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Chelonicola and *Poulinea*, two new gomphonemoid diatom genera (Bacillariophyta) living on marine turtles from Costa Rica

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Abstract

Marine mammals such as whales and dolphins have been known for a long time to host a very specific epizoic community on their skin. Less known however is the presence of a similar community on the carapaces of sea turtles. The present study is the first describing new taxa inhabiting sea turtle carapaces. Samples, collected from nesting olive ridley sea turtles (*Lepidochelys olivacea*) on Ostional Beach (Costa Rica), were studied using light and scanning electron microscopy. Two unknown small-celled gomphonemoid taxa were analysed in more detail and are described as two new genera, closely related to other gomphonemoid genera with septate girdle bands, such as *Tripterion*, *Cuneolus* and *Gomphoseptatum*. *Chelonicola gen. nov.* has a flat valve face, uniseriate striae composed of more than three areolae, simple raphe external endings, internally a siliceous flap over the proximal raphe endings and lives on mucilaginous stalks. *Poulinea gen nov.* has at least one concave valve, uniseriate striae composed of only two elongated areolae, external distal raphe endings covered by thickened siliceous flaps and lives attached to the substrate by a mucilaginous pad. *Chelonicola costaricensis sp. nov.* and *Poulinea lepidochelicola sp. nov.* can be separated based on stria structure, girdle structure composed of more than 10 copulae, raphe structure and general valve outline. A cladistics analysis of putative members of the Rhoicospheniaceae indicates that the family is polyphyletic. *Chelonicola* and *Poulinea* are sister taxa, and form a monophyletic group with *Cuneolus* and *Tripterion*, but are not closely related to *Rhoicosphenia*, or other genera previously assigned to this family. Features used to help diagnose the family such as symmetry and presence of septa and pseudosepta are homoplastic across the raphid diatom tree of life.

Keywords: Bacillariophyta, cladistics, Costa Rica, epizoic diatoms, marine turtles, new genus, phylogenetic analysis.

Introduction

During a survey of the epizoic flora on marine olive ridley sea turtles (*Lepidochelys olivacea* Eschscholtz 1829), several small, unknown gomphonemoid diatom taxa were observed that could not be identified using the currently available (though sparse) literature about these genera. At present, several small-celled gomphonemoid genera are known from the marine environment. *Cuneolus* Giffen (1970: 90) was described in 1970 from the African coast. Two others were split off in 1986 by Medlin and Round from the freshwater genus *Gomphonema* Ehrenberg (1832: 87): *Gomphonemopsis* Medlin in Medlin & Round (1986: 207) and *Gomphoseptatum* Medlin & Round (1986: 212). An interesting feature of *Cuneolus* and *Gomphoseptatum* is the presence of septa on the valvocopulae (lacking in *Gomphonemopsis*), usually only found in araphid genera (Van de Vijver *et al.* 2012). Holmes *et al.* (1993a) described a third gomphonemoid genus bearing similar septa, living epizoically on the skin of porpoises: *Tripterion* R.W.Holmes *et al.* (1993a: 7). So far, these septa-bearing genera are rather species-poor with only two species known in *Gomphoseptatum* (Medlin & Round 1986, Witkowski *et al.* 2000), one in

Cuneolus (Giffen 1970, Medlin & Round 1986) and three in *Tripterion* (Holmes *et al*. 1993a, b, Fernandes & Sar 2009).

In this paper, we focus on two taxa that were recently observed living epizoically on the carapaces of sea turtles in Costa Rica. For a long time, epizoic diatom taxa were only known living either on bird feathers (Holmes & Croll 1984: genus *Pteroncola* R.W.Holmes & Croll 1984: 267) and the skin and teeth of whales and dolphins (Denys 1997, Denys & Van Bonn 2001 and references therein). Apart from some occasional observations of diatom taxa in samples scraped off from whales that normally prefer other habitats, a limited number of genera seemed to be restricted to this particular habitat such as for instance *Epiphalaina* R.W.Holmes *et al*. (1993a: 4), *Bennetella* R.W.Holmes (1985: 48) and *Plumosigma* T.Nemoto (1956: 111). Almost all recorded taxa were only known from the marine environment as most whales and dolphins are restricted to a marine life. Recently however, several new epizoic diatoms were described from a freshwater turtle in the Rio Negro (Wetzel *et al*. 2010, 2012), including one taxon belonging to the presumably exclusively marine ceticolous genus *Tursiocola* R.W.Holmes *et al*. (1993a: 5). The discovery of these epizoic diatoms on aquatic turtles raised interesting research opportunities for the study of epizoic diatoms on other aquatic and marine animals such as marine turtles. In 2010, some preliminary results were presented during the 21st IDS conference in St. Paul (USA) (Brady 2010, pers. comm.), although no follow-up paper on this research was published afterwards. Recently, three new *Tursiocola* taxa were described from West Indian manatees (Frankovich *et al*. 2015).

Based on light microscopical observations, it was almost impossible to separate the two taxa living on turtles but detailed analysis of their ultrastructure revealed important morphological differences, excluding not only conspecificity but also the position of both taxa within the same genus. Careful comparison of the features of both taxa with all small-celled gomphonemoid genera known so far (see above), led to the conclusion that both taxa cannot be attributed to either of them and should be placed in two new genera. The present paper describes therefore these two new genera *Poulinea* Majewska, De Stefano & Van de Vijver, *gen. nov*., typified by *P. lepidochelicola* Majewska, De Stefano & Van de Vijver, *sp. nov*., and *Chelonicola* Majewska, De Stefano & Van de Vijver, *gen. nov*., typified by *C. costaricensis* Majewska, De Stefano & Van de Vijver, *sp. nov*. Both genera possess a unique combination of morphological features, compared to other, similar, small-celled gomphonemoid genera.

Material & methods

Epizoic samples used in this study were collected in October 2013 from the turtles in Ostional Beach on the Pacific coast of Costa Rica during their nesting event (*arribada*). Approximately 20 cm^2 of arbitrarily chosen carapace pieces of several olive ridley sea turtles were scraped off when the turtles came ashore to lay eggs. Although olive ridley sea turtles are a protected species, they breed with success in Ostional and are currently not endangered there. A collection of epizoic diatoms, epibionts, and ectoparasites was made by scraping individual turtle carapaces with a razor. The method is not invasive, as it is limited to the most external part of the turtle carapace scutes, and it does not harm or cause the animal suffering. All sampling procedures took place as approved by MINAE under close supervision of SINAC park rangers. All procedures involved respect the ethical standards in the Helsinki Declaration of 1975 (revised in 2000 and 2008), as well as all applicable national laws.

Samples were kept in seawater and preserved immediately with 4 % formaldehyde. In order to remove all organic material, carapace sub-samples were digested following a slightly modified method by von Stosch (Hasle & Syvertsen 1997) using a mixture of boiling concentrated acid (64 % nitric acid and 97 % sulphuric acid added at a 1:3 volume ratio). Following digestion and centrifugation, cleaned material was rinsed and diluted with deionized water. For light microscopy (LM) analysis, cleaned material was mounted permanently on glass slides using Naphrax® and observed using an Olympus BX53 microscope, equipped with Differential Interference Contrast (Nomarski) and the Olympus UC30 Imaging System. Samples and slides are stored at the Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, II University of Naples, and the BR-collection, property of the Belgian federal government and given in permanent loan to the Botanic Garden Meise (Belgium). For scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered through a 1-μm Isopore™ polycarbonate membrane filter (Merck Millipore).

The second part of the collected material was cut into *ca*. 2 cm^2 squares and dehydrated by immersion in alcohol series at increasing gradation (20, 30, 40, 50, 60, 70, 80, 90, 95, 100 % alcohol solutions in distilled water). Subsequently, carapace pieces were treated with a Critical Point Drier (K850 EMITECH), placed on aluminum stubs with carbon tape. The stubs were sputter-coated with a Gold-Palladium layer of 20 nm and

studied in a ZEISS Supra 40 SEM microscope at 5 kV (Centro Grandi Apparecchiature, II University of Naples, Naples, Italy).

Diatom terminology follows Ross *et al*. (1979), Medlin & Round (1986), Round *et al*. (1990), Fernandez & Sar (2009) and Van de Vijver *et al*. (2012). The morphology of the new taxa has been compared with the ultrastructure of known epizoic species described worldwide (Nemoto 1956, Giffen 1970, Medlin & Round 1986, Holmes *et al*. 1993a, b, Witkowski *et al*. 2000, Fernandez & Sar 2009).

The discriminating features of both new taxa are hardly discernible in the light microscope making it impossible to separate both taxa in LM. Scanning electron microscopy was essential to clarify the morphological characteristics of both taxa. Therefore the scanning electron microscopy stub was designated as holotype for both new taxa.

The phylogenetic position of the taxa considered herein was determined through a cladistic analysis of morphological features. The 23 taxa included other genera assigned to the Rhoicospheniaceae by Round *et al*. (1990), Lange-Bertalot (1995), and Fernandes & Sar (2009), as well as taxa thought to be close allies of this group. These include representatives of the 'monoraphid' Achnanthidiaceae and Cocconeidaceae) and the Cymbellales, shown to be close allies of *Rhoicosphenia* (Jones *et al*. 2005, Nakov *et al*. 2014, Thomas *et al*. in review). Based on previous phylogenetic analyses of the raphid diatoms, *Achnanthes brevipes* Agardh (1824: 1) and *Mastogloia smithii* Thwaites *in litt*. ex W.Smith (1856: 65), are both positioned as early branches in the naviculoid diatoms (Ruck & Theriot 2011; Kociolek *et al*. 2013) were identified as the outgroups for this analysis. The analysis included 28 characters and character state definitions for valve morphology as suggested by Cox & Williams (2006) and Kociolek & Stoermer (1993) and are presented in Table 1. The data matrix of terminal taxa, characters and character states is found in Table 2.

A Branch-and-Bound Search for most parsimonious trees was completed in PAUP*4.0a146 (Swofford 2003) and character state data were analyzed as unordered and unweighted. The four most equally parsimonious trees were used to build a strict consensus tree which is presented in figure 32.

Observations

Division Bacillariophyta
Class Bacillariophyceae
Subclass Bacillariophycidae

Chelonicola **Majewska, De Stefano & Van de Vijver,** *gen*. *nov*.

Frustules isovalvar, wedge-shaped in girdle view. Girdle composed of a large number (>10) of open, perforated bands of equal width. Valvocopula bearing a septum at the headpole and the second copula with a septum at the footpole. Valves heteropolar with a broadly rounded headpole and an acutely rounded footpole. Pseudosepta and apical pore field absent. Raphe straight to very weakly curving. Proximal raphe endings unilaterally weakly deflected towards the primary side. Distal raphe fissures elongated, deflected to the secondary side, continuing on both poles shortly onto the mantle. Internal proximal raphe endings covered by silica flap and distal raphe endings straight, terminating on weakly developed helictoglossae. Striae uniseriate, composed of several small, rounded areolae. Areolae internally occluded by hymenes.

Type:—*Chelonicola costaricensis* Majewska, De Stefano & Van de Vijver, *sp*. *nov*.

Etymology:—The generic name refers to the epizoic habitat where it was found: living (Latin: *-cola* = living on) on the carapaces of sea turtles (Latin: *Chelonia* = turtle)

Chelonicola costaricensis **Majewska, De Stefano & Van de Vijver,** *sp*. *nov*. **(Figs 1–12)**

Frustules wedge-shaped in girdle view showing conspicuous septa at both poles. Valves small, heteropolar, typically clavate with a broadly rounded, non-protracted headpole and an acutely terminating footpole. Septa visible in LM and SEM on both poles. Valve dimensions (n=50): length 6.0–17.5 μm, width 1.7–3.1 μm. Axial area very narrow, not discernible in LM. Central area very small. Raphe filiform, straight with simple, indistinct proximal raphe endings. Distal raphe endings not discernible in LM. Striae almost parallel throughout the entire valve, very faintly visible in LM, 36–47 in 10 μm.

Type:—COSTA RICA. Olive ridley sea turtle, 9º 59' 23.7" N, 85º 41' 52.6" W, *M. de Stefano*, *27 October 2013* (holotype BR! stub 4420).

Scanning Electron Microscopy:—(Figs 1–12) Frustules isovalvar, clavate in girdle view, attached by the footpole on short mucilaginous stalks (Fig. 3). Valve face flat in both valves with a clear angle to the very shallow mantle (Figs 3, 7). The mantle is equally high in its distal and proximal part but larger in the central part (Fig. 7). Pseudosepta absent (Figs 9, 10, 12). Axial area very narrow, linear (Figs 4–6). Central area very small, bordered on one or both sides by one slightly shortened central stria (Figs 4–7). Fascia never present (Fig. 5). External raphe branches almost straight to very weakly curving (Figs 4–6). External proximal raphe endings slightly expanded, unilaterally weakly deflected (Fig. 5). External distal raphe fissures elongated, weakly deflected, continuing shortly onto the mantle on both poles (Figs 6, 7). Striae uniseriate, equally spaced throughout the entire valve, composed of a series of 3–5 slightly transapically elongated areolae (Figs 4–7). Areolae bordering the axial area being the largest (Fig. 6). Striae continuing without interruption onto the shallow mantle (Figs 4–7). Apical pore field absent on both poles (Figs 4, 6, 8). Internally, raphe straight to weakly curved, positioned asymmetrically in a raised raphe sternum (Figs 9–12). Primary side of the sternum thickened, opening the raphe in a lateral position (Figs 9–12). Proximal raphe endings covered by a silica flap and distal raphe endings straight, terminating on weakly developed helictoglossae (Figs 9–12). Areolae internally slightly sunken between thickened interstriae, covered by hymenes (Figs 11, 12). Cingulum composed of a large number (up to 12) of open copulae, each with one row of apically elongated, slit-like poroids in the advalvar position (Figs 3, 4 & 7). First band, the valvocopula, with a small, but distinct septum at the head pole (Fig. 9). Second copula with a small septum at the footpole (Fig. 10). Other copulae lacking a septum.

Etymology:—The specific epithet refers to the geographical locality, Costa Rica, where the species was first observed.

Poulinea Majewska, De Stefano & Van de Vijver, *gen. nov.*

Frustules wedge-shaped to rectangular in girdle view. One valve typically concave while other straight. Girdle composed of a large number (>10) of open, perforated bands of different width with occasionally two irregular rows of poroids. Valvocopula bearing a septum at the headpole and the second copula with a septum at the footpole. Valves heteropolar with a broadly rounded headpole and a more acutely rounded footpole. Pseudosepta absent. Apical pore field absent but several more closely-spaced areolae surrounding the distal raphe endings. Raphe straight to very weakly curving. Raphe branch in the headpole shorter than in the footpole. Proximal raphe endings straight to weakly unilaterally deflected. External distal raphe fissures elongated, deflected, located in a shallow groove, covered by a large silica flap extending from both valve apices. Internal proximal raphe endings covered by a silica flap and distal raphe endings straight, terminating on weakly developed helictoglossae. Striae uniseriate, composed of two elongated areolae, clearly separated by the valve face/mantle junction. Areolae occluded in the areolar canal by hymenes.

Type:—*Poulinea lepidochelicola* Majewska, De Stefano & Van de Vijver, *sp. nov.*

Etymology:—The genus is named in honour of our colleague and dear friend Dr. Michel Poulin (Canadian Museum of Nature, Ottawa, Canada) in recognition of his important research on marine diatoms.

Poulinea lepidochelicola Majewska, De Stefano & Van de Vijver, *sp. nov.* (Figs 13–31)

Frustules wedge-shaped in girdle view showing conspicuous septa at both poles. One valve slightly concave while other valve flat. Valves small, heteropolar, typically clavate with acutely rounded, non-protracted headpole and footpole. Septa visible in LM and SEM on both poles. Valve dimensions (n=50): length 5.2–10.0 μm, width 1.6–2.8 μm. Axial area very narrow, not discernible in LM. Central area forming a wide fascia. Raphe filiform, curved with expanded proximal raphe endings. Distal raphe endings not discernible in LM, typically covered by a silica flap on both poles, only visible in SEM. Striae weakly radiate near the central area, almost parallel throughout the rest of the valve, very faintly visible in LM, 25–36 in 10 μm, composed of only two, transapically elongated areolae, only discernible in SEM.

Type:—COSTA RICA. Olive ridley sea turtle, 9º 59' 23.7" N, 85º 41' 52.6" W, *M. de Stefano*, *27 October 2013* (holotype BR! stub 4421).

Scanning Electron Microscopy:—(Figs 13–31) Frustules heterovalvar, wedge-shaped in girdle view (Fig. 15), attached by the footpole to the substrate by a mucilaginous pad (Figs 13, 14). Valve face flat in one valve and slightly concave in the other (Fig. 15). Valve face gently sloping towards the mantle margin (Fig. 26). Mantle height largest near the valve middle becoming shallower towards both poles (Figs 15, 16, 26). Pseudosepta absent (Figs 27–29). Axial area narrow, linear, narrowing towards the apices (Fig. 21). Central area small, forming a rectangular fascia that widens towards the valve margins (Figs 21, 24, 26, 27). Occasionally shortened striae present in the central area (Figs 15, 16, 21). External raphe branches differing in length with branch in upper half (headpole) shorter than in lower halve of the valve (Figs 16, 21). Branches almost straight to curving (Fig. 21). External proximal raphe endings spatulate, unilaterally weakly deflected (Fig. 24). Distal raphe fissures elongated, unilaterally bent, terminating near the valve poles, covered on the headpole and footpole by silica flaps, conspicuously thickened on the footpole (Figs 21–23). Striae uniseriate, equally spaced on most of the valve, but somewhat denser near the poles (Figs 21–23), composed of 1–2 (very rarely 3, Figs 27–29) transapically elongated areolae (Fig. 25). Both rows of areolae separated by a larger hyaline area, formed by the valve face/mantle junction (Figs 25, 26). Apical pore field absent on both poles, but one series of elongated areolae surrounding the distal raphe ending present at the footpole (Fig. 23). Internally, raphe straight, located on a raised raphe sternum (Fig. 27). Proximal raphe endings covered by a silica flap (Figs 30, 31). Evident in oblique view, proximal raphe endings terminating on a slightly raised central nodule (Fig. 31). Distal raphe endings straight, terminating on weakly developed helictoglossae (Figs 28, 29). Areolae internally slightly sunken between interstriae, covered by hymenes located in the middle of the areolar canal (Figs 28–30). Cingulum composed of a large number (up to 12) of open copulae (Fig. 20), each with one row of apically elongated, slit-like poroids in the advalvar position (Figs 15–18). Near the footpole, a double row of poroids often present on the copulae (Fig. 17, arrow). First band, the valvocopula, with a small, but distinct septum at the head pole (Figs 19 & 20). Second copula with a small septum at the footpole (Fig. 19). Other copulae lacking a septum.

Etymology:—The specific epithet *lepidochelicola* refers to the habitat of the new species, living (Latin -*cola*) on *Lepidochelys olivacea*.

Phylogenetic analysis:—A total of four most parsimonious trees of 97 steps was recovered in the cladistics analysis. From these four trees, a strict consensus tree was computed and is presented in figure 32 and had a consistency index of 0.4433 and retention index of 0.5970. The strict consensus tree shows a monophyletic clade with *Chelonicola* sister to *Poulinea*. This group is sister to *Cuneolus*, and together that group of three genera is sister to *Tripterion*. Other taxa suggested to be part of the Rhoicopheniaceae are found in widely divergent places in the tree, either sister to gomphonemoid diatoms (*Rhoicosphenia* and *Gomphoseptatum*) or 'monoraphid' diatoms (*Gomphonemopsi*s and *Gomphosphenia* are sisters and then related to a clade of *Achnanthidium* and *Cocconeis*).

Discussion

A comparison of morphological features of both new taxa (*Chelonicola costaricensis* and *Poulinea lepidochelicola*) with that of similar known small-celled gomphonemoid genera including *Gomphonemopsis*, *Gomphosphenia*, *Gomphoseptatum*, *Tripterion*, and *Cuneolus* (Table 1) reveals important combinations of differences, justifying the description of the two new genera. These significant morphological differences include the presence/absence of septate girdle bands, striae structure, the presence/absence or development of apical pore fields, raphe structure, and cingulum structure. *Rhoicosphenia* Grunow (1860: 511) is similar to these genera in having pseudosepta and valves bent along the transapical axis, but it is excluded from further comparisons because the reduced raphe structure and distinct striae structure clearly differentiate this genus from the others.

Only a few genera show the presence of septate girdle bands. Van de Vijver *et al*. (2012) discussed the structure of septate girdle bands in both raphid and araphid diatoms and concluded that in most cases the term 'septum' was erroneously used in raphid diatom morphology, reducing the number of raphid genera with a septum to only a handful: *Cuneolus*, *Gomphoseptatum*, *Tripterion*, *Chelonicola* and *Poulinea* all possess one (*Cuneolus*) or two septate girdle bands (Round *et al*. 1990, Holmes *et al*. 1993a, present study). Some *Rhoicosphenia* taxa possess siliceous flaps on their valvocopula (septa-like structure) although real septa in the sense of araphid diatoms never have been observed (E.Thomas, pers. ~~comm~~obs.) *Gomphonemopsis* and *Gomphosphenia* do not possess septa. *Cuneolus*, *Rhoicosphenia* and *Gomphoseptatum* have pseudosepta at one (*Gomphoseptatum*) or two (*Cuneolus*, *Rhoicosphenia*) poles (Round *et al*. 1990), contrary to both new genera that lack pseudosepta.

Based on stria structure, two separate groups of genera can be formed. A first group contains those genera having striae with three or more areolae: *Cuneolus*, *Rhoicosphenia*, *Tripterion*, and *Chelonicola*, whereas a second group is formed by all gomphonemoid genera with maximum of two, rarely three, areolae per stria: *Gomphoseptatum*, *Gomphosphenia*, and *Gomphonemopsis*, and *Poulinea* (Medlin *et al*. 1986, Round *et al*. 1990, present study). *Cuneolus* can be further separated based on differences in the structure of the internal proximal raphe endings (being clearly hooked, not covered by siliceous flap) and a much lower number of girdle bands (Medlin *et al*. 1986, Round *et al*. 1990). *Gomphoseptatum* differs in the presence of a well-developed apical pore field at the footpole (absent in both new taxa), the presence of short projections constricting the areolae into several sections (see Round *et al*. 1990, p. 477, fig. f) (never observed in both new taxa), a girdle containing a lower number of copulae, simple internal proximal raphe endings, and the lack of a siliceous flap (Medlin *et al*. 1986, Round *et al*. 1990). It should be noted however that the presence of the siliceous flap on the central nodule is not a very discriminating feature as in several larger genera such as *Pinnularia* or *Cymbella*, species can be found with and without this siliceous covering (Round *et al*. 1990) making this feature less important in separating both new taxa from either *Cuneolus* or *Gomphoseptatum*.

Based on the morphological comparison, only *Tripterion* shows sufficient morphological similarity with the two new taxa to warrant further morphological analysis. Three species of *Tripterion* are known: *T. kalamensis* (Holmes *et al*. 1993a: 8), *T. philoderma* Holmes *et al*. (1993b: 130) and *T. margaritae* (Frenguelli & Orlando 1958: 98). *Tripterion philoderma* is most similar to *P. lepidochelicola*, with both having septate girdle bands, fascia, radial to parallel (at apices) striae, trans-apically elongated punctae, and closely-spaced punctae in rows at the footpole (Table 1). However, some important differences can be noted, seen particularly, in the two other *Tripterion* species. *Tripterion kalamensis* and *T. margaritae* possess circular to oval punctae in greater number in each stria (Holmes *et al*. 1993a, Fernandes & Sar 2009). All *Tripterion* species have at least three, usually 4–5 areolae per stria, a feature never observed in *Poulinea* but present in *Chelonicola*. All *Tripterion* species (Holmes *et al*. 1993a, b, Fernandes & Sar 2009) show a clear increase in stria density near the footpole compared to the valve central area. In both new taxa, this was not observed. Moreover, the areolae become smaller in *Tripterion* close to the footpole (Holmes *et al*. 1993a), whereas in both new taxa, the shape of the areolae does not seem to change. In *T. margaritae* the areolae near the footpole almost form an apical pore field (Fernandes & Sar 2009: Figs 36 & 37). In *T. philoderma* rows of closely-spaced elongate puncta, resembling an apical pore field, are present along the edge of the valve at the apices (Holmes *et al*. 1993b: fig. 6). Similar arrangements of puncta at the apices was not found in *C. costaricensis*, but a single row of closely spaced puncta was observed at the footpole of *P. lepidochelicola*. The external raphe structure of *Tripterion* resembles *Poulinea* in having a thickened siliceous flap on the distal raphe fissures but differs from *Chelonicola* that is lacking this feature (Holmes *et al*. 1993b, plate 2, figs 1, 2). Both new genera also differ from *Tripterion* in the structure of the internal proximal raphe endings since the latter lacks a siliceous flap over the endings (see for instance Fernandes & Sar 2009, fig. 37), typical for both *Chelonicola* and *Poulinea*. *Tripterion* has a much lower number of copulae in its girdle, whereas both new genera have at least 10 separate girdle elements.

In terms of the systematic placement of the two new genera described here, cladistic analysis shows that they are closely allied to one another, as sister taxa within a branch of naviculoid diatoms that includes also *Cuneolus* and *Tripterion*. Synapomorphies to support this overall clade include heteropolar valve symmetry and presence of septa (characters 1 and 26, respectively) diagnose this clade. The synapomophies of external polar raphe ends being straight and presence of pseudosepta (characters 23, 27, respectively) suggest *Cuneolus* is more closely related to *Poulinea* and *Chelnicola*, the latter two sharing presence of hymenate occlusions, the deflection of the proximal and polar raphe ends (characters 21, 23) as synapomorphies. Features thought to be shared amongst members of the Rhoicospheniaceae, such as heteropolar symmetry, and presence of septa-like structures and pseudosepta are seen to be homoplasic in this lineage plus gomphonemoid diatoms and in the group closely allied with the 'monoraphid' diatoms.

Based on the results presented here, the Rhoicospheniaceae, as circumscribed originally by Chen and Zhu (1983) to accommodate the unique features of *Rhoicosphenia*, may be quite limited in the taxa it represents, possibly containing only *Rhoicosphenia* and *Gomphoseptatum* which are more closely related to the Cymbellales than other taxa considered here. Other putative members of the family are not shown to be closely related, however, there does appear to be a monophyletic clade of the genera of epizooic and attached diatoms, including *Poulinea* and *Chelonicola*, for which some higher Linnaean category might be proposed in the future.

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Table & Figure captions

TABLE 1. Listing of characters, character states used in the cladistics analysis. Definition of the characters and character states is based on Cox & Williams (2006) and Kociolek & Stoermer (1993).

TABLE 2. Data matrix used in cladistics analysis, based on the data in Table 3 as well as Cox & Williams (2006) and Kociolek & Stoermer (1993).

FIGURES 1–6. *Chelonicola costaricensis*. SEM pictures taken from the holotype population from the olive ridley sea turtle carapace collected on Ostional Beach, Costa Rica. 1. Three frustules of *Chelonicola* attached to the substrate together with one frustule (upper one) of *Poulinea*. 2. Several valves of Chelonicola attached to the substrate showing their valve face and the girdle composition. 3. One frustule attached to the substrate by a mucilaginous stalk. The girdle structure composed of at least 10 bands is shown. 4. Valve face view showing the head pole with the distal raphe fissure continuing onto the mantle. 5. External detail of the central area showing the lack of fascia and the stria structure. 6. External detail of a head pole showing the distal raphe fissure. Scale bar represents 10 μm except for Figs 5, 6 where scale bar = 1 μm.

FIGURES 7–12. *Chelonicola costaricensis*. SEM pictures taken from the holotype population from the olive ridley sea turtle carapace collected on Ostional Beach, Costa Rica. 7. External valve face view showing the mantle and the areolae. 8. External detail of a footpole showing the absence of an apical pore field and the distal raphe fissure continuing onto the mantle. 9 & 10. Internal views of an entire valve showing the raphe sternum,

the septate girdle bands and the covered areolae. 11. Internal detail of the central area. 12. Internal detail of the footpole with helictoglossa. Scale bar represents 1 μm.

FIGURES 13–20. *Poulinea lepidochelicola*. SEM pictures taken from the holotype population from the olive ridley sea turtle carapace collected on Ostional Beach, Costa Rica. 13. Several frustules attached to the substrate showing how the population is organised. 14. Five frustules attached to the substrate showing the headpoles and the girdle bands. 15. External girdle view of an entire frustule showing the girdle bands and the mantle. 16. External girdle view of an entire valve showing the mantle, the distal raphe fissures and the doubling of the perforation of the girdle bands near the footpole. 17. External detail of the footpole. The arrow shows the doubling of the poroids. 18. External detail of the headpole. 19. Internal view of an entire valve showing the raphe sternum, the septate girdle bands and the covered areolae. 20. Second copula with the typical septum at the footpole. Scale bar represents 10 μm, except for Figs 17, 18 where scale bar = 1 μm.

FIGURES 21–25. *Poulinea lepidochelicola*. SEM pictures taken from the holotype population from the olive ridley sea turtle carapace collected on Ostional Beach, Costa Rica. 21. External view of an entire valve showing the raphe structure, the striae composed of two enlarged areolae and the fascia with only one reduced stria. 22. External detail of a headpole with the siliceous covering of the distal raphe fissure and the lack of an apical pore field. 23. External detail of a footpole with the thickened siliceous covering of the distal raphe fissure and the presence of several areolae surrounding the apex. 24. External detail of the central area showing the spatulate proximal raphe endings. 25. External detail of the striae. Note the hymenate coverings in the areolae canals. Scale bar represents 1 μm except for Fig. 21 where scale bar = 10 μm.

FIGURES 26–31. *Poulinea lepidochelicola*. SEM pictures taken from the holotype population from the olive ridley sea turtle carapace collected on Ostional Beach, Costa Rica. 26. External oblique view of an entire valve showing the shape of the mantle. 27. Internal view of two entire valves without girdle elements showing the raphe sternum and the covered areolae. 28. Internal detail of the headpole showing the helictoglossae and a few areolae surrounding the headpole. 29. Internal detail of the footpole with the helictoglossae and several rows of areolae surrounding the footpole. 30. Internal detail of the central area with the siliceous covering of the proximal raphe endings. 31. Internal detail of the central area. The siliceous flap covering the proximal raphe endings has been removed showing the raphe endings. Scale bar represents 1 μm except for Fig. 27 where scale bar = 5 μm.

FIGURE 32. Strict consensus tree of four most parsimonious cladograms.

Character #	Character	Character States	Character state
1	Valve symmetry 1	Isopolar	0
		Heteropolar	1
2	Valve symmetry 2	Bilaterally symmetrical	0
		Dorsiventral - primary side ventral	1
		Dorsiventral - primary side dorsal	2
3	Frustule symmetry	Isovalvar	0
		Heterovalvar	1
4	Striae 1	Simply areolate	0
		Chambered - external surface areolate	1
		Chambered - internal surface areolate	2
5	Striae 2	Uniseriate throughout	0
		Biseriate (at least partly)	1
		Multiseriate	2
6	Areola occlusions 1	With cribra	0
		Without cribra	1
7	Areola occlusions 2	Without hymenes	0
		With hymenes	1
8	Areola occlusions 3	With volae	0
		Without volae	1
9	Areola type	Poroid	0
		Loculate	1
10	Areola openings (external) 1	More or less circular	0
		Elongate	1
		Reniform	2
11	Areola openings (external) 2	Openings discrete	0
		Openings confluent	1
12	Areola openings (external) 3	Opening perpendicular to stria direction	1
		Opening parallel to stria direction	2
13	Girdle bands 1	With two rows of pores	0
		With one row of pores	1
		Without pores	2
14	Girdle bands 2	Pores like valve pores	0
		Pores unlike valve pores	1
15	Internal raphe sternum	Absent	0
		With central fissure	1
		With lateral fissure	2
16	Accessory rib	Absent	0
		On primary side only	1
		On primary and secondary sides	2
17	Internal central raphe fissures 1	Unilaterally deflected	0
		Straight	1
		Oppositely deflected	2
18	Internal central raphe fissures 2	Simple	0
		Hidden (+ intermissio)	1
		Helictoglossa	2
19	Internal polar helictoglossae 1	Straight	0
		Twisted	1
		Hooded	2
20	Internal polar helictoglossae 2	Discrete	0
		Fused with sternum	1
		Forming porte-crayon ending	2

Character #	Character	Character States	Character state
21	External central raphe endings	Straight	0
21	External central raphe endings	Deflected to primary side	1
22	External raphe endings (central v. polar)	Deflected to secondary side	2
		Different	0
22	External raphe endings (central v. polar)	Similar	1
23	External polar raphe endings	Deflected to secondary side	0
23	External polar raphe endings	Straight	1
24	Apical pore fields	Deflected to primary side	2
		Opposite	3
		Absent	0
24	Apical pore fields	At both poles	1
25	Stigmata	At one pole	2
		None	0
25	Stigmata	One	1
26	Septa	More than one	2
		Absent	0
26	Septa	Present	1
27	Pseudosepta	Absent	0
27	Pseudosepta	Present	1
28	Growth Form	Free-living	0
28	Growth Form	Attached without a stalk	1
		Attached with a stalk	2
		Tube dwelling	3

Taxon/Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Achnanthes brevipes	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	2
Achnanthidium minutissimum	0	0	1	0	0	1	0	1	0	0	0	0	2	1	0	0	2	0	0	0	0	1	1	0	0	0	0	2
Caloneis amphisbaena	0	0	0	1	2	1	1	1	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Chelonicola	1	0	0	0	0	1	1	1	0	0	0	0	1	1	1	0	1	1	1	1	2	0	2	0	0	1	1	2
Cocconeis placentula	0	0	1	0	0	1	1	1	0	1	0	2	2	1	0	0	2	0	0	1	0	0	1	0	0	0	0	0
Craticula ambigua	0	0	0	0	0	1	1	1	0	1	0	1	1	1	0	0	1	0	0	0	2	0	0	0	0	0	0	0
Cuneolus	1	0	1	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	1	1	1
Cymbella affinis	0	1	0	0	0	1	0	0	0	1	0	1	1	1	0	0	0	1	2	0	1	0	0	1	1	0	0	2
Encyonema caespitosum	0	2	0	0	0	1	0	1	0	1	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	3
Gomphonema acuminatum	1	0	0	0	1	1	0	0	0	2	0	1	1	1	0	0	0	0	0	0	1	0	0	2	1	1	1	2
Gomphonemopsis	1	0	0	0	0	1	1	1	0	1	0	2	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	2
Gomphoseptatum	1	0	0	0	0	1	0	1	0	1	0	2	1	1	1	0	0	2	0	1	1	0	1	2	0	1	1	2
Gomphosphenia	1	0	0	0	0	1	1	1	0	1	0	2	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Mastogloia smithii	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	1	2	0	0	0	0	0	0	1
Navicula gregaria	0	0	0	0	0	1	1	1	1	1	0	1	2	1	2	1	1	0	1	1	1	0	0	0	0	0	0	0
Pinnularia gibba	0	0	0	1	2	1	1	1	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Placoneis placentula	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	0	2	0	0	0
Poulinea	1	0	0	0	0	1	1	1	0	1	0	2	1	0	2	0	1	1	0	1	2	0	2	0	0	1	1	1
Reimeria sinuata	0	2	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	2
Rhoicosphenia curvata	1	0	1	0	0	1	1	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	2	0	1	1	2
Sellaphora pupula	0	0	0	0	0	1	1	1	0	0	0	0	2	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Stauroneis anceps	0	0	0	0	0	1	1	1	1	1	0	2	1	1	0	0	1	0	0	0	2	0	0	0	0	0	0	0
Tripterion	1	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0	1	0	0	1	1	0	0	2	0	1	0	2