. *Nagumoea serrata* Majewska et Van de Vijver sp. nov.: (1–23) Light micrographs, (1–11) Girdle view, (12–23) Valve view. Arrows indicate the barely detectable raphe. (24) Complete frustules in girdle view. Arrows indicate the last-formed copula with serrated margin. Loose girdle bands in the lower part of the picture belong to *Pteroncola inane* (Giffen) F.E.Round. (25) Intact frustule attached to the seagrass surface through a mucilaginous pad produced by one of the apices. Arrows indicate the last-formed copula with serrated margin. (26) Valve in girdle view with partially detached girdle bands (external view). Arrows indicate the digitate margin of valvocopula. Scale bars 10 μ m (1–24, 26), 5 μ m (25).



Figs 27–32. *Nagumoea serrata* Majewska et Van de Vijver sp. nov.: (27, 28) External valve view. (29, 30) Internal valve view. (31) Detail of the central area (internal view). Arrows indicate the siliceous elevations connecting the lower parts of the fibulae. (32) Detail of the apex (internal view). Arrows indicate the siliceous elevations connecting the lower parts of the fibulae. Scale bars 10 µm (27, 29), 5 µm (28, 30), 1 µm (31, 32).

edge of the last girdle band.

Ecology: Abundant (up to 35% of the total relative diatom abundance) on *Cymodocea* sp. collected from the intertidal area on the sandy/rocky beach. Attaching to the substratum via one pole of the cell or motile. Accompanied by *Pteroncola inane* (Giffen) F.E.Round and an unidentified eunotioid diatom.

DISCUSSION

The placement of the new species within the genus *Nagumoea* is justified based on the combination of its morphological features. The presence of the typical scalariform valvocopula, the shape and structure of the fibulae, the structure of the striae and the areolae and the structure of the raphe are consistent with the genus

Table 1. Comparison of *Nagumoea serrata* with the currently known members of the genus.

	<i>N. neritica</i>	<i>N. africana</i>	<i>N. livingstonensis</i>	<i>N. vallus</i>	<i>N. serrata</i>
Reference	WITKOWSKI et al. (2011)	WITKOWSKI et al. (2011)	VAN DE VIJVER & KOCIOLEK (2018)	NIKOLAEV (1969), SULLIVAN (2010)	this paper
length (µm)	6–11	6–18.3	7–13	8–20	7–29
width (µm)	1.8–2.6	1.3–2.3	1.8–2.2	1.5–2.0	1.0–1.5
valve outline	narrowly elliptical	narrowly elliptical	linear to linear–lanceolate	linear–lanceolate	linear to linear–lanceolate
apices	broadly rounded	broadly rounded	broadly rounded	acutely rounded	acutely rounded
number of fibulae (in 10 µm)	6–11	2–6	3–6	4–9	8–12
number of areolae per stria	3–4	1–2	2–3	2(3?)	4–5
number of striae (in 10 µm)	41–51	36–46	ca. 40	36–50	44–50
valvocopula perforations	uniseriate	uniseriate	unknown	uniseriate	biseriate
valvocopula margin	undulate	undulate	unknown	crenate	digitate

description (WITKOWSKI et al. 2011). Worldwide, only three *Nagumoea* species were described. Table 1 shows the morphological characteristics of these species in comparison with *N. serrata*. The new species is generally larger, with the valve length of 7–29 µm, whereas *N. africana*, the largest of the previously described species, reaches a maximum length of 18 µm. Valve width, on the contrary, is within the range of or smaller than in the three other *Nagumoea* taxa. *Nagumoea serrata* is the only species possessing acutely rounded apices since the other three species have broadly rounded apices. The most important difference is the striation pattern and structure. Whereas the three previously described *Nagumoea* species have maximum three areolae per stria, the new *N. serrata* has up to five areolae per stria with two complete rows of areolae on the valve face and 2–3 areolae on the mantle. *Nagumoea livingstonensis* and *N. africana* have reduced rows of areolae on the valve face (WITKOWSKI et al. 2011, figs 87–89; VAN DE VIJVER & KOCIOLEK 2018, figs 7–8). Only *N. neritica* has one complete series of areolae on the valve face (HOLMES & CROLL 1984, figs 40, 41, 47; WITKOWSKI et al. 2011, figs 58, 59). Moreover, remarkable differences between *N. serrata* and other members of the genus can be observed in the cingulum structure. The new species is the only one that possesses valvocopula with two rows of perforations (one row in other species) and a digitate margin (undulate in remaining taxa). It is also the first *Nagumoea* species in which the distinctly serrated last-formed copula was observed. These morphological differences separate convincingly the new species from the other three *Nagumoea* species.

The generic type of *Nagumoea* was originally described as *Denticula neritica* Holmes et Croll. TOTTI et al. (2009) observed *N. neritica* on intertidal seaweeds in Iceland but as *Nagumoea* was only described two years later, the species was reported as *Denticula neritica*. It is therefore highly likely that other reports of *Denticula* species on seaweeds and seagrasses might in fact represent *Nagumoea* observations. Unfortunately, none of these records were illustrated and are therefore hard to verify. One of the *Denticula* species that shows some similarity with the new *Nagumoea* species is *Denticula vallus* (Nikolaev) Sullivan, an epiphytic species that was originally described as an *Anaulus* species living on red and brown algae in the Sea of Japan (NIKOLAEV 1969; SULLIVAN 2010). The species shows all morphological features that are observed in the genus *Nagumoea* such as the scalariform valvocopula, the similar raphe structure and the structure of the striae. SULLIVAN (2010) already noticed the similarity between *Anaulus vallus* and *Denticula neritica* and transferred the species to *Denticula* (as *Nagumoea* was only described in 2011). Based on its structure, the species should actually be transferred to the genus *Nagumoea*:

***Nagumoea vallus* (Nikolaev) Majewska et Van de Vijver comb. nov.**

Basionym: *Anaulus vallus* Nikolaev 1969, Novitates Systematicae Plantarum non Vascularium p. 30, pl. 1, figs 3–8.

Synonym: *Denticula vallus* (Nikolaev) M.J.Sullivan, 2010, Vie et Milieu p.175.

Nagumoea vallus differs from the new *Nagumoea* species in having wider valves (1.5–2.0 µm compared to 1.0–1.5 µm in *N. serrata*), only one row of areolae on the valve face (instead of two rows in *N. serrata*), and a lower number of fibulae per valve (max. nine, whereas it is up to 24 in *N. serrata*; Table 1). Therefore, conspecificity between these two *Nagumoea* species has to be excluded. CARPENTER (1970) observed and illustrated a *Nagumoea* species in his samples collected from *Sargassum* algae in the Sargasso Sea but named it *Navicula* sp. commenting that it could not belong to *Denticula* because of its raphe structure. This example shows that the genus *Nagumoea* might be more widespread but due to force-fitting and misidentifications, the records are not easily retraced in the literature.

The description of the new *Nagumoea* species and the transfer of *Anaulus vallus* to the genus, brings the total number of *Nagumoea* species known so far up to five. It is likely that this current number is an underestimation of the total *Nagumoea* diversity. Based on all *Nagumoea* observations worldwide (South Africa, Mozambique, Japan, Antarctic Region), it is safe to state that the genus has a broad biogeographical distribution. Most *Nagumoea* species were found living epiphytically (SULLIVAN 2010; MAYOMBO et al. 2019, this study) or epizoically (WITKOWSKI et al. 2011). Only *Nagumoea livingstonensis* was found in a littoral sediment sample of a coastal pool frequented by elephant seals and marine birds. These animals that could have been the vector by which the diatom was transferred from the marine realm to the pool (VAN DE VIJVER & KOCIOLEK 2018). It is also possible that the species might be living as an epiphyte on the numerous marine macroalgae that were blown into the coastal pool by wind or seaspray, a conclusion already made by WITKOWSKI et al. (2011).

ACKNOWLEDGEMENTS

The authors thank Paul Hamilton for his valuable comments on the previous version of this manuscript. Alex Ball and the staff of the IAC laboratory at the Natural History Museum are thanked for their help with the scanning electron microscopy. This study was supported by the BELSPO MICROBIAN project to visit the National History Museum in London, UK.

REFERENCES

- CHEMANE, D.; MOTTA, H. & ACHIMO, M. (1997): Vulnerability of coastal resources to climate changes in Mozambique: a call for integrated coastal zone management. – *Ocean & Coastal Management* 37: 63–83.
- CHUNG, M. & LEE, K. (2008): Species composition of the epiphytic diatoms on the leaf tissues of three *Zostera* species distributed on the southern coast of Korea. – *Algae* 23: 75–81.
- FRANKOVICH, T.A.; GAISER, E.E.; ZIEMAN, J.C. & WACHNICKA, A.H. (2006): Spatial and temporal distribution of epiphytic diatoms growing on *Thalassia testudinum* Banks ex König: relationships to water quality. – *Hydrobiologia* 569: 259–271.
- HASLE, G.R. & SYVERTSEN, E.E. (1997): Marine diatoms. – In: TOMAS C.R. (ed.): *Identifying Marine Phytoplankton*. – pp. 5–385, Academic Press, San Diego.
- HASEGAWA, N.; HORI, M. & MUKAI, H. (2007): Seasonal shifts in seagrass bed primary producers in a cold-temperate estuary: dynamics of eelgrass *Zostera marina* and associated epiphytic algae. – *Aquatic Botany* 86: 337–345.
- 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: MANN, D.G. (ed.): *Proceedings of the Seventh International Diatom Symposium*. – pp. 265–277, O. Koeltz, Koenigstein.
- KANJER, L.; MUCKO, M.; CAR, A. & BOSAK, S. (2019): Epiphytic diatoms on *Posidonia oceanica* (L.) Delile leaves from eastern Adriatic Sea. – *Natura Croatica* 28: 1–20.
- MABROUK, L.; BRAHIM, M.B.; HAMZA, A.; MAHFOUDHI, M. & BRADAI, M.N. (2014): A comparison of abundance and diversity of epiphytic microalgal assemblages on the leaves of the seagrasses *Posidonia oceanica* (L.) and *Cymodocea nodosa* (Ucria) Asch in Eastern Tunisia. – *Journal of Marine Biology* 2014, Article ID 275305, DOI: <http://dx.doi.org/10.1155/2014/275305>
- MAJEWSKA, R.; D'ALELIO, D. & DE STEFANO, M. (2014): *Cocconeis* Ehrenberg (Bacillariophyta), a genus dominating diatom communities associated with *Posidonia oceanica* Delile (monocotyledons) in the Mediterranean Sea. – *Aquatic Botany* 112: 48–56.
- MAJEWSKA, R.; KUKLINSKI, P.; BALAZY, P.; YOKOYA, N.S.; MARTINS, A.P. & DE STEFANO, M. (2015): A comparison of epiphytic diatom communities on *Plocamium cartilagineum* (Plocamiales, Florideophyceae) from two Antarctic areas. – *Polar Biology* 38: 189–205.
- MAYOMBO, N.A.S.; MAJEWSKA, R. & SMIT, A.J. (2019): Diatoms associated with two South African kelp species: *Ecklonia maxima* and *Laminaria pallida*. – *African Journal of Marine Science* 41: 221–229.
- NIKOLAEV, V.A. (1969): Species novae Bacillariophytorum epiphyticorum marinorum. – *Novitates Systematicae Plantarum non Vascularium* 6: 29–34.
- SULLIVAN, M.J. (2010): Transfer of the benthic marine diatom *Anaulus vallus* to the genus *Denticula*. – *Vie et Milieu – Life and Environment* 60: 173–176.
- SULLIVAN, M.J. (1979): Epiphytic diatoms on three seagrass species in Mississippi Sound. – *Bulletin of Marine Science* 29: 459–464.
- ROUND, F.E., CRAWFORD R.M. & MANN D.G. (1990): *The Diatoms. Biology and Morphology of the Genera*. – 747 pp, Cambridge University Press, Cambridge.
- STERRENBURG, F.A.S.; ERFTEMEIJER, P.L.A. & NIENHUIS, P.H. (1995): Diatoms as epiphytes on seagrasses in South Sulawesi (Indonesia). Comparison with growth on inert substrata. – *Botanica Marina* 38: 1–7.
- TOTTI, C.; POULIN, M.; ROMAGNOLI, T.; PERRONE, C.; PENNESI, C. & DE STEFANO, M. (2009) Epiphytic diatom communities on intertidal seaweeds from Iceland. – *Polar Biology* 32: 1681–1691.
- VAN DE VIJVER, B. & KOCIOLEK, J.P. (2018): A new species of *Nagumoea* (Bacillariophyta) from Antarctica, and a further consideration of the systematic position of the genus. – *Phytotaxa* 349: 152–158.
- VAN DE VIJVER, B.; ECTOR, L. & COX, E.J. (2012): Ultrastructure of *Diatomella balfouriana* with a discussion of septum-like structures in diatom genera. – *Diatom Research* 27: 213–222. DOI:10.1080/0269249X.2012.720612
- WITKOWSKI, A.; KOCIOLEK, J.P. & KURZYDŁOWSKI, K.J. (2011): Valve ultrastructure of two new genera of marine canal-bearing diatoms (Bacillariophyceae). – *Phycologia* 50: 170–181.