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C.E. Wetzel, B. van De Vijver, L. Ector. *Luticola deniseae* sp. nov. A new epizoic diatom from the Rio Negro (Amazon hydrographic basin). *Vie et Milieu / Life & Environment*, 2010, pp.177-184. hal-03262158

HAL Id: hal-03262158

<https://hal.sorbonne-universite.fr/hal-03262158>

Submitted on 16 Jun 2021

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LUTICOLA DENISEAE SP. NOV. A NEW EPIZOIC DIATOM FROM THE RIO NEGRO (AMAZON HYDROGRAPHIC BASIN)

C. E. WETZEL^{1,3*}, B. VAN DE VIJVER², L. ECTOR³

¹ Instituto de Botânica, Seção de Ecologia, Post-graduate program on 'Biodiversidade Vegetal e Meio Ambiente', Av. Miguel Stéfano 3687, CEP 04301-012, São Paulo, SP Brazil

² National Botanic Garden of Belgium, Department of Bryophyta & Thallophyta, Domein van Bouchout, B-1860 Meise, Belgium

³ Department of Environment and Agro-biotechnologies (EVA), Public Research Centre - Gabriel Lippmann, Rue du Brill 41, L-4422 Belvaux, Grand-Duchy of Luxembourg

* Corresponding author: catiwetzel@yahoo.com.br

AMAZON BASIN
BACILLARIOPHYCEAE
DIATOMS
EPIZOIC ALGAE
FRESHWATER TURTLES
PODOCNEMIS ERYTHROCEPHALA
BRAZIL

ABSTRACT. – *Luticola deniseae* sp. nov. is described from the Rio Negro basin (Amazon, Brazil), where it was found in abundance living, epizoically on the carapaces of the freshwater turtle *Podocnemis erythrocephala*. The detailed morphology of this epizoic diatom is examined using both light (LM) and scanning electron microscopy (SEM) and compared to similar *Luticola* species. The main morphological features of *L. deniseae* include the presence of thickened valve apices, a transapically elongated stigma and a rather low number of areolae per stria (3-5).

INTRODUCTION

Despite several recent floristic studies on freshwater diatoms of South America (Metzeltin & Lange-Bertalot 1998, 2007, Rumrich *et al.* 2000, Kociolek *et al.* 2001, Metzeltin *et al.* 2005, Morales & Vis 2007, Morales *et al.* 2007, 2008) many species remain undescribed and thus information about their occurrence and distribution is poorly documented (Ferrari *et al.* 2009, Wetzel *et al.* 2010). Although there have been relatively few taxonomic investigations of South American diatoms, every study has revealed a significantly greater species diversity than previously perceived (Kociolek *et al.* 2001).

The Amazon River drains the ancient eroded surface of the Brazilian Shield, a drainage pattern that dates from the Mesozoic Era. This ancient river dates from the rifting of the South Atlantic Ocean and the separation of the African and South American continents in the Cretaceous Period. Similar to ancient lakes, its basin has a large number of endemic species and closely related species groups (Kociolek & Spaulding 2000). The region is subject to a wide range of climatic, geographical and geomorphologic conditions which cause its considerable variety of freshwater ecosystems and biodiversity.

Diatoms are present in nearly every possible freshwater habitat and display several life forms ranging from strictly planktonic to benthic. The substrata with which diatoms may be associated include a diverse mix of biological and non-biological surfaces. Freshwater turtles with flat large carapaces provide a suitable habitat for algal communities (Yoneda 1952, Edgren *et al.* 1953, Belusz & Reed 1969, Arndt 1975, Colt *et al.* 1995). Thus far, the genus *Bacillaria* (Chlorophyta, Cladophorales) has been reported most frequently on freshwater turtles (Garbary *et*

al. 2007). As summarized by Burkholder (1996), epizoic algae are commonly found on many animals from estuaries, freshwaters and marine coastal habitats. Studies on epizoic flora on turtles, however, rarely mention diatoms. If present, their absence in the taxa list does not seem consistent, making diatoms a neglected, but nevertheless important, component of these epizoic assemblages.

The genus *Luticola* was established in 1990 by DG Mann in Round *et al.* (1990) to accommodate all species of *Navicula* s.l. with the following combination of features: uniseriate striae with more-or-less round poroids on the valve face; presence of a single stigma; filiform raphe with deflected, bent or hooked central and terminal endings as well as a longitudinal canal positioned between the valve wall.

Most species in the genus *Luticola* occur on soils or other subaerial habitats such as semi-wet, terrestrial mosses; wet slopes; permanently moist stones, and cave walls (Denys & De Smet 1996, Van de Vijver & Beyens 1997, Poulíčková & Hašler 2007, Van de Vijver *et al.* 2002, 2006, Van de Vijver & Mataloni 2008). Within the Neotropical region, the genus *Luticola* is quite diverse, with 16 species recorded by Metzeltin & Lange Bertalot (1998, 2007). Some of these species have peculiar ecological characteristics, such as *Luticola muticoides* (Hustedt) DG Mann, which lives in symbiosis with the lichen *Coenogonium linkii* Ehrenberg (Lakatos *et al.* 2004).

The present paper describes a new species found on the carapace of the freshwater turtle *Podocnemis erythrocephala* (Spix, 1824), captured during an expedition across the River Negro in the Amazon hydrographical basin. The species was dominant in the diatom communities sampled on every animal; it was, however, not recorded from any other benthic or planktonic habitat.

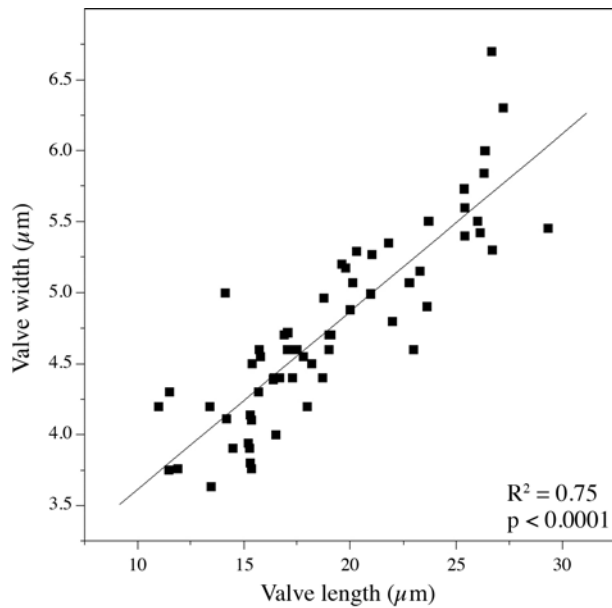


Fig. 1. – Scatter plot and linear regression of valve length *versus* valve width (n = 61).

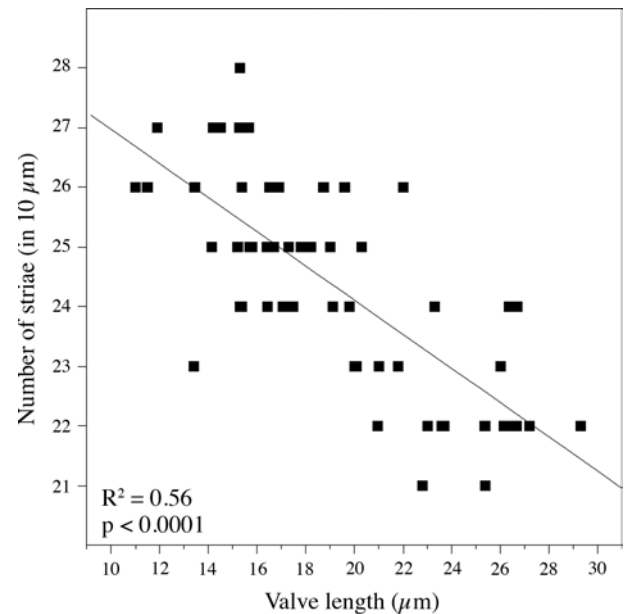


Fig. 2. – Scatter plot and linear regression of valve length *versus* number of striae in 10 µm (n = 61).

MATERIAL AND METHODS

During an expedition in 2005 into the Amazon hydrographical basin, twelve *Podocnemis erythrocephala* turtles from the upper courses of the Rio Negro, a black water tributary of the Amazon River were captured and released after sampling. The Rio Negro basin is characterized by intensely coloured (due to high concentrations of humic compounds) waters of very low pH (3.8–4.9) and low concentrations of both dissolved salts and suspended matter (Sioli 1984, Küchler *et al.* 2000).

Diatom samples were obtained from the turtle's carapaces that were scrubbed off using a toothbrush in order to carry out qualitative and semi-quantitative diatom analysis. Small aliquots of raw samples were digested with concentrated H₂O₂ (30 %) and hydrochloric acid (HCl, 1 M) for four hours. Following digestion and decantation, cleaned material was diluted with deionized water and mounted on permanent slides with Naphrax[®]. Optical observations, identifications and measurements were performed; and photomicrographs were taken with a light microscope Leica[®] DMRB equipped with a DC500 high-resolution digital camera.

For scanning electron microscopy (SEM), part of the cleaned suspension was filtered and washed through polycarbonate membrane filters of 3 µm pore diameter, fixed to aluminium stubs with double-sided tape and sputtered with gold (40 nm). Observations were performed and photographs were taken using a Leica[®] Stereoscan 430i, operated at 15–20 kV.

Holotype permanent slides, as well as raw and cleaned samples, were deposited at the 'Herbário Científico do Estado Maria Eneyda P Kauffmann Fidalgo, Secretaria do Meio Ambiente do Estado de São Paulo', Brazil (SP). Isotype slides were deposited at the National Botanic Garden of Belgium (BR, Meise, Belgium).

RESULTS

Luticola deniseae CE Wetzel, Van de Vijver & Ector sp. nov. (Figs 3–75)

Descriptio:

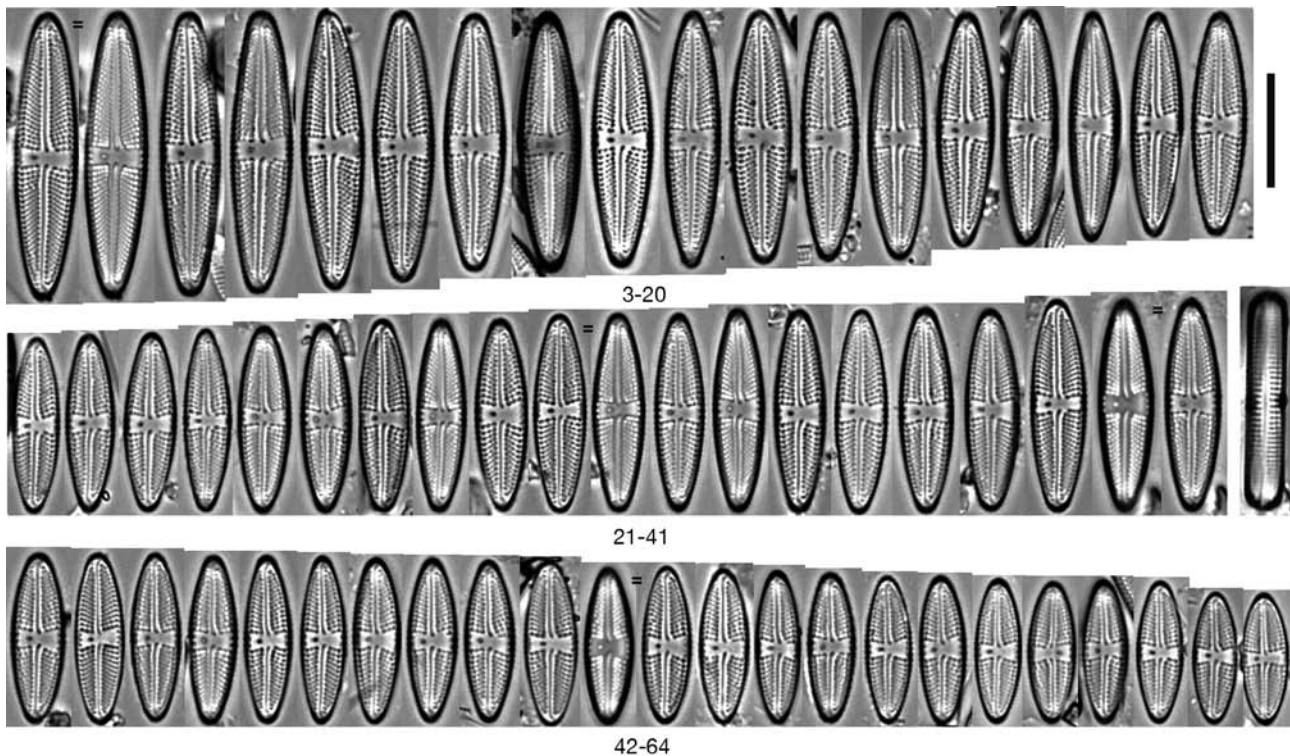
Valvae late lanceolatae ad lineares-lanceolatas in speciminibus maximis apicibusque anguste rotundatis. Margines valvae circum apices incrassati. Longitudo 11.0–29.5 µm, latitudo 3.5–7.0 µm. Area axialis angusta, linearis. Area centralis formans fasciam cuneiformem, asymmetricam. Rami raphis recti, terminationibus centralibus deflexis distalibusque uncinatis. Striae transapicales moderate radiatae apud aream centram, magis radiatae ad apices, 21–28 in 10 µm, constantes ex seriebus tribus vel quattuor pororum rotundatorum. Zona hyalina adest in apicibus. Stigma transapicaliter elongata isolataque adest in aream centram.

Holotype: slide SP-400477, deposited at the 'Herbário Científico do Estado Maria Eneyda P. Kauffmann Fidalgo', São Paulo, Brazil.

Isotypes: National Botanic Garden of Belgium, Meise, Belgium (BR-4166).

Additional material observed: samples collected from different specimens of *Podocnemis erythrocephala* from Rio Negro hydrographical basin: SP-400478, SP-400479, SP-400480, SP-400481, SP-400482, SP-400483, SP-400484, SP-400485, SP-400487, SP-400488, SP-400489, leg. Ector L & Wetzel CE, 11/III/2005.

Type locality: carapaces of the freshwater turtle *Podocnemis erythrocephala* (Spix, 1824) captured in the upper courses of the Rio Negro basin, central Amazon, Brazil (64°49'45"W, 0°30'43"S).



Figs 3-64. – *Luticola deniseae*. Light Microscopy. Several epizoically specimens from *Podocnemis erythrocephala*. Type population showing size variation. Fig. 32. Holotype specimen (SP-400477). Scale bar = 10 μ m.

Etymology: the specific epithet is given in honour of Dr Denise de C Bicudo, algologist at the ‘Instituto de Botânica’, São Paulo, Brazil.

LM observations (Figs 3-64)

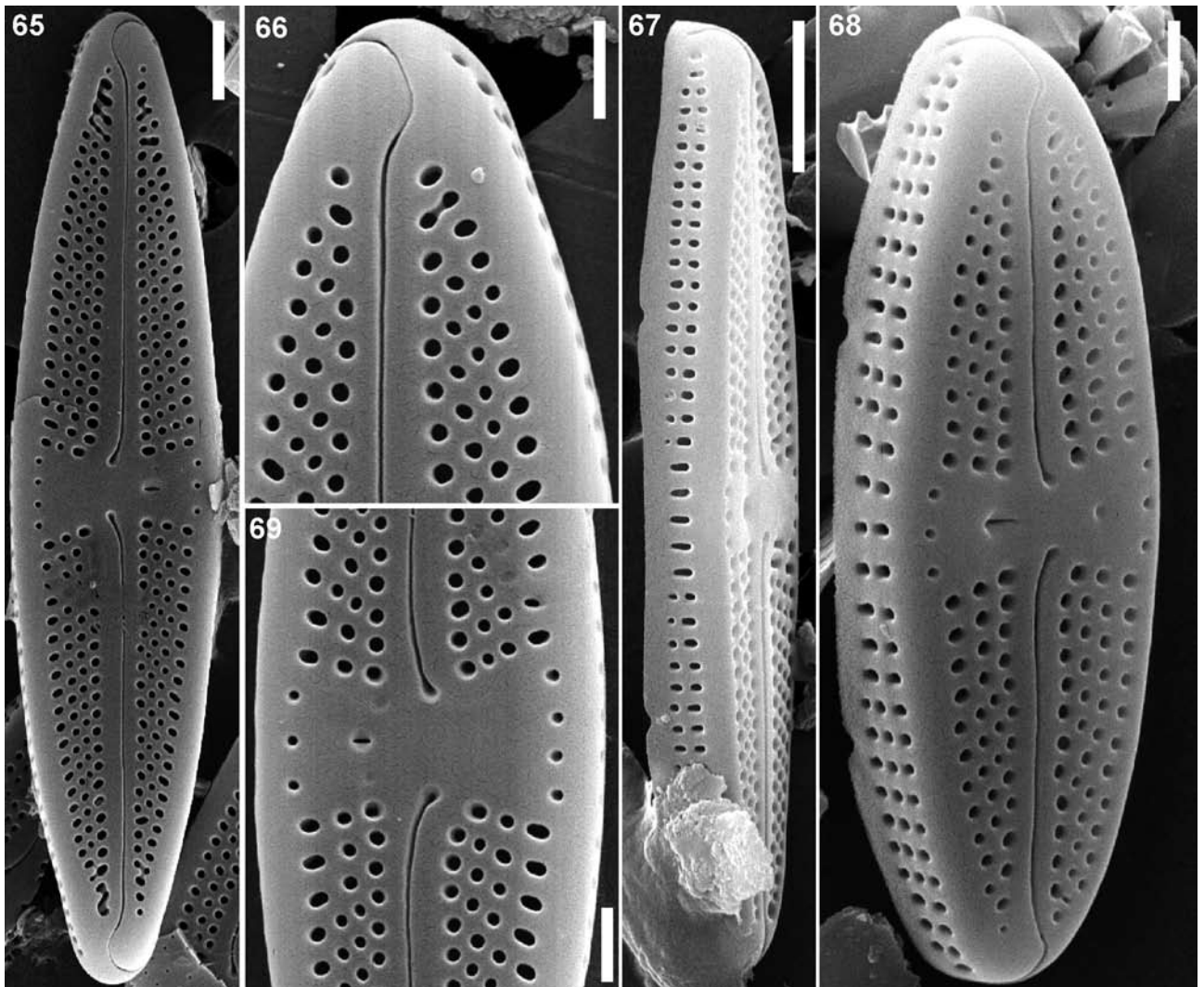
Frustules are rectangular in girdle view (Fig. 41) and frustule height varies between 4.4 and 5.4 μ m. The individual areolae on the mantle striae can be distinguished. The valve outline is mostly broadly lanceolate or, in the largest specimens, slightly linear-lanceolate. The valve apices are narrowly round, non-protracted and more strongly silicified resulting in rather thick valve margins at the apices (see Fig. 9). The valve length varies between 11.0 and 29.5 μ m with average length of 18 μ m ($n = 61$). Width varies between 3.5 and 7.0 μ m (average 4.6 μ m, $n = 61$). As in the other *Luticola* species, there is a significant relationship between valve length and valve width (Fig. 1). The axial area is rather narrow and linear with a simple filiform raphe. The raphe branches are almost straight. The central endings are clearly deflected to the side opposite to the stigma and bear slightly expanded central pores. The terminal fissures are strongly hooked towards the stigma and in most cases quite easy to see in LM. The central area is formed by a typically asymmetrical, usually wedge-shaped to panduriform stauros, although in some valves it may have a rectangular outline on one side of the central area (opposite to the stigma). The side opposite to the stigma is usually wider; close

to the axial area as well as to the valve margin. On both valve margins near the central area, three, sometimes four, isolated areolae may be present. A large, transapically elongated, sometimes round, stigma is present between the valve margin and the valve centre, closer to the axial area. The stigma is never associated with a stria, being always entirely isolated. Spines are absent at the valve margin. The transapical striae are moderately radiate near the middle of the valve, becoming more strongly radiate, even slightly curved or geniculate towards the valve apices. The number of striae in 10 μ m varies between 21 and 28 in 10 μ m depending on the length of the valve. As shown in Fig. 2, striae density decreases significantly with increasing length. Specimens with a length below 20 μ m tend to have 23-28 striae in 10 μ m (average = 25) and valves longer than 20 μ m have 21-26 (average = 22). The striae end at some distance from the cell apices, leaving a rather large hyaline area at the valve apices.

Each stria consists of three to four rather small, round areolae. In larger specimens, striae with five areolae may be occasionally observed. As far as the areolae are concerned, it seems that there is no difference in size throughout the entire valve.

SEM observations (Figs 65-75)

The mantle shows no special features, apart from two to three rows of large round areolae (Figs 67, 68), continuing without interruption from one valve apex to the



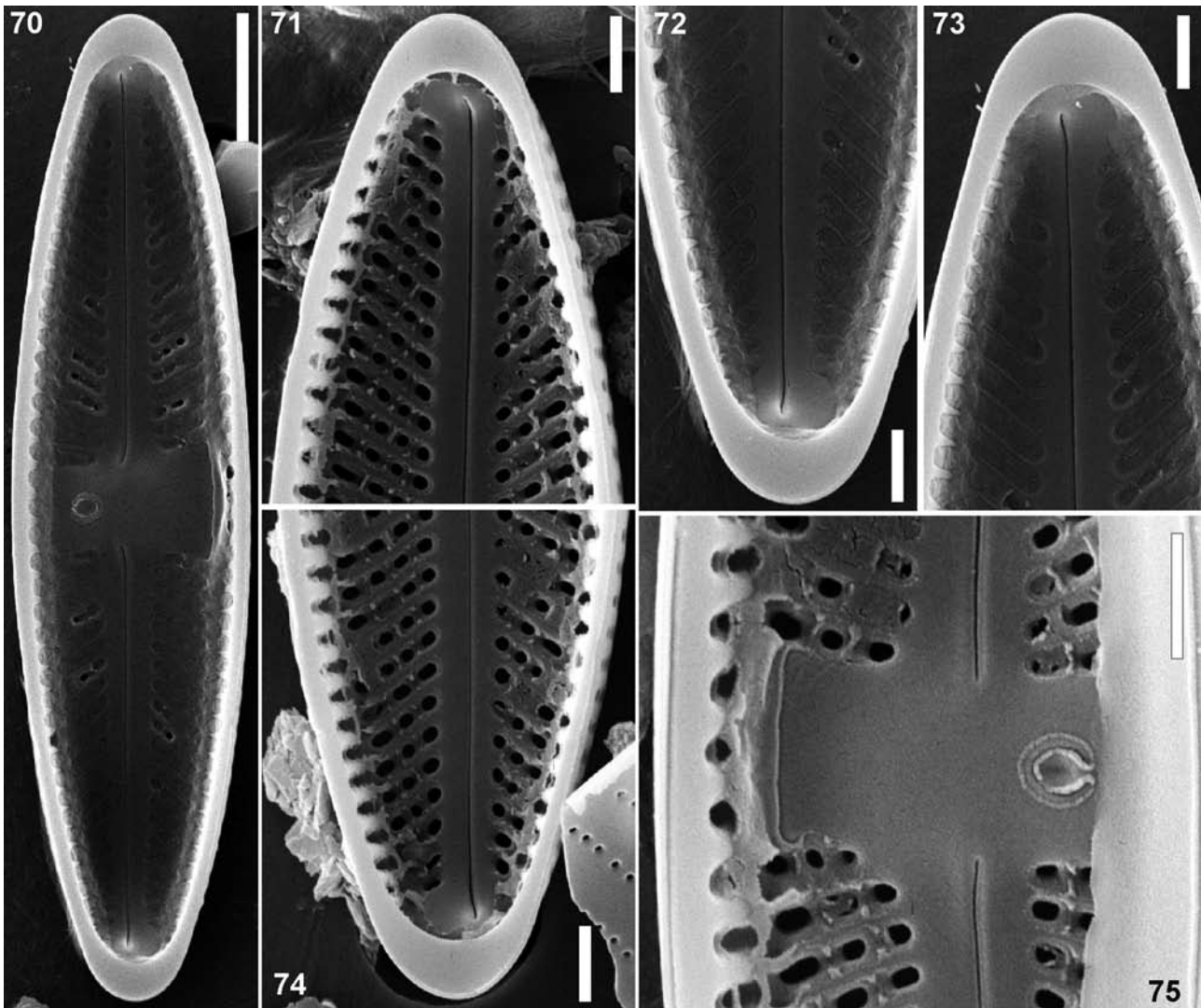
Figs 65-69. – Scanning electron microscopy of *Luticola deniseae* CE Wetzel, Van de Vijver & Ector in external view. Scale bars = 1 μm , except for Fig. 67 where scale bar = 2 μm .

next. Near the central area, the number of rows of areolae may be reduced to a single elongated areola (Fig. 67). No mantle areolae are present around the valve apices. On the juncture of valve face and mantle, a large hyaline zone is observed separating the mantle areolae from the valve face areolae. As in most *Luticola* species, the valve edge is notched in each valve quadrant, halfway between the apices and the valve centre.

The external valve face is rather flat with only a very weakly raised sternum on which the external raphe slit is situated (Figs 65, 68). The central raphe endings, bearing expanded central pores, are clearly deflected away from the stigma (Fig. 69) whereas the terminal raphe fissures are strongly hooked, first weakly deflected towards the same side as the central endings, then abruptly hooked towards the opposite side, continuing onto the valve mantle, terminating only shortly before the valve edge (Figs 66, 67).

The external opening of the stigma is linear, slit-like (Figs 65, 68), well-isolated and positioned in the central area, close to the axial area, aligned with the areolae bordering the axial area. The size and shape of the areolae show only little variation throughout the valve. Round or slightly elongated areolae are the most common. The largest, most elongated areolae seem to be located near the valve margins whereas the areolae in the middle rows are usually slightly smaller and almost always round. Near the valve apices, some areolae tend to fuse, forming an irregular pattern. The striae terminate near the start of the terminal fissures.

Internally, the poroids of the striae are occluded by hymens, forming a continuous strip across the valves (Figs 70, 72, 73). When eroded, the round poroids are visible (Figs 71, 75). The central area is formed by a thickened stauros (Figs 70, 75). The internal raphe slit is very simple and straight, with no marked terminal and central endings (Fig. 70). The central raphe endings are rather



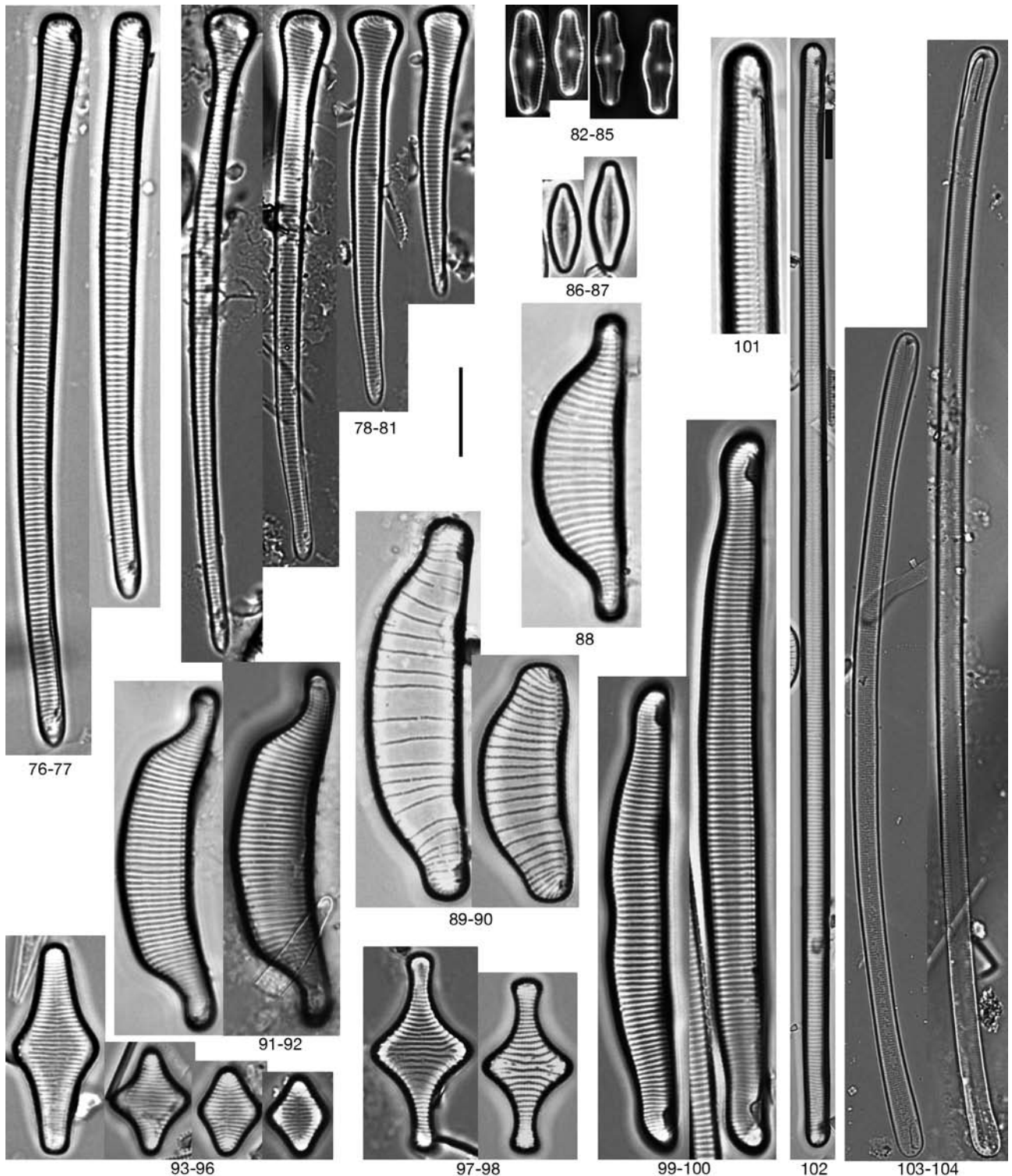
Figs 70-75. – Scanning electron microscopy of *Luticola deniseae* C.E. Wetzel, Van de Vijver & Ector in internal view. Scale bars = 1 μm , except for Fig. 70 where scale bar = 2 μm .

inconspicuous and very weakly deflected towards the internal stigma opening (Fig. 75). The distal raphe endings terminate on a very small helictoglossa (Figs 71-74). The internal opening of the stigma is covered by a typical circular and lipped structure (Fig. 75). The thickenings of the valve margin near the valve apices are quite obvious. Compared to the rest of the valve, the margin is almost twice as thick around the apices (Figs 72, 73).

DISCUSSION

Based on its main morphological features, *Luticola deniseae* may be easily separated from every other *Luticola* species. A characteristic feature is the presence of the thickened valve margins near the apices in the entire height of the mantle (Figs 70, 72, 73), giving the idea of small polar caps. Within the genus *Luticola*, this has never been observed before, except in *Luticola grupcei*

(Pavlov *et al.* 2009). A similar feature is often found in taxa belonging to the genus *Hippodonta* such as *H. avitata* (Cholnoky) Lange-Bertalot *et al.* (Lange-Bertalot 2001). A large number of endemic *Luticola* taxa is present in tropical South America (Metzeltin & Lange-Bertalot 1998, 2007) but they all show a different combination of morphological characteristics. Several of these taxa show a similar transapically elongated stigma. *Luticola monita* (Hustedt) DG Mann has larger valve dimensions, a more elliptical-lanceolate outline with broadly instead of acutely round apices and a greater number of areolae per stria (6-8 versus 3-5 in *L. deniseae*). *Luticola dapaalis* (Frenguelli) DG Mann, *L. frenguelli* Metzeltin & Lange-Bertalot and *L. dapaaliformis* (Hustedt) DG Mann are more broadly lanceolate, much wider (> 10 μm) with 5-7 areolae per stria whereas *L. deniseae* only shows 3-4 (5) areolae. *Luticola uruguayensis* Metzeltin *et al.* is much larger (width 17.0-24.0 μm versus 3.5-7.0 μm in



Figs 76-104. – Light microscopy of some species found in diatom communities on the carapaces of *Podocnemis erythrocephala* where *Luticola deniseae* CE Wetzel, Van de Vijver & Ector was the dominant species. Figs 76, 77: *Actinella supeperonioides* Metzeltin & Lange-Bertalot; Figs 78-81: *A. peronioides* Hustedt; Figs 82-85: *Chammaepinnularia brasilianopsis* Metzeltin & Lange-Bertalot; Figs 86, 87: *Nupela rumrichorum* Lange-Bertalot; Fig. 88: *Eunotia convexa* Hustedt; Figs 89, 90: *E. hirudo* Metzeltin & Lange-Bertalot; Figs 91, 92: *E. reflexa* Hustedt; Figs 93-96: *Fragilariforma hamiltonii* Metzeltin & Lange-Bertalot; Figs 97, 98: *F. lata* (Cleve-Euler) DM Williams & Round; Figs 99-100: *Eunotia sulcatoides* Metzeltin & Lange-Bertalot; Figs 101, 102: *E. baculus* Hustedt; Figs 103, 104: *E. conversa* Hustedt. Scale bar = 10 μ m.

L. deniseae) with an undulating valve outline (Metzeltin & Lange-Bertalot 2007).

The most similar *Luticola* species is the presumably cosmopolitan *L. goeppertiana* (Bleisch) DG Mann.

The latter has a more elliptical-lanceolate outline, larger valve dimensions and somewhat smaller central raphe pores (Pavlov *et al.* 2009). The main differences are the central area that is typically asymmetrical in *L. deniseae* while *L. goeppertiana* has a symmetrical central area and the higher number of areolae per stria in *L. goeppertiana* (> 5). The orientation of the striae near the central area is less radiate in *L. deniseae* compared to *L. goeppertiana*. Finally, *L. goeppertiana* lacks the typical polar thickenings and its mantle shows only one row of areolae, whereas *L. deniseae* has 2-3 rows of large round areolae.

Luticola dismutica (Hustedt) DG Mann sensu Metzeltin & Lange-Bertalot (1998, figs 87: 22-24) is another comparable taxon, but its main differences from *L. deniseae* are the larger cells (width 8.5-10.0 μm versus 3.5-7.0 μm in *L. deniseae*) and coarser areolae. Other South American *Luticola* species such as *L. peguana* (Grunow) DG Mann and *L. simplex* Metzeltin *et al.* have a different (i.e. circular) stigma and a larger number of areolae per stria. In the Antarctic region, two recently described taxa (Van de Vijver & Mataloni 2008) present a similar valve outline but both *L. nelidae* Van de Vijver and *L. australomutica* Van de Vijver have a different raphe structure and an almost round stigma.

Ecology and distribution

The chelonian family Podocnemididae, known to have existed since the Cretaceous, comprises many extinct and eight extant large freshwater turtle species from South America and Madagascar (Lapparent de Broin 2001, Carvalho *et al.* 2002). Because of the pressure put on this species by local inhabitants *Podocnemis erythrocephala* is classified by the International Union for Conservation of Nature (IUCN) as vulnerable (Hilton 2000, Vogt 2001, Batistella & Vogt 2008, IUCN 2009).

Luticola deniseae has only been found so far in the Rio Negro living epizoically on small freshwater *Podocnemis erythrocephala* turtles. Mittermeier & Wilson (1974) described this turtle species as mainly herbivorous, feeding on aquatic plants and fallen fruit, but also on lines baited with fish; furthermore, animals in captivity do accept fish. Although a large number of samples were collected from aquatic plants, and hard substrates (e.g. rocks, pebbles and sediment) as well as phytoplankton (CE Wetzel, unpublished results), *L. deniseae* was never observed in any of these samples. The geographical range of these turtles is restricted to the Orinoco and Negro rivers. Although *L. deniseae* entirely dominated (> 80 % of all counted valves) the diatom community on the turtle carapaces, several other freshwater diatom species have also been found, such as *Actinella peronioides* Hustedt, *A. superperonioides* Metzeltin & Lange-Bertalot, *Chamaepinnularia brasilianopsis* Metzeltin & Lange-Bertalot, *Eunotia baculus* Hustedt, *E. conversa* Hustedt, *E. hirudo* Metzeltin & Lange-Bertalot, *E. reflexa* Hustedt,

Fragilariforma lata (Cleve-Euler) Williams & Round, *F. stevensonii* Metzeltin & Lange-Bertalot, *Frustulia* spp., *Luticola permuticoides* Metzeltin & Lange-Bertalot, and *Nupela rumrichorum* Lange-Bertalot. All referred species are commonly found in other habitats and are not exclusively found on the turtle carapaces, as it is the case of *Luticola deniseae*. Some of the listed species are shown in Figs 76-104.

ACKNOWLEDGEMENTS. – CEW thanks the Public Research Centre - Gabriel Lippmann (Belvaux, Luxembourg) for support and FAPESP - 'Fundação de Amparo à Pesquisa do Estado de São Paulo' (Process n° 07/51360-6) for a PhD grant. Financial support for this work was also provided by FINEP (Financiadora de Estudos e Projetos, Ministério da Ciência e Tecnologia, Governo Federal, Brazil). We are grateful to the herpetologists Dr R Vogt and to R Bernhardt (Instituto Nacional de Pesquisas da Amazônia, INPA, Brazil) for capturing the Rio Negro turtles. M A Oliveira and J Sayell kindly revised the English.

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Received September 17, 2009
 Accepted November 12, 2009
 Associate Editor: C Gobin