

A new sediment dwelling and epizoic species of *Olifantiella* (Bacillariophyceae), with an account on the genus ultrastructure based on Focused Ion Beam nanocuts

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Abstract: The marine diatom *Olifantiella* Riaux–Gobin et Compère predominantly occurs in tropical Indo–Pacific coral reef environments. The genus has about a dozen validly published taxa and is characterized by transversally elongate striae composed of a macroareola, broad perforated girdle, and tubular buciniportula process of trumpet–shape. The valve mantle of taxa related to the generitype, *O. mascarenica*, have a canal–shaped structure around the valve at the face / mantle junction, while those to *O. gorandiana* have a simple mantle or strongly narrow canal and complex buciniportula composed of hollow processes. The geographic distribution of *Olifantiella* is expanded in this study to include species observed in loggerhead sea turtles scrapes from the Aegean Turkish coast and samples from the Turkish Black Sea and Adriatic Sea coasts. *Olifantiella* has also been identified in Martinique Island, Caribbean Sea. A transfer of *Navicula infirmitata* is proposed due to the areola and buciniportula structure, as *Olifantiella infirmitata*. Further, an application of the Focused Ion Beam (FIB) to a sequential cutting of frustules allows resolution of *Olifantiella mascarenica* valve ultrastructure.

Key words: diatoms, epizoic, Focused Ion Beam technique, marine coasts, new species, *Olifantiella*, sediment dwelling, ultrastructure

INTRODUCTION

Small–sized biraphid pennate diatoms of genus *Olifantiella* Riaux–Gobin et Compère have been described from coral reefs of Rodrigues Island, Indian Ocean, with *O. mascarenica* Riaux–Gobin et Compère (RIAUX–GOBIN & COMPÈRE 2009) selected as generitype. *Olifantiella gorandiana* Riaux–Gobin, *O.*

paucistriata Riaux–Gobin, *O. pilosella* Riaux–Gobin, *O. pseudobiremis* Riaux–Gobin and *O. rodriguensis* Riaux–Gobin were described in RIAUX–GOBIN & AL–HANDAL (2012) from Western Indian Ocean. In addition *O. pilosella* var. *rhizophorae* Riaux–Gobin and *O. societatis* Riaux–Gobin were described from tropical waters of Indian and Pacific Oceans by RIAUX–GOBIN (2015). More recently, *Olifantiella elisabethiana* Van

De Vijver was described from Antwerp, Belgium by VAN DE VIJVER et al. (2016). Taxa belonging in *Olifantiella* are small and difficult to identify under light microscopy (LM) and only observations with scanning electron microscopy (SEM) can differentiate between particular taxa (LOBBAN et al. 2012; RIAUX–GOBIN & AL–HANDAL 2012; RIAUX–GOBIN 2015). Following characters have been listed as characterizing *Olifantiella* (RIAUX–GOBIN 2015, and emended definition in RIAUX–GOBIN & AL–HANDAL 2012, p. 216):

- Transapically elongate striae composed of a single areola (the so-called macroareola)
- Presence of remarkable tubular process – buciniportula, raised or not
- Broad girdle composed of numerous perforated bands
- Marginal ridge, more or less deep, stepping over the striae

Based on the early identifications of *Olifantiella*, observed species were confined to tropical waters of the Indopacific (RIAUX–GOBIN 2015). However, recent findings in the Martinique Island littoral zone (DESROSIERS 2014) and description of *O. elisabethiana* from the North Sea coast (Antwerp Harbour in Belgium) expands the geographic distribution of *Olifantiella* to tropical and temperate Atlantic waters (Fig. 1).

The establishment of genus *Olifantiella* was preceded by *Labellicula* Van de Vijver et Lange–Bertalot (VAN DE VIJVER et al. 2005). The latter was monotypic as *L. subantarctica* Van de Vijver et Lange–Bertalot until description of a second species, *L. lecohuiana* Majewska et Van de Vijver in MAJEWSKA et al. (2017). Both genera possess a process with simple stigmata structure in *Labellicula* (VAN DE VIJVER et al. 2005) and rather complex, tubular design in *Olifantiella* (RIAUX–GOBIN & COMPÈRE 2009; RIAUX–GOBIN 2015). Despite a strong similarity between the two genera, process structures of *Labellicula* and *Olifantiella* are easily distinguished in SEM micrographs. The buciniportula show some variation in established *Olifantiella* species. Three different types of buciniportula are observed: 1) Buciniportula showing raised tubular structure in *O. mascarenica*, 2) double tubular and raised structure in *O. rodriguensis*, and 3) double or multiple and flattened tubular structures in *O. gorandiana*.

If the process is not observed, the major difference is that *Olifantiella* has external central raphe endings always bent into the primary valve side, but towards the valve secondary side for *Labellicula*. This means that in *Labellicula* external proximal raphe endings tend to bend towards the valve face process opening, whereas proximal raphe endings externally bend outwards the process opening in *Olifantiella* (VAN DE

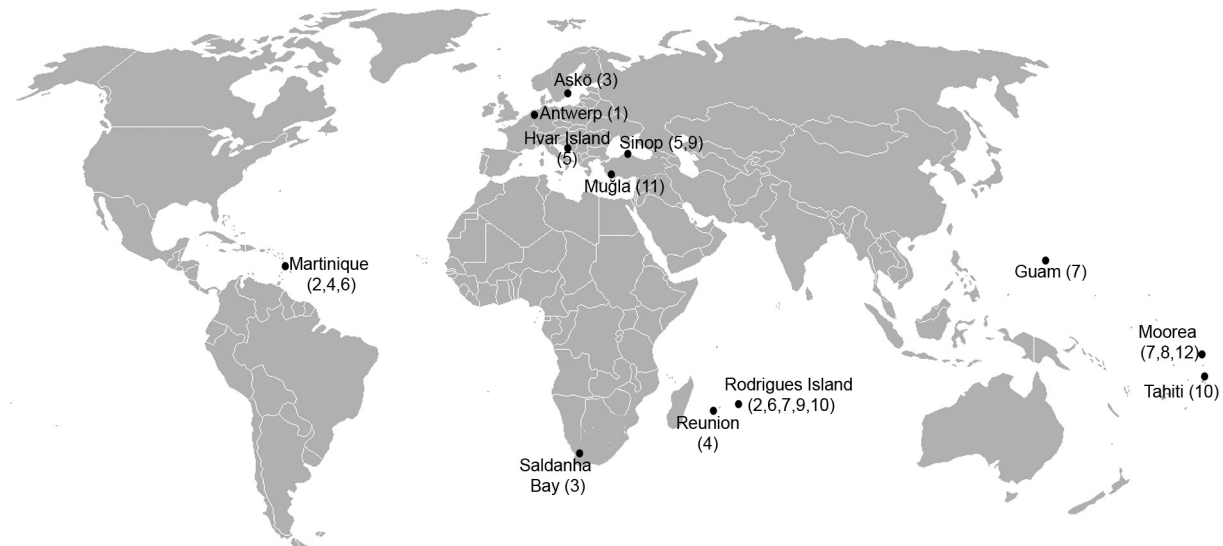


Fig. 1. Distribution of *Olifantiella* Riaux–Gobin et Compère: (1) *Olifantiella elisabethiana* Van de Vijver – Antwerp (VAN DE VIJVER et al. 2016); (2) *Olifantiella gorandiana* Riaux–Gobin – Rodrigues Island, Martinique Island (RIAUX–GOBIN & AL–HANDAL 2012; DESROSIERS et al. 2014; RIAUX–GOBIN 2015); (3) *Olifantiella infirmitata* (Giffen) Witkowski et Snoeijis comb. nov. – Saldanha Bay, South Africa, Askö, Sweden (GIFFEN 1975; SNOEIJIS & KAUTSKY 1989; SNOEIJIS & NOTTER 1993); (4) *Olifantiella mascarenica* Riaux–Gobin et Compère – Reunion, Martinique Island (RIAUX–GOBIN & COMPÈRE 2009; DESROSIERS 2014; RIAUX–GOBIN 2015), Martinique Island; (5) *Olifantiella* cf. *mascarenica* Sinop, Stari Grad Bay, Hvar Island, Central Adriatic, Croatia – This study; (6) *Olifantiella paucistriata* Riaux–Gobin – Rodrigues Island (RIAUX–GOBIN & AL–HANDAL 2012), Martinique Island (DESROSIERS 2014); (7) *Olifantiella pilosella* Riaux–Gobin – Rodrigues Island, Moorea Island & Guam (LOBBAN et al. 2012; RIAUX–GOBIN & AL–HANDAL 2012; RIAUX–GOBIN 2015); (8) *Olifantiella pilosella* var. *rhizophorae* Riaux–Gobin – Moorea Island (RIAUX–GOBIN 2015); (9) *Olifantiella pseudobiremis* Riaux–Gobin – Rodrigues Island (RIAUX–GOBIN & AL–HANDAL 2012; RIAUX–GOBIN 2015), also in Moorea Island (C.R. – G. Pers. obs.), Sinop – This study; (10) *Olifantiella rodriguensis* Riaux–Gobin – Rodrigues Island & Tahiti Island (RIAUX–GOBIN & AL–HANDAL 2012; RIAUX–GOBIN 2015); (11) *Olifantiella seblae* Kaleli, Krzywda, Witkowski et Solak sp. nov. – Dalyan, Muğla, Turkey – This study; (12) *Olifantiella societatis* Riaux–Gobin – Moorea Island (RIAUX–GOBIN 2015).

VIJVER et al. 2005; RIAUX–GOBIN & COMPÈRE 2009; RIAUX–GOBIN 2015).

In this paper, *Olifantiella seblae* sp. nov. is described as an epizoic species on loggerhead sea turtles (*Caretta caretta* LINNAEUS 1758) from Aegean Sea coasts of Turkey. Furthermore, transfer of *Navicula infirmitata* GIFFEN is proposed into *Olifantiella infirmitata* comb. nov., with ultrastructural observations detailing the buciniportula and macroareola. Occurrences of several *Olifantiella* taxa are reported from the Adriatic, Aegean and Black Seas. Also, we present for the first time nanocuts of *Olifantiella mascarenica* by means of Focused Ion Beam (FIB): The siliceous frustules have been cut at nanoscale and imaged.

MATERIAL AND METHODS

Loggerhead sea turtle scrapes originated from Dalyan–İztuzu on the Aegean Sea coast (36°42'02"N, 28°41'31"E) in southwest Turkey. Dalyan–İztuzu beach is an important Mediterranean nesting site (MARGARITOU et al. 2003) for loggerhead sea turtles. The sample location is a protected area with conservational studies made by DEKAMER (Sea Turtle Research, Rescue and Rehabilitation Center, CANBOLAT 2004; BAŞKALE & KASKA 2005). During the nesting season (late April to early September), sea turtles that come to lay eggs are measured (BOLTEN 1999), tagged and nests are protected with cages by authorized staff at the rescue center. The protection center has responsibility for treatment and rehabilitation of injured turtles.

Epizoic diatom samples were collected from the nesting loggerhead sea turtles in Dalyan–İztuzu, Muğla during the nesting season between May – August, 2012–2016. Samples were brushed from sea turtle carapaces at night.

The holotype slide of *Navicula infirmitata* and unmounted material studied in this paper originated from Malcolm H. GIFFEN collection and have been provided by Colin ARCHIBALD (CSIR, Durban, South Africa). Currently, the collection is maintained by Dr. Jonathan TAYLOR at North–West University, Research Unit for Environmental Sciences, in Potchefstroom, South Africa under custody of the South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa (NIWR).

A diatom sample from the invasive alga *Caulerpa taxifolia* (M. VAHL) AGARDH No. SZCZ 14361, was collected on Hvar Island (Stari Grad Bay, Central Adriatic) on April 20, 2009. This area (43°10'54"N, 16°35'00"E) has been affected by *C. taxifolia* at depths ranging from 5 to 8 m. *C. taxifolia* was observed in Stari Grad Bay for the first time in 1994 on hard, sandy and muddy substrates without vegetation or within meadows of *Posidonia oceanica* (ŽULJEVIĆ & ANTOLIĆ 2002). The diatom samples were also collected from epipelagic and epilithic habitats from Aklıman Bay, Sinop, located in Southern Black Sea coast (42°02'36"N, 35°02'46"E) in December 2012.

A series of samples from Martinique Island (14°4' N, 61° W), surrounded by the Atlantic Ocean and Caribbean Sea, have been analysed by means of LM and SEM. Samples were collected from the littoral zone, from Plexiglas® plates submerged at 3 m depth for five weeks. *Olifantiella* was found on sites located on the Caribbean sea side, with

often high concentration of suspended matter and turbidity (DESROSIERS 2014). Part of the research on Martinique Island diatoms including SEM has been performed at Warsaw University of Technology in fall 2012 (cf. DESROSIERS 2014). During this time also FIB cutting of *Olifantiella mascarenica* has been made, however, the results have not been published thus far.

Samples were washed with 10% HCl, boiled in 30 % H₂O₂ and rinsed with distilled water to remove organic material (SWIFT 1967). Permanent slides were air dried and mounted in Naphrax® and prepared for LM.

Samples from sea turtles, *Caulerpa taxifolia*, Sinop Aklıman Bay, Giffen collection and Martinique Island were examined by Olympus BX–51 and Nikon Eclipse_Ci microscopes (×1000 magnification) at Dumlupınar University Biology Department in Turkey and Zeiss Axio Imager 2 (Carl Zeiss Microscopy GmbH, Jena, Germany) at the Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin in Poland. ImageJ was used for LM and SEM image measurements.

Ultrastructural analysis was performed with a Hitachi SU–8000 scanning electron microscope at Warsaw University of Technology, Faculty of Materials Science and Engineering, Hitachi S–4500 at Goethe University in Frankfurt am Main, and Hitachi S–4500 at University of Perpignan. Observations on nanostructure of *Olifantiella mascarenica* striae were performed by means of Hitachi NB5000 integrated system that consists of ultra–high performance FIB (40 kV) and high resolution field emission (FE–SEM, 30 kV). This dual beam system enabled high–throughput specimen preparation, high–resolution imaging and analysis of frustule nanostructures. That construction enables SEM imaging during and after FIB cutting. The special low–damage cutting technique has been applied during diatom processing dedicated for materials sensitive to electron beam (e.g., VASILE et al. 1999). The process of cutting was performed at accelerating voltage of 10 kV for FIB. The siliceous valves of *O. mascarenica* and the transapical striae in particular have been cut at nanoscale and imaged prior to and during particular steps of cutting. The valve exterior and interior have been primarily cut along the transapical axis to image the canal–like structure located along the mantle, however, a few cuts along the valve surface apical axis have also been made.

Slides and processed material are deposited at the Department of Biology, Dumlupınar University, Kütahya (Turkey) and diatom collection (SZCZ) of the Palaeoceanology Unit, University of Szczecin, Szczecin (Poland).

RESULTS

Abundant populations of *Olifantiella* have been observed during examination of species composition in scrapes from the loggerhead sea turtle carapaces sampled at Turkish coast nesting grounds. Analysis of *Olifantiella* morphology growing on *C. caretta* revealed the presence of one characteristic taxon, described below as a new species. Some similarity to *O. gorandiana* and *O. mascarenica* was observed by SEM–based analysis of ultrastructure. This is the first report of epizoic populations of *Olifantiella* from loggerhead sea turtles.

We also propose transfer of *Navicula infirmitata* GIFFEN as *Olifantiella infirmitata* (Giffen) Witkowski et Snoeijs comb. nov., with morphological data based on LM and SEM observations.

New *Olifantiella* taxa

Olifantiella seblae Kaleli, Krzywda, Witkowski et Solak sp. nov. (Figs 2–19; LM 2–7, SEM 8–19)

Description: Valves elliptic to lanceolate with rounded to slightly protracted endings. 4.5–14.5 µm long, 1.9–3.2 µm broad. Raphe straight, filiformis. Central raphe endings straight to slightly deflected toward the valve primary side in LM. Transapical striae in LM not resolvable.

Holotype: Slide SZCZ 19776, A.W. collection housed in the University of Szczecin, Poland., leg. Erhan KABUK

Isotype: NHM London, Slide BM 101 921

Type locality: İztuzu Beach, Dalyan (36°42'02"N, 28°41'31"E), Turkey.

Etymology: This species is dedicated to Sebla SOLAK, wife of our friend Dr. Cüneyt SOLAK.

Distribution: Thus far only observed in the type habitat, 177 specimens scrapped from 14 loggerhead sea turtles.

Type habitat: Carapace of nesting Loggerhead Sea turtles from İztuzu Beach, Turkey.

Morphology

LM observations (Figs 2–7)

Valves elliptic to lanceolate, with rounded to very slightly protracted ends (Fig. 2). Striae not detectable in LM, buciniportula may be visible as isolated point (Figs 2–7).

SEM external view (Figs 8–11)

Each transapical stria is composed of one macroareola, parallel at the valve center, becoming radiate and finally again parallel at apices, 40–46 in 10 µm (Figs 8, 9, 11). Process opening oblong to sub-quadrangular (Fig. 10, arrow), close to valve margin (marinal structureless band). External proximal raphe endings expanded, straight to slightly deflected towards the valve primary side (Fig. 10). Striae on valve mantle composed of solitary oblong short macroareola (Fig. 11, arrow), at apices becoming smaller (Fig. 9). Terminal raphe endings terminate below the apices and are slightly deflected (Figs 9, 11), towards the valve primary side (Fig. 9). Girdle composed of several perforated bands, with two rows of apically elongate puncta (Fig. 11).

SEM internal view (Figs 12–19)

Valve interior flat with transapical striae, each composed of one macroareola. Raphe straight, filiformis. Internal central raphe endings simple, expanded, separated with double helictoglossa. Buciniportula composed of two flattened tubes with lateral opening,

elevated (arrows) and located on mid-valve (Figs 14, 17, 19). Macroareola covered by hymenes, here corroded (Figs 14–19).

Taxonomic remarks: *Olifantiella seblae* observed in the epizoic samples and described as new to science revealed high morphologic plasticity. The smallest valves are elliptic– lanceolate with obtusely rounded apices, whereas the largest ones, narrowly lanceolate with somewhat protracted apices. In general this species shares characteristics with *Olifantiella* and pertains to the group that includes *O. gorandiana*. The two taxa are characterized by strongly narrow canal and complex buciniportula. In details the newly described taxon is similar to *Olifantiella gorandiana* in terms of overlapping valve size [length 5–8 µm in *O. gorandiana* (RIAUX–GOBIN & AL–HANDAL 2012, p. 201) versus 4.5–14.5 µm in *O. seblae*] with *O. gorandiana* valves rarely protracted. The two taxa differ in the shape of buciniportula, which in *O. seblae* is composed of 2 fan-like flattened tubes, while there are two supplementary small satellites in *O. gorandiana*. Also one other difference is the process opening position on the valve exterior. In *O. seblae* the process opening is located close to the structureless band along the valve margin, whereas in *O. gorandiana* the opening is always positioned within this band. Terminal raphe endings are slightly bent in the same direction and associated with strongly bent shallow groove in *O. seblae* whereas simple and slightly expanded in *O. gorandiana*. In RIAUX–GOBIN (2015), transapical striae of *O. gorandiana* are parallel throughout becoming radiate at apices, whereas *O. seblae* has transapical striae radiate in the middle, becoming strongly radiate towards apices, with lower stria density (40–46 in 10 µm) compared to *O. gorandiana* (60–63 in 10 µm).

Olifantiella infirmitata (Giffen) Witkowski et Snoeijs comb. nov. (Figs 20–41; LM 20–34, SEM 35–41)

Basionym: *Navicula infirmitata* GIFFEN in GIFFEN (1975). An account of the littoral diatoms from Langebaan, Saldanha Bay, Cape Province, South Africa. *Botanica Marina* 18: 71–95. p. 83, pl. 2, pp. 69–73 reproduced in Figs 6–10.

Type Material: slide 629, Giffen Collection North–West University, Research Unit for Environmental Sciences, South Africa (NIWR)

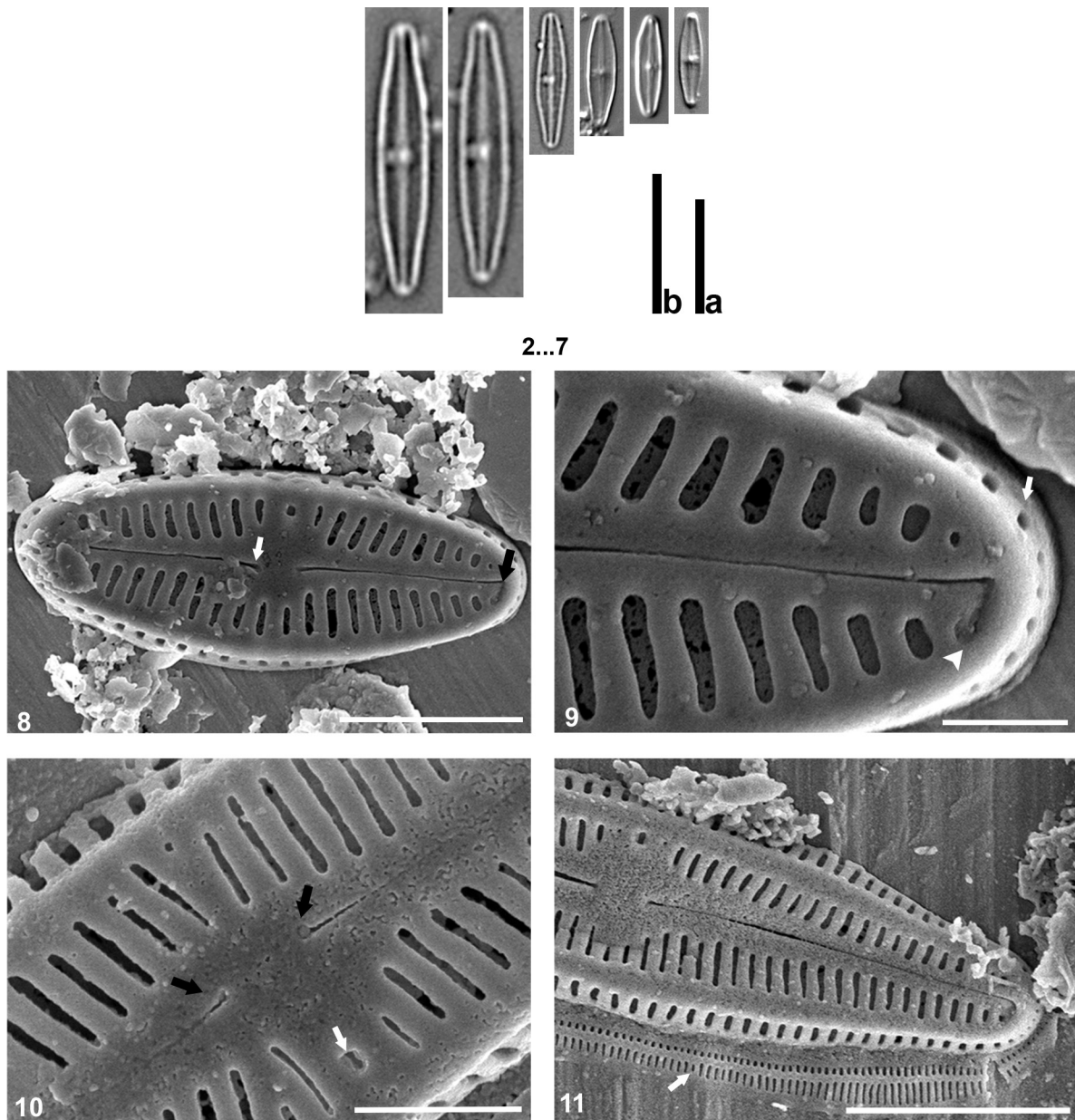
Type habitat: Langebaan Lagoon (33°05'S, 18°02'E), Saldanha Bay, South Africa.

Description: Valves lanceolate with protracted, broadly rounded endings, 9.5–12.0 µm long, 1.9–3.1 µm wide. Raphe straight, external central endings barely observed in LM, approximate. Transapical striae barely resolvable with light microscope, here, only in Figs 13–16.

Morphology

SEM external view (Figs 35–37, 41)

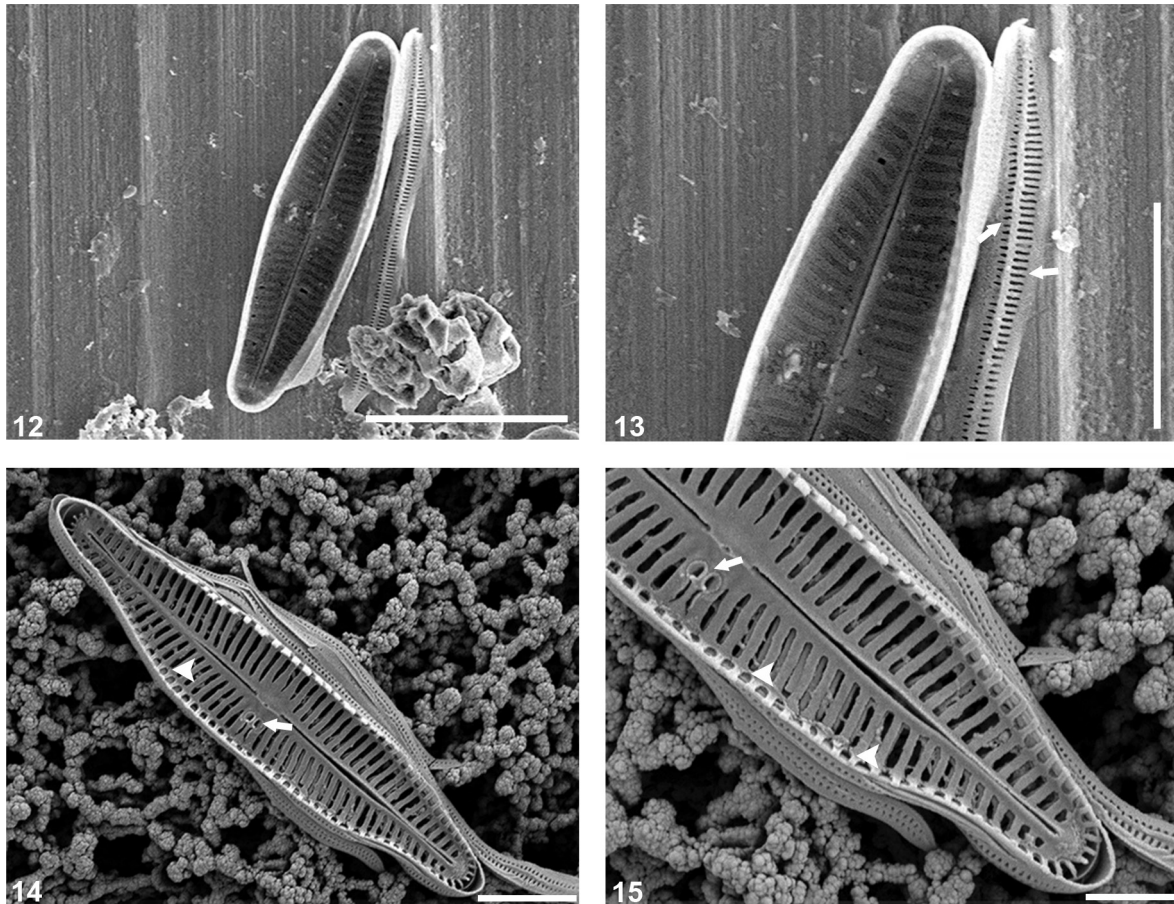
Frustules rectangular in girdle view with rounded



Figs 2–11. *Olifantiella seblae* Kaleli, Krzywda, Witkowski et Solak sp. nov.: (2–7) LM images of *O. seblae*., (5) Holotype image; (8–11) SEM external view of *O. seblae*., (8) Relatively small specimen showing proximal raphe endings bent towards the valve primary side (arrows) and terminal raphe endings (black arrow), (9) Detailed view of apical part of specimen illustrated in Fig. 8, note the distinct groove accompanying terminal raphe endings (arrowhead) and stria on mantle becomes smaller at apices (arrow), (10) Detailed view of central area, note the presence of process opening (arrow), external central raphe endings bent towards the valve primary side (black arrows), (11) Half of specimen showing distinct decrease in size of valve mantle areolae towards apex, note the cingular band with two rows of apically elongate punctae (arrow). Scale bars 10 μm (a, 4–7); 5 μm (b, 2, 3); 2 μm (8); 500 nm (9); 1 μm (10); 3 μm (11).

apices, girdle broad, composed of numerous bands. Girdle bands perforated with several rows of punctae. Valves elliptic–lanceolate in smallest to linear–lanceolate in longest specimens. Apices slightly set off to distinctly protracted, obtusely rounded. In SEM, striae composed of transapically positioned macroareola (BUKHTIYAROVA 2006), parallel in the middle, becoming slightly radiate at apices, 36–40 in 10 μm . In SEM, transapical striae parallel in the middle becoming radiate at protracted apices (Fig. 35). In Fig. 36, process opening is observed (arrowhead). Striae forming

macroareolae are depressed below valve face. The areola are covered with hymenes (Fig. 36, arrows). Valve face flat, with an opening of the buciniportula viewed within the central area (note the marginal remaining portion of the stria). In LM (Figs 31–34) and in the drawings of GIFFEN (1975) this structure is indicated as distinct punctum or stigma (Figs 20–24). The transapical striae depression below the valve face have a small opening (or fenestrulae, ca. 100 nm in diameter) penetrating towards the marginal canal. A large structureless area occurs in the transition from valve face



Figs 12–15. *Olifantiella seblae* Kaleli, Krzywda, Witkowski et Solak sp. nov.: (12–13) A series of images of same specimen illustrating valve internal structure. Fig. 12. Whole specimen internal view, (13) Close up of a specimen illustrated in Fig. 12, detailed view of girdle band with two rows of narrowly elongated puncta, note the shorter puncta on advalvar part of cingular band (arrows); (14, 15) Corroded specimen showing the internal valve view areolae, buciniportula and narrow canal, (15) Close up of the specimen illustrated in Fig. 14, note the structure of the buciniportula (arrow) and the narrow canal through the valve (arrowheads). Scale bars 5 μm (12); 3 μm (13); 2 μm (14); 1 μm (15).

to mantle, being the roof of a canal-like structure (see internal view, Fig. 38, arrows).

The marginal canal opens externally by small and round fenestrulae (Fig. 40, arrowheads). The striation on the valve mantle ends by solitary round areolae or fenestrulae. Buciniportula is solitary (Figs 39, 40, arrowheads) with mantle areola at the apices becoming apically elongate (Fig. 37, arrowhead). Raphe straight, filiform with external central endings very slightly expanded and slightly deflected towards valve primary side (white arrow). Terminal raphe endings are simple, slightly expanded and terminate at the transition between valve face and mantle (Fig. 37). In SEM, the process opening is oblong (arrow in Fig. 36).

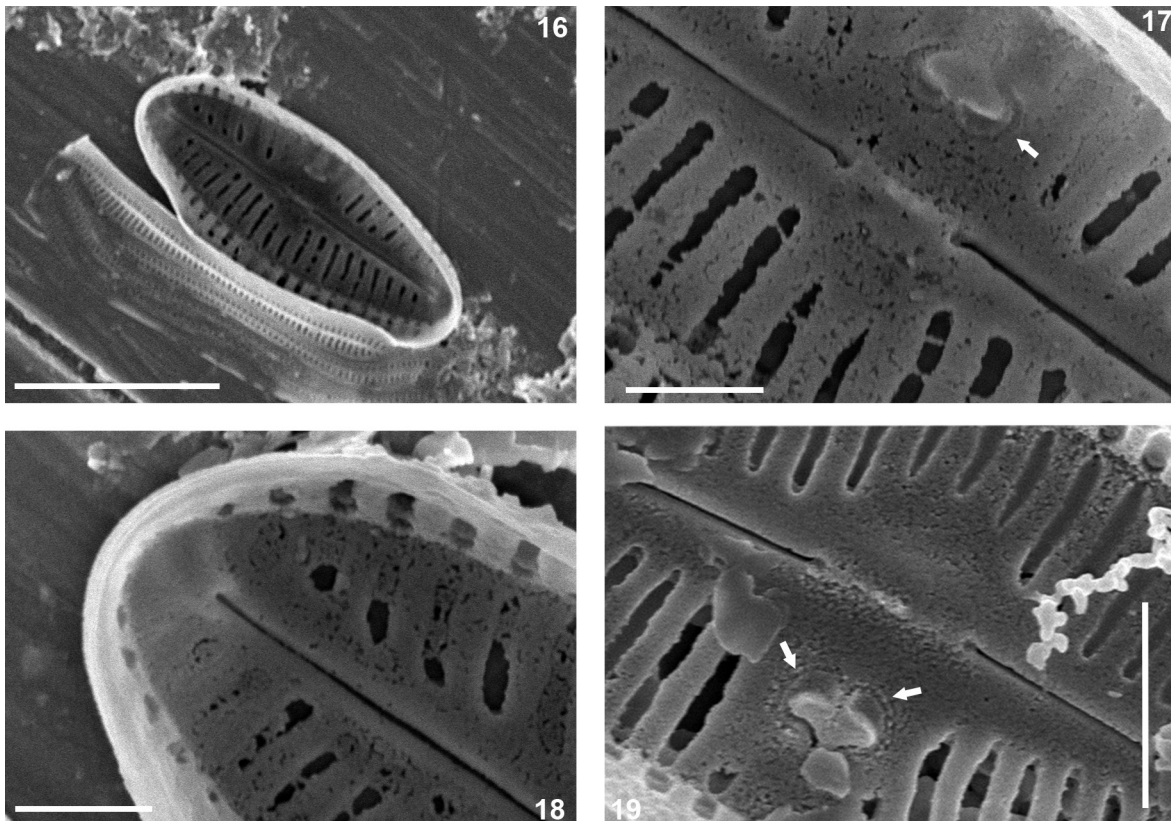
SEM internal view (Figs 38–40)

Valve interior flat with transapical striae composed of macroareola slightly elevated and virgae slightly depressed. Raphe straight and slit-like with internal proximal endings simple (Note a nodule in between these endings). Buciniportula solitary, trumpet-like, elevated above the valve surface. Internal side of valve mantle possess elevated, marginal canal structure continues

along the valve mantle between both apices (Fig. 38).

Taxonomic remarks: Despite a small size and hyaline valve structure, GIFFEN (1975) observed the presence of a stigma or punctum but stated that this species is easily overlooked, with LM images often not informative enough to make consistent measurements. Ultrastructure of *N. infirmitata* indicates that this species belongs in *Olifantiella*. Specimens possess transapically elongate striae composed of single areola and tubular buciniportula. In terms of valve ultrastructure this taxon pertains to the group of taxa that includes *Olifantiella mascarenica*. The measurements of both taxa are similar (Table 1), with differences observed in the proximal raphe endings in external view.

The perforation of cingular bands, as well as fenestrulae, are also smaller in *O. infirmitata*. The striation is more or less similar being somewhat denser in *O. mascarenica* than in *O. infirmitata* (Table 1) and also quite parallel in *O. mascarenica*. The major difference between the two taxa is the marginal structureless area, which is narrow in *O. mascarenica* and broad in



Figs 16–19. *Olifantiella seblae* Kaleli, Krzywda, Witkowski et Solak sp. nov.: (16) View of valve interior; (17, 19) Buciniportula composed of two flattened fan-like tubes (arrows); (18) Internal terminal raphe ending in the form of simple helictoglossa. Scale bars 3 μ m (16); 500 nm (17, 18); 1 μ m (19).

O. infirmitata. Fenestrulae in *O. infirmitata* are significantly smaller than in *O. mascarenica*. Likewise the mantle striae are elliptical in *O. mascarenica* whereas small and oblong in *O. infirmitata*.

Established *Olifantiella* taxa

Olifantiella cf. *mascarenica* Riaux–Gobin et Compère (Figs 42–57)

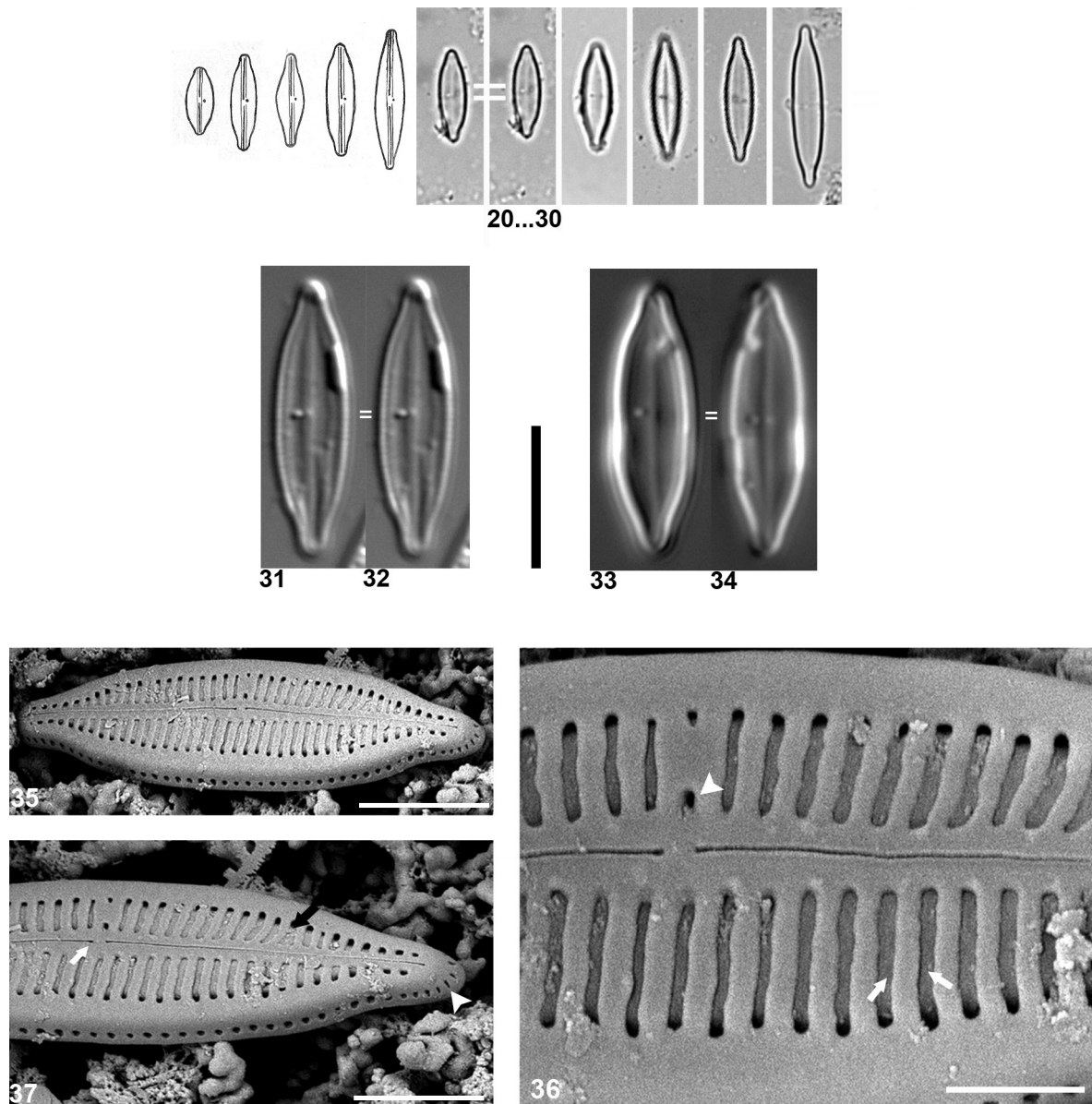
Specimens observed from Black Sea samples possess the characteristics of *O. mascarenica* described from Rodrigues Island by RIAUX–GOBIN & AL–HANDAL (2012). Specimens collected from Sinop Bay, Black Sea coast have valves 6.0–10.3 μ m in length, 2.0–2.7 μ m in width and 40 striae in 10 μ m. The Black Sea specimens have somewhat more elongate ends with solitary tubular buciniportula. Adriatic Sea specimens have slightly elongated endings with buciniportula tubular and closer to central area in internal view, and were slightly longer, 7.8–11.1 μ m, width 2.3–2.5 μ m and with somewhat higher stria density 40–50 in 10 μ m. Some valves resemble *O. mascarenica* illustrated in RIAUX–GOBIN & AL–HANDAL (2012; Figs 4, 5) with distinctly elongated endings, 9.1–10.8 μ m in length, 1.9–2 μ m in width and again with higher stria density, 45–55 in 10 μ m. The transapical striae are parallel throughout and become slightly convergent at apices

in all populations. Due to the fact that the Black Sea and Adriatic Sea specimens possess somewhat more elongated valves and lower stria density we decided to keep this species tentatively as *O. cf. mascarenica* until further SEM studies results will be available.

Olifantiella pseudobiremis Riaux–Gobin (Figs 58, 59)

Only one valve of *O. pseudobiremis* was observed in sample from Sinop, Akliman Bay. The valve was 6.0 μ m in length and 2.0 μ m in width with 40 striae in 10 μ m. This species was relatively rare in the type locality (Rodrigues Island, Indian Ocean, in RIAUX–GOBIN 2015), but has been later observed at Nosy Be Island (Madagascar) and in the South Pacific (Moorea Island, Society Archipelago, RIAUX–GOBIN pers. obs.).

Taxonomic Remark: *Olifantiella pseudobiremis* (one specimen observed during the present study) is described as rare and the probable tubular process (as previously observed through the valve in RIAUX–GOBIN & AL–HANDAL 2012, fig. 69) has not previously been detailed. The oblong external opening of the process is located in the central area of the frustule, near the proximal raphe endings, as in the *O. mascarenica* group.



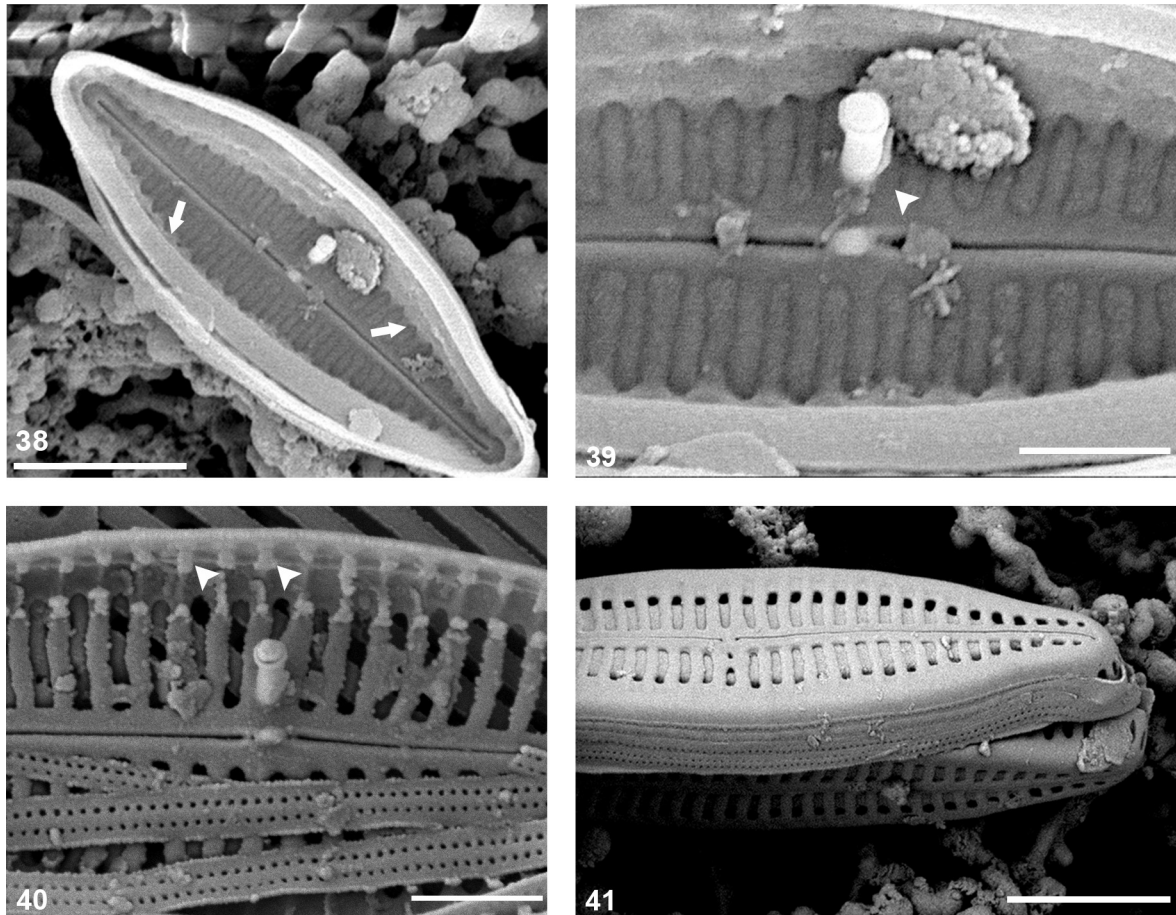
Figs 20–37. *Olifantiella infirmitata* (Giffen) Witkowski et Snoeijs comb. nov.: (20–24) Original drawings of *Navicula infirmitata* published by GIFFEN (1975, pl. 2, pp. 69–73); (25–34) LM images from the holotype slide in Giffen collection, sample 629; (35–37) External valve view, (35) External view of the whole specimen, (36) Close up of specimen illustrated in Fig. 35. Note the process opening (arrowhead) and hymenes covering the macroareolae (white arrows), (37) Close up of valve middle part of specimen illustrated in Fig. 35. Note the external proximal raphe endings slightly bent towards valve primary side (white arrow), and presence of Voigt discontinuity shown on valve secondary side (black arrow). Areola become apically elongate at the apices (arrowhead). Scale bars 10 μm (20–30), 5 μm (31–34), 3 μm (35), 900 nm (36), 2 μm (37).

Cutting *Olifantiella mascarenica* valves via FIB technique (Figs 60–63)

Specimens of *O. mascarenica* for nanocuts originated from Martinique Island (see above). Nanocuts were made on external and internal valve views and along apical axis in external position. Descriptions were clarified with traditional SEM (RIAUX–GOBIN 2015), processing of valves revealed presence of a canal (similar to a bridge) along the valve mantle. The presence of septa delineating short chambers was not observed. The canal-like structure has no transverse subdivisions and entire over the valve periphery. The canal opens externally *via* fenestrulae ca. 100 nm in diameter (see

definition in RIAUX–GOBIN 2015). The buciniportula opening on valve exterior is single for *O. mascarenica*, perpendicular to the valve internal surface. The external elliptical opening is close to the raphe and an inlet to buciniportula tube. Nanocuts through the valve face openings show that the second, elongate structure appears not connected to buciniportula tube inside the cell (Fig. 60, arrow). The larger opening appears to be modified striae and connected to the channel-like structure. The cingular bands have two rows of puncta (Fig. 60, black arrows).

Remarks: Attempts to nanocut frustules from the *O.*



Figs 38–41. *O. infirmitata* (Giffen) Witkowski et Snoeijns comb. nov.: (38) Internal valve view with marginal canal (arrows); (39) Detail of specimen illustrated in Fig. 38, internal valve view with focus on buciniporcula closed by a plug (arrowhead); (40) Internal valve view, note the corroded canal and hymenate areola occlusions. Well developed is the simple slit-like raphe with internal proximal raphe endings and small double helictoglossa. (41) Oblique view of the valve face and mantle with displaced, perforated girdle bands. Scale bars 2 μ m (38, 41), 700 nm (39), 900 nm (40).

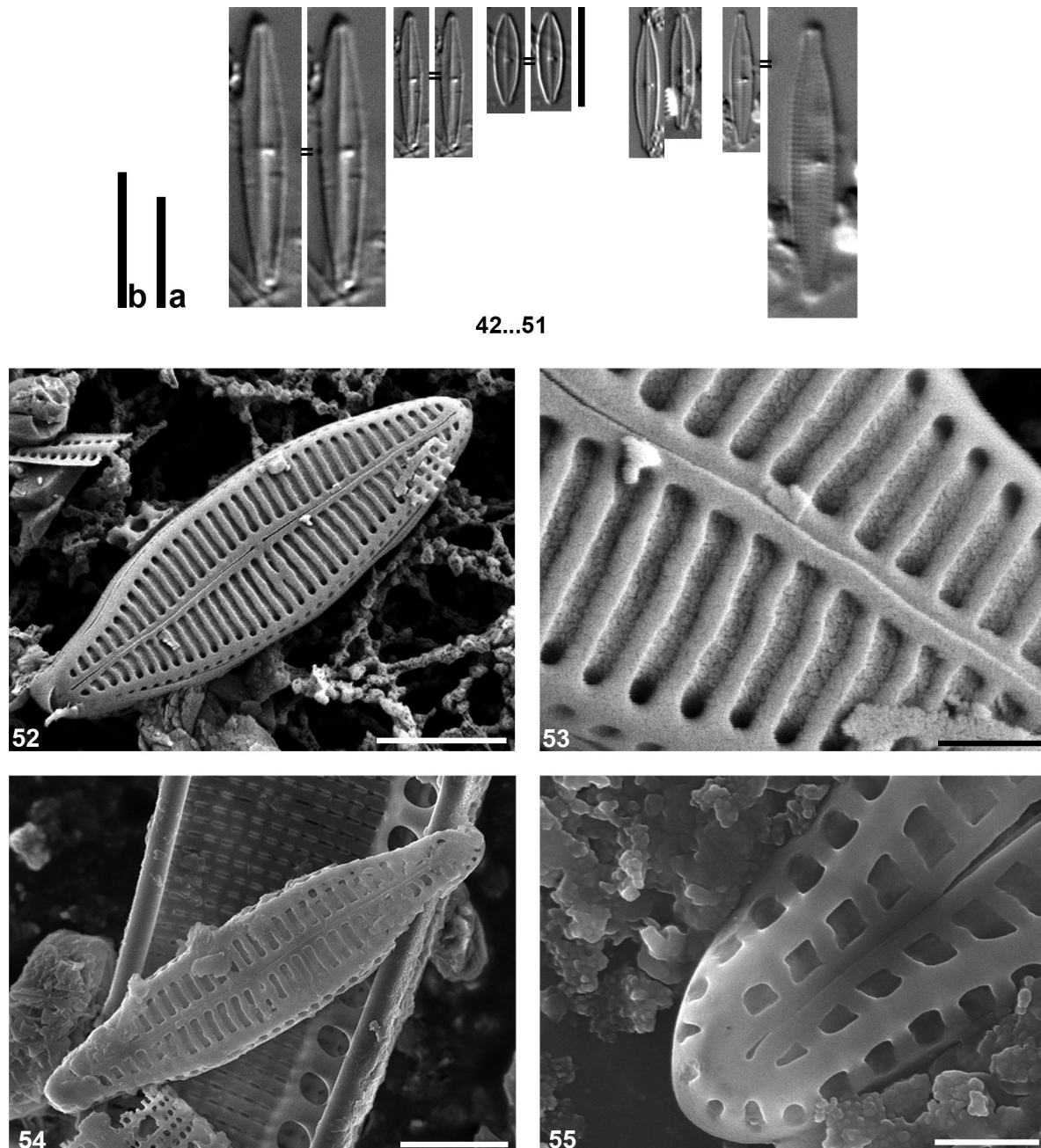
gorandiana group failed. We did not observe the presence of extra space within the valve interior, while RIAUX–GOBIN & AL–HANDAL (2012, Figs 30–31) clearly show small spaces that they interpreted as narrow fenestrulae. Possibly corroborating the latter observation, under SEM, a strongly narrow canal was observed in the internal side of a corroded specimen of *O. seblae* (see arrowheads in Figs 14–15).

DISCUSSION

The genus *Olifantiella* was established relatively recently (RIAUX–GOBIN & COMPÈRE 2009); Taxa belonging in this genus are small and have structures barely resolvable in LM and are thus difficult to identify. The use of electron microscopy is essential for reliable identification. The generic position of taxa belonging in *Olifantiella* is well determined by genus-specific processes – buciniporcula and girdle bands (e.g., RIAUX–GOBIN 2015). However, species identification

requires a thorough ultrastructure analysis of valve external and internal surfaces including external proximal and terminal raphe endings, transapical striae and valve mantle.

Based on SEM observations, the following characters have been established as typical for *Olifantiella*: 1) presence of a more or less raised buciniporcula, 2) each stria composed of one macroareola, 3) external proximal raphe endings bent towards the valve primary side, 4) hyaline band present along the valve–mantle boundary and internally the canal is more or less developed and present along the mantle interior, and 5) broad cingulum composed of numerous perforated bands (generally with two rows of puncta). In all established species, macroareolae are positioned below the valve surface and covered by finely perforated hymenes (RIAUX–GOBIN & COMPÈRE 2009; RIAUX–GOBIN 2015). Despite these consistent characters, some variation in *Olifantiella* species is observed. Our observations confirm two groups of taxa belonging in *Olifantiella*. The first taxon treated in this survey *O. infirmitata* is closely related to the generitype *O. mascarenica*, and is here presented in the *O. mascarenica*

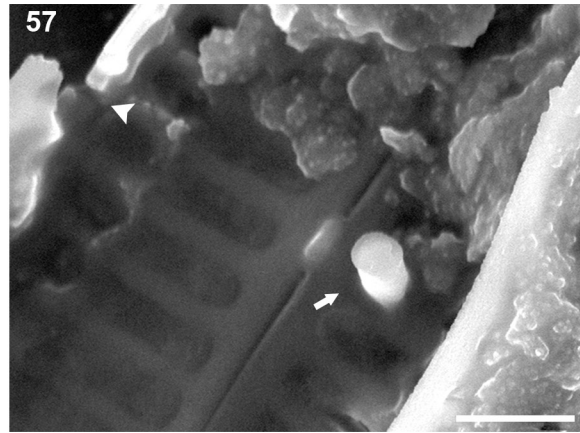
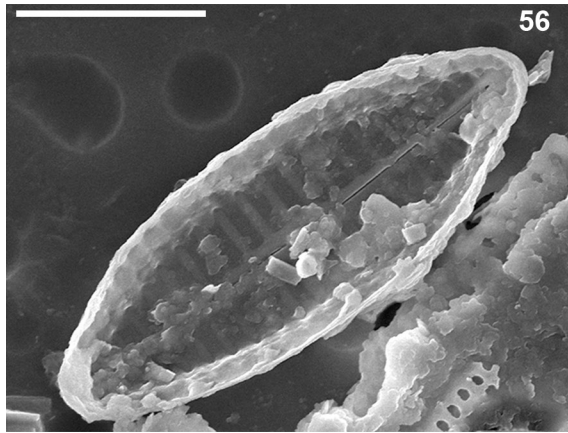


Figs 42–55. *Olifantiella* cf. *mascarenica* Riaux–Gobin et Compère: (48–51) Specimens from Sinop, Black Sea coast; (42–47, 52, 53) Specimens from Hvar Island, Adriatic Sea; (54, 55) Specimens from Sinop, Black Sea coast; (51) External valve view of *O.* cf. *mascarenica*; (52) Illustration of terminal raphe ending. Scale bars 10 μm (a, 44–50), 5 μm (b, 42, 43, 51); 2 μm (52, 54), 400 nm (53), 500 nm (55).

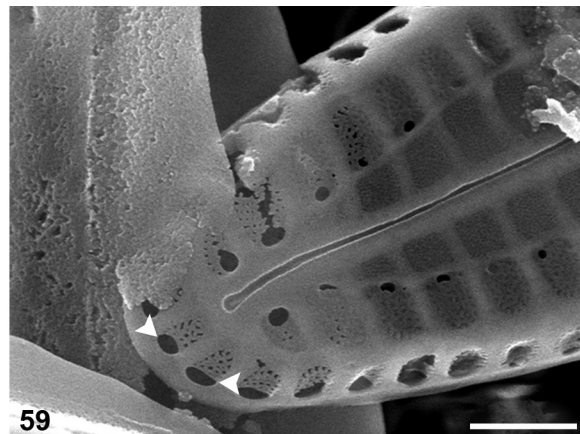
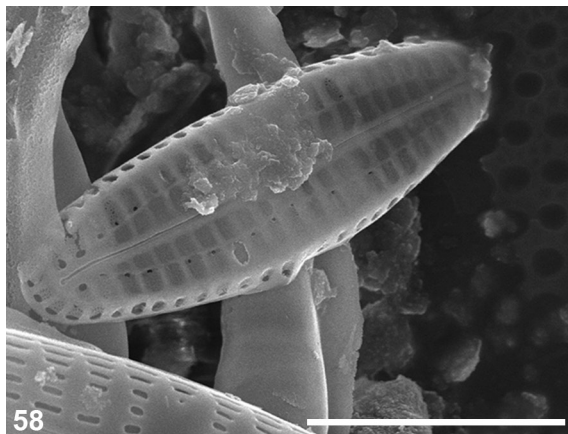
group. However, the two taxa differ in terms of marginal hyaline area at the valve face and mantle junction. The latter structure is narrow in *O. mascarenica* and broad in *O. infirmitata*. The fenestrulae are narrower in *O. infirmitata*. The mantle macroareolae are elliptical in *O. mascarenica* whereas small and oblong in *O. infirmitata* (Table 1).

In most taxa, including the generitype *O. mascarenica*, a canal spreading over the whole valve occurs at the mantle and valve face junction. This character, at first sight, is missing in other species belonging in the *Olifantiella gorandiana* group, but closer examination

of *O. gorandiana* reveals a very narrow canal (RIAUX–GOBIN & AL–HANDAL 2012, see also SEM images of *O. seblae*, Figs 14 and 15). The separation of the two above groups of taxa are also principally supported by buciniportula structure and apical raphe endings. Variations in buciniportula structure include: 1) group conforming to *O. mascarenica* and *O. rodriguensis* with one or two tubular processes lying perpendicular/upright to the valve surface, and 2) group that includes *O. gorandiana* with complex buciniportula of tubular structures lying close to the valve. Terminal raphe endings in *O. mascarenica* group are simple and



Figs 56–57. *Olifantiella* cf. *mascarenica*. Specimens from Sinop, Black Sea coast: (56) Internal valve view of *O.* cf. *mascarenica*; (57) Close up view of buciniportula. Scale bars 1 μ m (56); 500 nm (57).



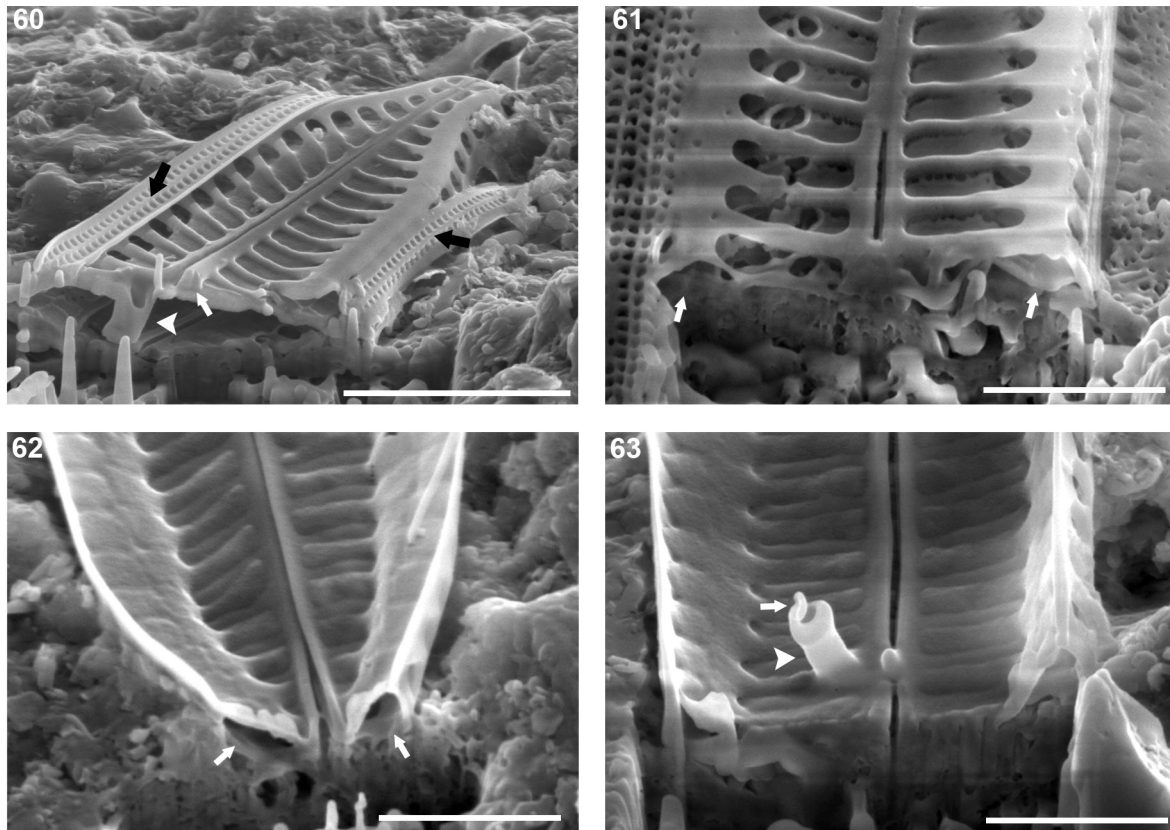
Figs 58–59. *Olifantiella pseudobiremis* Riaux–Gobin from Sinop, Black Sea coast: (59) Detailed view of specimen illustrated in Fig. 58, note the short striae composed of macroareolae covered by complex and granulated hymenes showing small free apertures (arrowheads). Terminal raphe ending simple and slightly bent (Fig. 59). Scale bars 3 μ m (58); 500 nm (59).

terminate somewhat below apices. Members of the *O. gorandiana* group also have raphe endings simple, that terminate below apices, but are slightly bent. Note that *O. seblae*, a taxon included in the *O. gorandiana* group, is characterized by a shallow groove that continues over the apex surface, in continuation of the terminal raphe ending. Morphological features comparison of these two groups of species with similar taxa is provided in Table 1.

Olifantiella was previously reported predominantly from tropical Indo–Pacific coral reef habitats (RIAUX–GOBIN & COMPÈRE 2009; LOBBAN et al. 2012; RIAUX–GOBIN & AL–HANDAL 2012; RIAUX–GOBIN 2015). The genus was subsequently observed in the tropical West Caribbean, from coral habitat built on volcanic boulders and from bays with silt bottom and high turbidity (DESROSIERS 2014). At Martinique Island several established species including *O. mascarenica*, *O.* cf. *paucistriata* and *O. gorandiana* have been identified, next to several more taxa determined as *Olifantiella* sp. (DESROSIERS 2014). More recently the occurrence of *Olifantiella* has been observed near

Antwerp (North Sea, VAN DE VIJVER et al. 2016) on the temperate Atlantic coast of Belgium. The present research shows *Olifantiella* to have a much broader geographic distribution that is a common component of temperate marine and brackish–water environments. *Olifantiella* species have been observed throughout the Mediterranean (Adriatic, Aegean) and abundant at places in the Black Sea. *O.* cf. *mascarenica* and *O. pseudobiremis* have been identified to a species level, however, analyses based on SEM are necessary to better estimate *Olifantiella* biodiversity of the genus in this geographic region.

Although habitats of the Mediterranean and Black Seas differ in terms of climatic conditions from the coral reef habitats, *Olifantiella* species established in sub–tropical to tropical Indo–Pacific, and Caribbean appear to tolerate fluctuations of marine water temperature and salinity. The Black Sea coast Akliman Bay, Sinop specimens of *Olifantiella* cf. *mascarenica* (Figs 42–55) have tubular buciniportula process and overall size dimensions that compare well to the RIAUX–GOBIN & AL–HANDAL (2012, Figs 4–10) illustrations



Figs 60–63. Nanocuts with FIB of *Olifantiella mascarenica* Riaux–Gobin et Compère from Martinique Island, Atlantic Ocean: (60) External valve view cut through central nodule (arrow) showing opening of the buciniportula (arrowhead), please note the cingular band with two rows of puncta (black arrow); (61) Marginal canal of cut valve external surface (arrows); (62) Cut of internal valve view showing canal (arrows) at the apex; (63) Cut of valve interior with hollow buciniportula close to the central nodule (arrowhead) and the canal. Note the small flap (plug) (arrow) partially closing the buciniportula (Fig. 63). Scale bars 2 μm (60, 61), 1 μm (62, 63).

from the original description of the species, except for more elongate shape and slightly lower stria density. *Olifantiella pseudobiremis*, described from Rodrigues Island as extremely rare by RIAUX–GOBIN & AL–HANDAL (2012), was observed as single specimen in multiple samples from Sinop, Akliman Bay, Black Sea coast (Figs 58, 59) is a new record for Turkish diatom flora, but this taxon was also recently reported from Nosy Be Island (Madagascar) and Society Archipelago in the South Pacific (RIAUX–GOBIN unpublished observations). A large marginal canal is well identifiable for the latter taxon, with complex fenestrulae partly closed by ornamented complex velum. As well as in the original description, terminal raphe endings are slightly deflected towards valve primary side and each fenestrulae is covered by a granular velum and possess a small opening with two small spines. In addition, several species of *Olifantiella* have been observed in Crete and Greek Aegean Sea Islands (ANNA ULANOVA, pers. comm. May 2017).

The present observations and transfer of *Navicula infirmitata* GIFFEN expand the geographical repartition of *Olifantiella infirmitata* comb. nov. with type habitat in SW South Africa (Langebaan Lagoon, GIFFEN 1975). The taxon was also recorded by SNOEIJIS & KAUTSKY (1989) as *Navicula infirmitata* in the Baltic

Sea during the spring ice–break up. This species was also abundant in nuclear power station cooling waters located on the Swedish east coast (SNOEIJIS & NOTTER 1993; SNOEIJIS & BALASHOVA 1998).

The FIB technique brings more precise observations in nanoscale. Recently published results of molecular phylogeny of *Olifantiella* sp. from the Yellow Sea (HAN et al. 2018) show this genus to be in close relationship with *Luticola* D.G. MANN in ROUND et al. (1990) are together placed in Diadesmidiaceae D.G. Mann. Although in gross morphology *Olifantiella* and *Luticola* are different in respect to striation and girdle formation, the two genera appear to share some morphological characters: e.g., the presence of an internal channel–like structure and internal complex and hollow process in both genera. Due to the usually robust structure of the valves in *Luticola* the channel positioned on the valve face / mantle transition can be observed in standard SEM examination or even in LM (LEVKOV et al. 2013). Recently BAĞ et al. (2017) have illustrated the channel ultrastructure of *L. galapagoensis* Witkowski, Bağ, Kociolek, Lange–Bertalot et Seddon performed with the FIB. Indeed the two genera share this character positioned between the valve face and mantle. Nanocuts of *Olifantiella mascarenica* valves reveal the ultrastructure of the valve/mantle transition

Table 1. Morphological characters of some species of genus *Olifantiella*. vps: Valve primary side.

Feature	<i>O. gorandiana</i> group		<i>O. mascarenica</i> group			
	<i>O. gorandiana</i> Riaux–Gobin	<i>O. seblae</i> sp. nov.	<i>O. mascarenica</i> Riaux–Gobin et Compère	<i>O. cf. mascarenica</i>	<i>O. infirmitata</i> comb. nov.	<i>O. pseudobiremis</i> Riaux–Gobin
Valve Shape	elliptical, slightly elongated endings	elliptic–lanceolate, rounded to protracted endings	elliptical, apiculate endings	elliptical, slightly elongated endings	lanceolate, protracted broadly rounded endings	naviculoid–elongate
Length μm	6.8–8.1	4.5–14.5	8.4–10.7	6.0–11.1	9.5–12	6–12
Width μm	1.8–2.3	1.9–3.2	2.1–2.7	1.9–2.7	1.9–3.1	1.6–3
Striae in 10 μm	60–63	40–46	42–49	40–55	36–40	28–40
Striae direction	parallel throughout, becoming slightly radiate at apices	parallel at the center, becoming slightly radiate, parallel at apices	sub–parallel	parallel throughout, becoming slightly convergent at apices	parallel in the middle, slightly radiate at apices	slightly radiate
Proximal raphe endings	inflated, slightly deflected away from process opening	expanded, straight to slightly deflected towards vps	inflated, very slightly deflected away from process opening side	inflated, very slightly deflected away from process opening side	very slightly expanded, deflects towards vps	slightly inflated, deflected away from the foramen
Terminal raphe endings	simple, straight	deflected towards vps, ends gently bent	simple, slightly deflected on foramen side	simple, slightly deflected on foramen side	straight, slightly expanded	simple
Buciniportula	multiple, flattened, (not erected), with two satellites	double, flattened tubular	simple, raised, plugged	simple, raised, plugged	double, covered with a plug like structure	not observed
External process, opening, shape	trapezoidal, deeper split into four sectors, close to margin	oblong, near margin	oblong, simple, close to central area	oblong, simple, close to central area	oblong, single pore	oblong, simple, close to central area
Girdle bands	several, two rows of more or less oblong poroids	two rows of puncta	several, opened, two rows of elliptical small poroids	several, opened, two rows of elliptical small poroids	several rows of small and oblong puncta	several, two rows of puncta
Marginal canal	strongly narrow	strongly narrow	present	present	present	present

Dichotomous key to distinguish the taxa observed in this study, from *Olifantiella mascarenica*, *O. gorandiana* and *Labellicula subantarctica*

- 1 marginal canal–shaped structure very high to moderately high.....2
 1 marginal canal very low or sub–absent.....3
 2 fenestrulae partially closed by an ornamented velum.....*Olifantiella pseudobiremis*
 2 open fenestrulae, more or less wide, raised tubular buciniportula.....4
 3 process as simple stigmata, sub–rostrate valve shape.....*Labellicula subantarctica*
 3 process otherwise.....5
 5 buciniportula composed of 2 flattened tubes and 2 satellites, terminal raphe endings simple
*Olifantiella gorandiana*
 5 buciniportula composed of 2 flattened hollow ‘fan–like’ structure, apical groove
*Olifantiella seblae*
 4 fenestrulae relatively large.....*Olifantiella mascarenica*
 4 fenestrulae narrow.....*Olifantiella infirmitata*

and allow to perform detailed observations on buciniportula and other valve characters of diatom species and genera.

CONCLUSIONS

Our observations indicate that *Olifantiella* taxa may be generally present on marine coasts with variable climatic conditions. *Olifantiella* diversity is best studied and highest in tropical marine coasts of Indo–Pacific coral reef habitats, but also reported in this first study from the Caribbean with somewhat less diversity in the Central and Eastern Mediterranean. Whereas water salinity is high in the Mediterranean, temperature is subject to annual oscillations from high in summer to much lower in winter. *Olifantiella* on Black Sea Turkish coasts appears to be abundant and includes warm water Indo–Pacific species and local elements, which require SEM examination. Seemingly, the oceanic *Olifantiella* species adapted to the Black Sea brackish–water conditions. *Olifantiella* seems to be a common component of the diatom assemblages of the temperate climate regions (SW Atlantic coasts of South Africa, the North Sea coast in Belgium, the Baltic Sea coast in Sweden). Finally, the abundant occurrence of epizoic *Olifantiella* on the marine turtles carapaces nesting in the Aegean east coast of Turkey is here described.

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