

***Nupela* (Bacillariophyceae) in littoral rivers from south Brazil, and description of six new species of the genus**

Priscila I. TREMARIN*, Arielli STRAUBE & Thelma A. V. LUDWIG

Botany Departament, Universidade Federal do Paraná, Centro Politécnico, P.O.Box 19031, 81531–990, Jardim das Américas, State of Paraná, Brazil; * Corresponding author e-mail: ptremarin@gmail.com

Abstract: The genus *Nupela* is characterized mainly by structure of the areola and raphe system. Many species were described from tropical regions; however, few studies have been conducted on Brazilian environments. The goal of this article is to identify and describe the morphology of *Nupela* species, under light and electron microscopy, found in the rivers of the Atlantic Forest from southern Brazil. Twelve species were found, including six described as new: *Nupela amabilis* TREMARIN et T. LUDWIG, *N. difficilis* STRAUBE, TREMARIN et T. LUDWIG, *N. kocioleckii* STRAUBE, TREMARIN et T. LUDWIG, *N. metzeltinii* TREMARIN et T. LUDWIG, *N. praecipuoides* TREMARIN et T. LUDWIG and *N. torganiae* TREMARIN et T. LUDWIG, and a new combination, *N. bicapitata* (HUSTEDT) TREMARIN et T. LUDWIG.

Key words: Atlantic Forest, diatom, freshwater, lotic, taxonomy, ultrastructure

INTRODUCTION

VYVERMANN & COMPÈRE (1991) established the genus *Nupela* based on *Nupela gihuensis* described as new to Papua New Guinea. *Nupela* comprises small freshwater diatoms with finely ornamented valves, difficult to resolve only by light microscopy. The areola with external opening covered by hymenes is larger than the internal aperture and is pointed as a very stable taxonomic and typical character (SIVER et al. 2007; SPAULDING & EDLUND 2008; KULIKOVSKIY et al. 2009). The external proximal ends of raphe usually are slightly expanded and internally somewhat curved or T-shaped (SPAULDING & EDLUND 2008).

The genus *Nupela* has been expanded in the last decade including heterovalvar species beyond the isovalvar forms based on the type material, referring to the degree of raphe development in both valve faces of the frustule. The raphe may be fully developed on both valves or fully developed on one valve and lacks or shortened on the other valve (MONNIER et al. 2003; POTAPOVA et al. 2003; SIVER & HAMILTON 2005; SIVER et al. 2007). Light microscopy is often insufficient for identification (POTAPOVA et al. 2003). Distinctive valve features are generally only observed by electron microscopy.

So far, about 50 *Nupela* species are known (CATALOGUE OF DIATOM NAMES 2013; BUCZKÓ et al. 2013; SALA et al. 2014). In Brazil, the *Nupela* species are documented mainly in floristic surveys (FARIA et al. 2010; CANANI et al. 2011; MORESCO et al. 2011; SANTOS

et al. 2011; BES et al. 2012; BARTOZEK et al. 2013). Seven species has been documented (TREMARIN et al. 2009) and a new species – *N. pardinhoensis* BES, TORGAN et ECTOR (BES et al. 2012) has been established. Despite the difficulty to distinguish species by optical microscopy, the number of studies is relatively broad worldwide. New species have been recorded to France (*N. exotica* MONNIER, LANGE–BERTALOT et BERTRAND), Poland (*N. marvanii* WOJTAŁ), United States (*N. neglecta* PONADER, LOWE et POTAPOVA, *N. carolina* POTAPOVA et CLASON, *N. scissura* SIVER, HAMILTON et MORALES, *N. potapovae* BAHLS, *N. elegantula* POTAPOVA, *N. frezelli* POTAPOVA), Russia (*N. matrioschka* KULIKOVSKIY, LANGE–BERTALOT et WITKOWSKI), among others (LANGE–BERTALOT 1993; LANGE–BERTALOT & MOSER 1994; MONNIER et al. 2003; POTAPOVA et al. 2003; SIVER & HAMILTON 2005; SIVER et al. 2007, 2010; KULIKOVSKIY et al. 2009; WOJTAŁ 2009; BAHLS 2011; POTAPOVA 2011; BUCZKÓ et al. 2013; SALA et al. 2014).

This paper aims to expand knowledge about the taxonomy of *Nupela* species based on samples collected in rheophilic environments from Brazilian Atlantic Forest, and well document the morphology of the taxa under light and electron microscopy.

MATERIAL AND METHODS

Diatom samples were collected from fifteen rivers along the Littoral basin of State of Paraná, located in the Atlantic Forest, Southern Brazil (Table 1). The headwaters of the selected rivers are located in the mountainous region or in

the plain, flowing toward the Atlantic Ocean. The upper courses, located at the Serra do Mar, have rapid and clear waters (São João, Mãe Catira, Cachoeira, Nhundiaquara, Nunes, Iporanga, Cacatu and Quintilha rivers). The lower courses are humic stained, and the rate of current is reduced (Guaraguaçu, Sertãozinho, da Onça, das Pombas, Cambará, do Salto and Colônia Pereira rivers) (BIGARELLA 2001).

The samples were collected and fixed with TRANSEAU solution (1:1) (BICUDO & MENEZES 2006). The phytoplankton were obtained with a plankton net (25 µm in mesh size) and periphyton samples were collected from stems of aquatic macrophytes, sand and stones submerged. Periphytic diatoms were obtained by scraping the biofilm with a brush or by vigorous agitation of the sandy sediment and supernatant was removed after settling for 30 seconds. Samples were cleaned with potassium permanganate (KMnO₄) and hydrochloric acid (HCl) (SIMONSEN 1974), rinsed and mounted in Naphrax® (R.I. = 1.74). Samples and slides are stored at the Herbarium of Universidade Federal do Paraná (UPCB) and at the Academy of Natural Sciences of Philadelphia (ANSP). Illustrations were performed using an optical microscope Olympus BX40 equipped with Olympus DP71 digital camera. Cleaned samples were prepared and analysed at scanning electron microscopy–SEM (JEOL JSM 6360LV and TESCAN VEGA3 LMU, 15 kV voltage and 8 mm working distance) and at transmission electron microscope–TEM (JEM microscope 1200EXII, voltage 80 kV), housed at the Electron Microscopical Center of Universidade Federal do Paraná. Abiotic variables (pH, electrical conductivity and water temperature) were measured using a Consort C535 equipment (Table 1). The terminology used in the descriptions followed ROUND et al. (1990), HENDEY (1964) and POTAPOVA et al. (2003).

RESULTS AND DISCUSSION

Nupela amabilis TREMARIN et T. LUDWIG sp. nov. (Figs 1–12)

Description: Frustules isovalvar, slightly asymmetric about apical plane. Valves linear–lanceolate with subrostrate apices, 11.5–12.2 µm long and 4.1–4.4 µm wide. Axial area linear. Central area asymmetric, reaching the margin on one side of valve, and limited by one or three areolae on the other side. Raphe slightly sinuous and complete on both valves. Proximal raphe externally expanded and internally hook–like. Terminal raphe ends curved to same side of the valve and internally ending in a small helictoglossa. Striae slightly radiate, ca. 50 in 10 µm, usually composed by discontinuous lines of areolae, 50 in 10 µm. Outer openings of areolae transapically elongate and the inner openings small, round to oval foramina.

Holotype: Slide UPCB 47513, Diatom collection of the Botany Department, Universidade Federal do Paraná, illustrated here in Figs 4–6.

Isotype: ANSP GC 26822.

Type material: Guaraguaçu river, Pontal do Paraná, State of Paraná, Brazil, 25°43'5.77"S, 48°33'26.81"W, October 2003.

Taxonomical remarks: The isovalvarity is not a common feature in the genus, only detected in some species, as *N. giluwensis* VYVERMAN, *N. mutabilis* SIVER, WOLFE et EDLUND, *N. potapovae* BAHLS, *N. subinvicta* (KRASSKE) LANGE–BERTALOT, *N. tristis* (KRASSKE) LANGE–BERTALOT, *N. vitiosa* (SCHIMANSKI) SIVER et HAMILTON, and for *N. amabilis* (LANGE–BERTALOT et al. 1996; SIVER & HAMILTON 2005; SIVER et al. 2010; BAHLS 2011). Among them, only *N. mutabilis* has valve outline similar to *N. amabilis*, but differs in the rostrate apices and the T–shaped internal proximal raphe ends (SIVER et al. 2010).

Nupela amabilis differs from most species of genus by the internal proximal raphe ends strongly curved in hook. Similar raphe was also described to *N. vitiosa*, *N. lapidosa* (KRASSKE) LANGE–BERTALOT, *N. neotropica* (HUSTEDT) LANGE–BERTALOT, *N. tenuicephala* (HUSTEDT) LANGE–BERTALOT, *N. acaciensis* VOUILLOUD et SALA and *N. catatumbensis* VOUILLOUD et PLATA–DÍAZ (LANGE–BERTALOT 1993; LANGE–BERTALOT & MOSER 1994; SIVER & HAMILTON 2005; WOJTAL 2009; SALA et al. 2014). Despite raphe ends coincidence among mentioned species, isovalvar feature is found only in *N. acaciensis* (SALA et al. 2014). The latter is distinguished from *N. amabilis* by lanceolate valve outline, cymbelloid symmetry, smaller central area and more elongated apices. *Nupela vyvermanii* LANGE–BERTALOT illustrated by WERUM & LANGE–BERTALOT (2004) has linear–lanceolate valve outline similar to *Nupela amabilis*, but has heterovalvar frustules with round central area, and its internal proximal ends of raphe are straight (WERUM & LANGE–BERTALOT 2004).

Thus, the proposition of *Nupela amabilis* is justified by a unique set of characters, mainly consisting of isovalvarity, linear–lanceolate valve outline and hook–shaped internal proximal raphe ends.

Nupela bicapitata (HUSTEDT) TREMARIN et T. LUDWIG comb. nov. (Figs 13–22)

Basionym: *Achnanthes bicapitata* HUSTEDT, Botaniska Notiser, p. 386, fig. 68–71, 1952.

Description: Frustules heterovalvar, slightly asymmetric about apical and transapical planes. Valves lanceolate with capitate apices, 19.2–22.9 µm long and 5.5–6.6 µm wide. Axial area lanceolate. Central area asymmetric, unilaterally expanded until the margin of the valve. Raphe slightly sinuous. One valve slightly convex, with long raphe slits. The other valve, slightly concave, with very short raphe slits, restricted to the apices, widely separated proximal endings, and with a linear depression along the axial area that resembles a raphe on LM. Proximal raphe ends externally simple. Terminal raphe ends curved to same side of the valve. Striae slightly radiate becoming convergent towards apices, ca. 44 in 10 µm.

Taxonomical remarks: Based on LM microscopy, the Brazilian material is exactly coincident to *Achnanthes*

Table 1. Localities in the State of Paraná – Brazil, data of sampling and their record number at the Herbarium of the Universidade Federal do Paraná (UPCB).

Register number	Locality	County	Date	Geographic coordinates	pH	Conductivity (µS.cm ⁻¹)	Temperature (°C)
47493–47503, 47506, 47509–47521, 47523–47526	Guaraguaçu river	Pontal do Paraná	16/04/2003, 24/10/2003	25°43'5.7"S, 48°33'26.8"W to 25°34'58.8"S, 48°28'43.5"W
72994, 72997	São João river	Morretes	02/12/2011	25°22'50.4"S, 48°51'50.1"W	6.9	21.1	19
72978, 72975	Mãe Catira river	Morretes	02/12/2011	25°21'52.7"S, 48°52'26.4"W	6.1	18.2	18.5
72984, 72985	do Nunes river	Antonina	02/12/2011	25°20'43.2"S, 48°46'14.0"W	6.3	16.7	19
72981, 72982	Cachoeira river	Antonina	02/12/2011	25°19'9.9"S, 48°42'26.8"W	6.9	39.4	20
76000, 73000	Cacatu river	Antonina	02/12/2011	25°19'26"S, 48°45'7.7"W	6.3	11.3	19
72979, 72980	Nhundiaquara river	Morretes	02/12/2011	25°26'01.7"S, 48°52'25.6"W	7.3	19.9	18.5
72972, 72973, 72974	Iporanga river	Morretes	02/12/2011	25°29'14.5"S, 48°51'9.6"W	9.7	17.0	19
72969, 72970	da Onça river	Matinhos	03/12/2011	25°43'54.3"S, 48°30'32.2"W	7.9	2.76	23
72971	Sertãozinho river	Matinhos	03/12/2011	25°48'0.58"S, 48°33'19.1"W	5.7	69.7	21
72965, 72966	Cambará river	Paranaguá	03/12/2011	25°43'59.8"S, 48°35'26.2"W	7.2	34.3	21
75227, 75226	Colônia Pereira river	Matinhos	03/12/2011	25°41'15.6"S, 48°34'30.3"W	6.2	151	21
73001, 73002	das Pombas river	Matinhos	03/12/2011	25°39'13.0"S, 48°35'12.7"W	6.1	167	22
73005	do Salto river	Paranaguá	03/12/2011	25°36'43.6"S, 48°36'18.6"W	6.6	78.4	22
72993, 72989	Quintilha river	Paranaguá	03/12/2011	25°38'28"S, 48°37'14.1"W	7.2	29.4	21

bicapitata described from a Norwegian sample as a monoraphid species by HUSTEDT (1952) and illustrated by SIMONSEN (1987, pl. 584, figs 1–11). The species is characterized by well developed raphe at one valve and an evident longitudinal linear depression at the other (Figs 13, 15, 18, 20). Nevertheless, SEM analyses of Brazilian material revealed a very reduced apical raphe at the supposed araphid valve. The new combination here performed is justified by the well documented typical *Nupela* areola and raphe.

This is the first subtropical record of this rarely reported species that besides type locality also occurred in North American low alkaline waters (CAMBURN & CHARLES 2000).

***Nupela decipiens* (REIMER) POTAPOVA (Figs 23–42)**

Basionym: *Achnanthes decipiens* REIMER, Notulae Naturae, p. 2, pl.1, figs 2–5, 1966.

Description: Frustules heterovalvar, slightly asymmetric about apical plane. Valves lanceolate with narrowly rostrate to subrostrate apices, 8.9–18.5 µm long and 4.1–5.9 µm wide. One valve with long raphe slits and the other valve araphid. Raphid valve with axial area lanceolate, central area widely rounded. Proximal raphe ends externally expanded and internally simple. Terminal raphe ends curved to same side of the valve and internally ending in a small helictoglossa. Araphid valve with axial area widely lanceolate, externally ornamented by several irregular depressions and a longitudinal one that resembles a raphe in LM; internally with slits occluded near the apices. Central area widely lanceolate, not reaching the valvar margin. Transapical striae radiate, 36–40 in 10 µm, composed by continuous lines of areolae, ca. 52 in 10 µm. Inner openings of areolae small, round to oval.

Taxonomical remarks: The morphometric variation of *N. decipiens* in the Brazilian specimens agrees with that given by POTAPOVA (2013) to USA. This register from Guaraguaçu river is the first record of the taxon to South America.

***Nupela difficilis* STRAUBE, TREMARIN et T. LUDWIG sp. nov. (Figs 43–54)**

Description: Frustules heterovalvar, slightly asymmetric about apical and transapical planes. Valves lanceolate with subrostrate apices, 8.8–14.8 µm long and 4.1–5.9 µm wide. Axial area lanceolate. Central area asymmetric, unilaterally reaching the valve margin. Raphe on both valves. One valve with long raphe slits. The other valve with shorter raphe slits and separated proximal endings. Proximal raphe ends internally simple. Terminal raphe ending in a small helictoglossa. Transapical striae slightly radiate, 40 in 10 µm, composed by continuous lines of areolae, 48–50 em 10 µm. Inner openings of areolae small–round.

Holotype: Slide UPCB 72984, Diatom collection

of the Botany Department, Universidade Federal do Paraná, illustrated here in Figs 48–49.

Isotype: ANSP GC26823

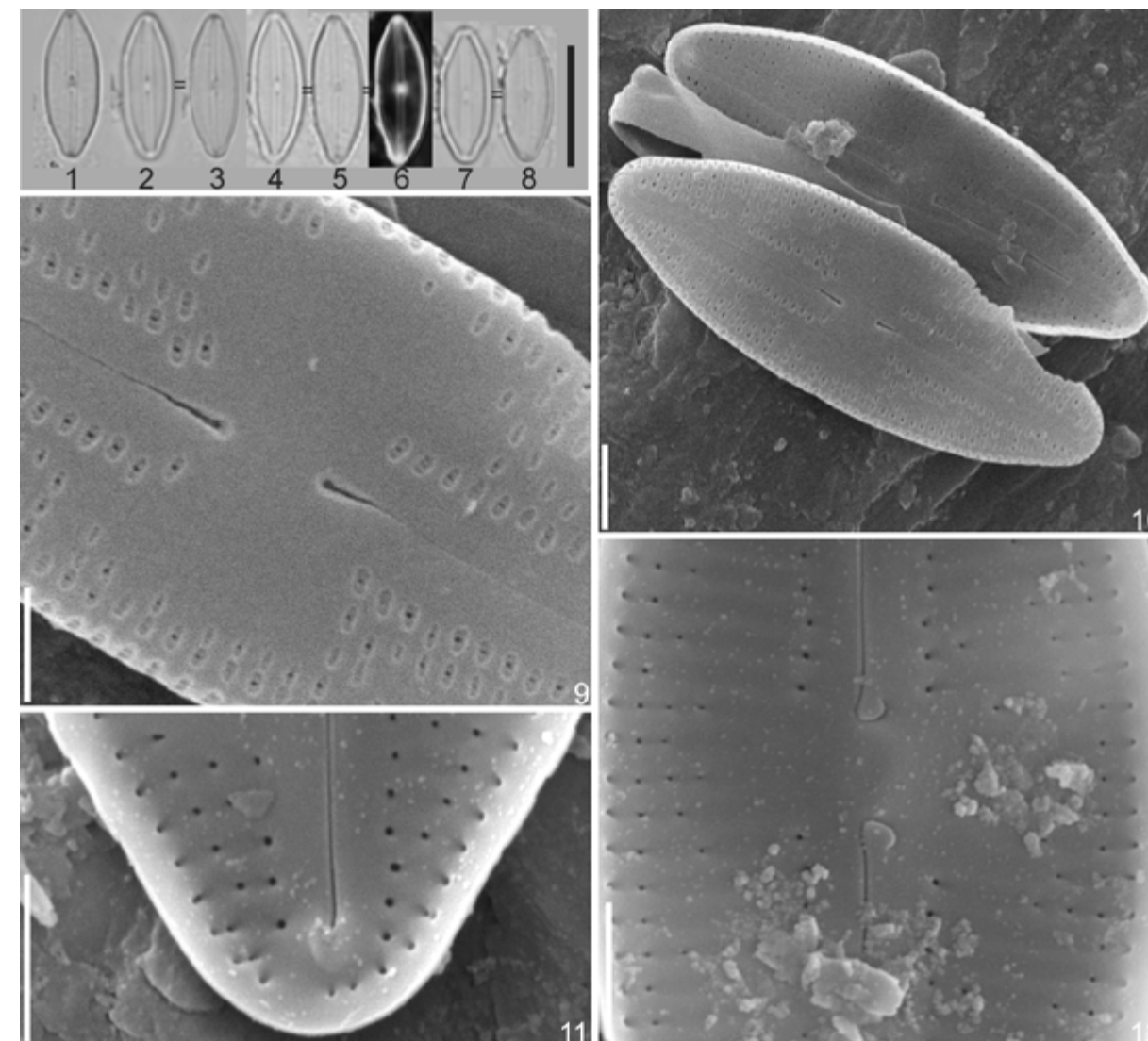
Type material: Do Nunes river, Antonina, State of Paraná, Brazil, 25°20'43.2"S, 48°46'14.0"W, December 2011.

Taxonomical remarks: *Nupela difficilis* is similar to *N. decipiens* in valve dimensions, shape of apices and raphe ends, but the latter differs by the monoraphid frustule and central area shape (POTAPOVA 2013). *Nupela vyvermanii* described and illustrated by LANGE–BERTALOT in MOSER et al. (1998) differs of *N. difficilis* by the smaller central area and lower density of striae (32 in 10 µm), besides convergent striae at the apices (WERUM & LANGE–BERTALOT 2004, pl. 25, figs 3–6). *Nupela* cf. *vyvermanii* LANGE–BERTALOT found by ALFINITO & LANGE–BERTALOT (2013, figs 65, 66) from Sierra Leone resembles *N. difficilis* in the valve size (length 14–15 µm and width 4–5 µm) and apices shape, distinguishing by the lower number of striae (ca. 30 in 10 µm) and central area not reaching to valve margin. *Nupela difficilis* is mainly characterized by the valve outline, apices and central area contour, also by the straight proximal raphe ends in the inside valve.

***Nupela exotica* MONNIER, LANGE–BERTALOT et BERTRAND (Figs 55–65)**

Description: Frustules heterovalvar, slightly asymmetric about apical plane. Valves elliptical–lanceolate with rounded to subrostrate apices, 10.4–15.2 µm long and 4.4–5.2 µm wide. Axial area straight and narrow. Central area reduced. Raphe on both valves, slightly sinuous. Raphe slits well developed along one valve and shortened at the other. Proximal raphe ends externally expanded and internally simple. Terminal raphe ends curved to same side of the valve and internally ending in a small helictoglossa. Transapical striae straight to slightly radiate, 38–40 in 10 µm, composed by discontinuous lines of areolae, ca. 23 in 10 µm. Outer openings of areolae transapically elongate and inner openings small, round to oval.

Taxonomical remarks: The exemplars of *N. exotica* analyzed in the Brazilian samples were wider than those recorded by MONNIER et al. (2003, width 3.0–4.1 µm), however other frustule features were identical to the type material. The irregular striation pattern of *N. exotica* resembles that of *N. pardinhoensis*, a recently species described to Southern Brazil. *Nupela pardinhoensis* differs to *N. exotica* mainly by the shortened raphe slits on one valve and more separated proximal endings (MONNIER et al. 2003, BES et al. 2012). In Brazil, this species was cited by TREMARIN et al. (2009).



Figs 1–12. *Nupela amabilis* TREMARIN et T. LUDWIG, LM (1–8) and SEM (9–12): (4–6) Holotype; (9) Detail of central region of valve in external view; (10) Overview of valves in external and internal view; (11) Distal end of raphe in internal view (12), Proximal ends of raphe in internal view. Scale bars 10 µm (1–8); 2 µm (9); 1 µm (10–12).

***Nupela kociolekii* STRAUBE, TREMARIN et T. LUDWIG sp. nov. (Figs 66–83)**

Description: Frustules heterovalvar, slightly asymmetric about apical plane. Valves elliptical–lanceolate with narrowly subrostrate apices, 9.4–14.1 µm long and 4.7–5.9 µm wide. Axial area lanceolate, sometimes with delicate depressions. Central area asymmetric, unilaterally reaching the valve margin, sometimes a c–letter area appears under LM. Raphe on both valves, slightly sinuous. One valve with long raphe slits. The other valve with shorter raphe slits and more separated proximal raphe endings. Proximal raphe ends externally expanded and internally hook–shape. Terminal raphe ends curved to same side of the valve and internally ending in small helictoglossa. Transapical striae radiate, 30 in 10 µm, composed by continuous lines of areolae, ca. 50 in 10 µm. Outer openings of areolae transapically elongate and inner openings small and round.

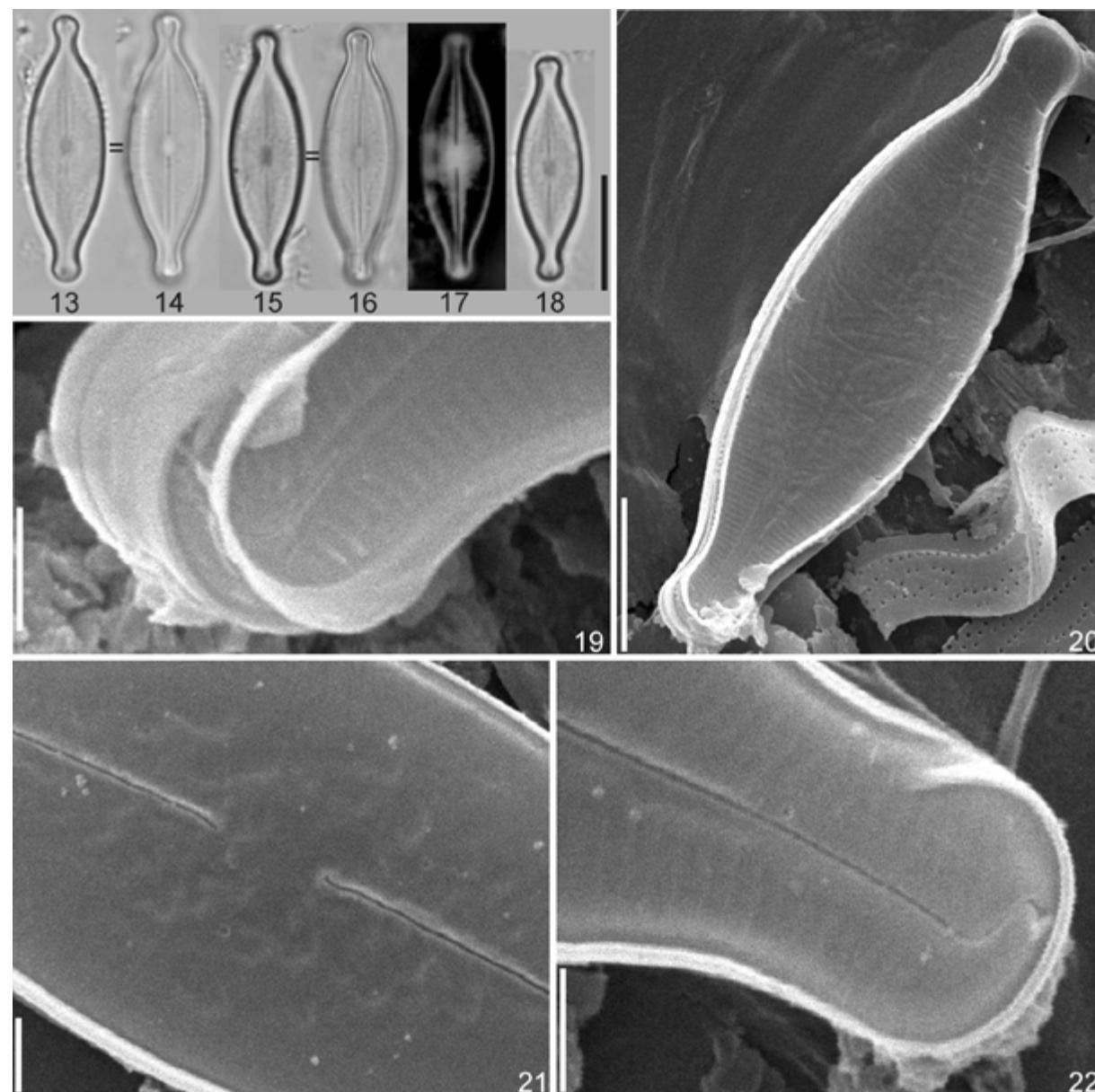
Etymology: This species is dedicated to Dr. Patrick KOCIOLEK, University of Colorado.

Holotype: Slide UPCB 72984, Diatom collection of the Botany Department, Universidade Federal do Paraná, illustrated here in Figs 66–67.

Isotype: ANSP GC26823.

Type material: Do Nunes river, Antonina, State of Paraná, Brazil, 25°20'43.2"S, 48°46'14.0"W, December 2011.

Taxonomical remarks: *Nupela kociolekii* is more closely related to *N. difficilis* with respect to the degree of development of the raphe and by the shape of apices. Although both species have occurred in the same samples, these can be differentiated by the proximal raphe ends, and number of striae. The striae of *N. difficilis* are almost inconspicuous in LM because of its denser arrangement (40 in 10 µm), furthermore the internal proximal raphe ends are straight.



Figs 13–22. *Nupela bicapitata* (HUSTEDT) TREMARIN et T. LUDWIG, LM (13–18) and SEM (19–22): (19) Extremities of valve showing the shortened raphe; (20) Overview of valve with shortened raphe; (21) Detail of central region of valve in external view; (22) External view of distal end of raphe. Scale bars 10 μm (13–18); 5 μm (20); 1 μm (19, 21, 22).

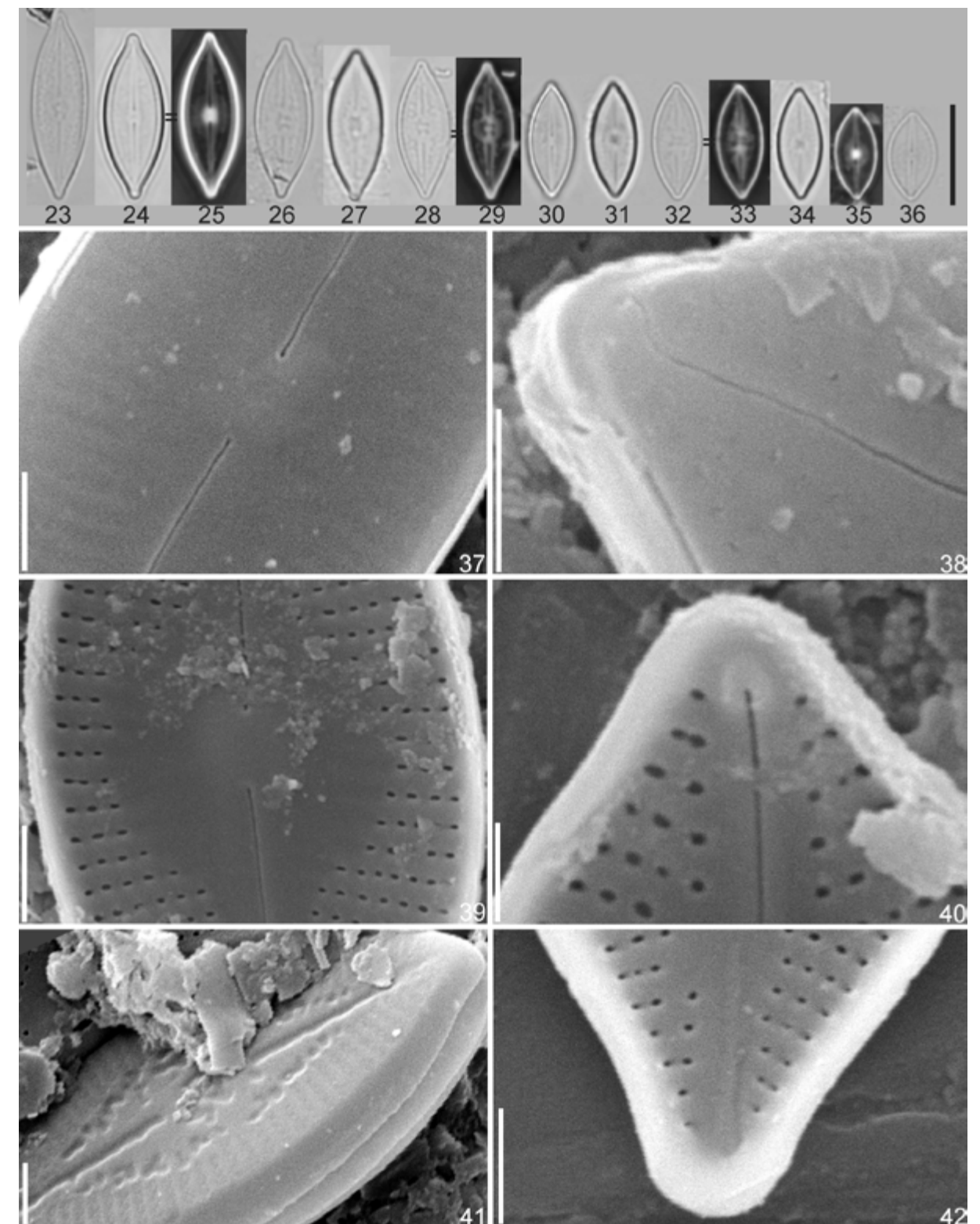
Nupela scissura SIVER, HAMILTON et MORALES resembles *N. kociolekii* in the shape of valves and apices, and by the central area unilaterally expanded until the margin with c-shaped area (SIVER et al. 2007). However, *N. scissura* differs by the smaller central area, higher number of striae (48–58 in 10 μm), straight proximal raphe ends and monoraphid frustules (SIVER et al. 2007).

***Nupela* cf. *matrioschka* KULIKOVSKIY, LANGE–BERTALOT et WITKOWSKI (Figs 84–86)**

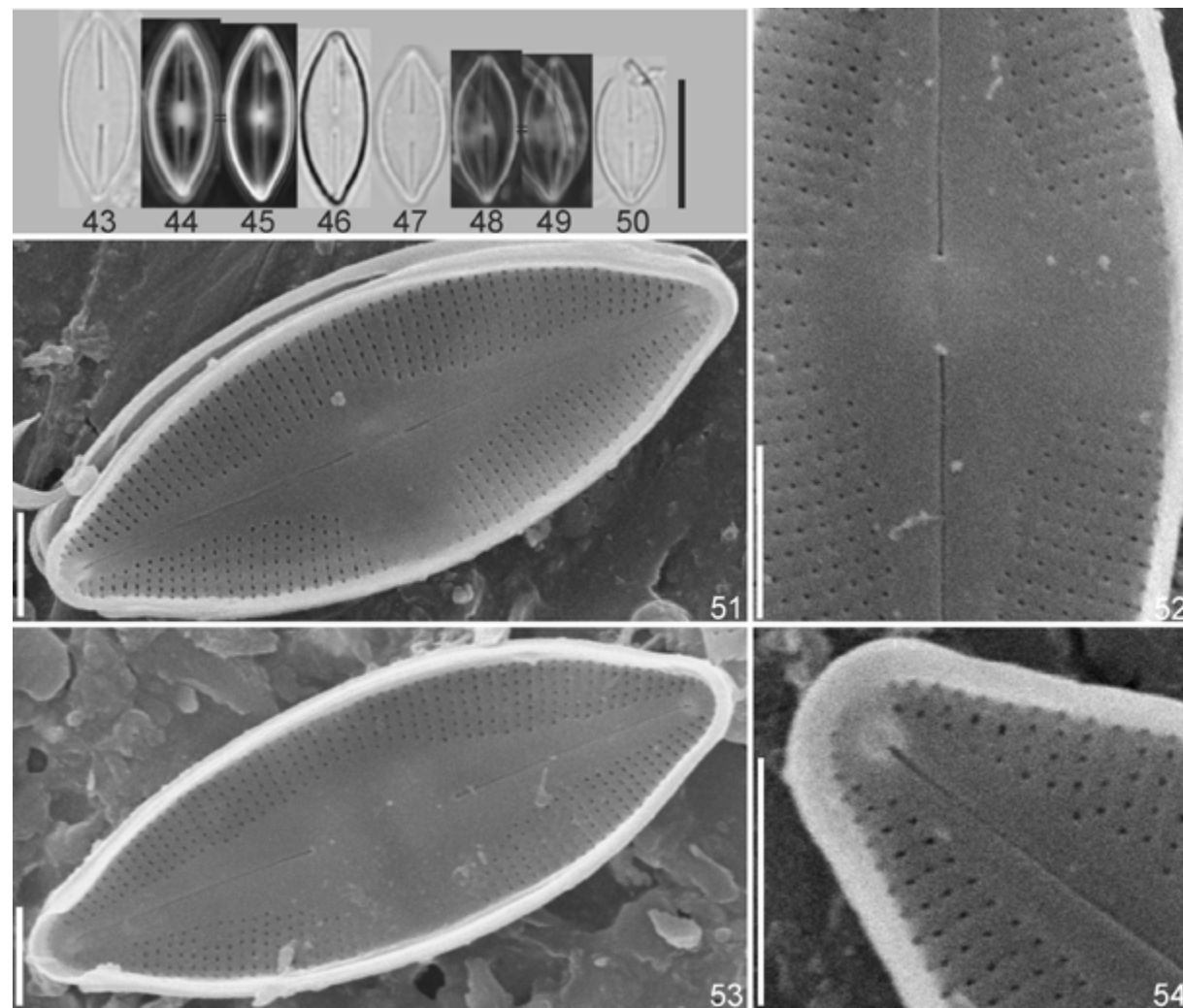
Description: Frustules heterovalvar, slightly asymmetric about apical plane. Valves elliptical to elliptical–lanceolate with substrate to rounded apices, 9.3–10.3 μm long and 4.6–5.1 μm wide. Axial

area linear and narrow. Central area reduced. Raphe on both valves, slightly sinuous. One valve with long raphe slits. The other valve with shorter raphe slits and slightly separated proximal endings. Proximal raphe ends externally expanded. Terminal raphe ends curved to the same side of the valve. Transapical striae radiate, ca. 35 in 10 μm , usually composed by discontinuous lines of areolae, ca. 39 in 10 μm . Outer openings of areolae transapically elongate.

Taxonomical remarks: *Nupela matrioschka* was described to *Sphagnum* bogs from Russia and has no subsequent records yet (KULIKOVSKIY et al. 2009). Few exemplars found in the Brazilian samples were similar to *Nupela matrioschka* in shape and size of the



Figs 23–42. *Nupela decipiens* (REIMER) POTAPOVA, LM (23–36) and SEM (37–42): (37) Central area of valve in external view; (38) External view of distal end of raphe; (39) Central region of valve with raphe in internal view; (40) Distal end of raphe in internal view; (41) External view of valve without raphe; (42) Detail of extremities of valve showing occluded fissure in internal view. Scale bars 10 μm (23–36); 1 μm (37–39, 41, 42); 0.5 μm (40).



Figs 43–54. *Nupela difficilis* STRAUBE, TREMARIN et T. LUDWIG, LM (43–50) and SEM (51–54): (48–49) Holotype; (51) Internal view of valve with longer raphe; (52) Central region of valve in internal view; (53) Internal view of valve with shortened raphe; (54) Distal end of raphe in internal view. Scale bars 10 µm (43–50); 2 µm (51–54).

valves and somewhat discontinuous striation pattern. However the valves have lower density of radiate striae (non 42–48 in 10 µm), not convergent at the apices (KULIKOVSKIY et al. 2009).

We did not find other *Nupela* species with similar features among the individuals studied. The scarce exemplars found in Brazilian material did not allow detailed analysis of the frustules and a possible proposal of a new taxon.

***Nupela metzeltinii* TREMARIN et T. LUDWIG sp. nov. (Figs 87–92)**

Description: Frustules heterovalvar, slightly asymmetric about apical plane. Valves narrowly lanceolate with subcapitate apices, 14.8–18.9 µm long and 3.7–4.1 µm wide. Axial area linear and narrow, with depressions along the raphe slits. Central area rounded. Raphe on both valves, straight. One valve with long raphe slits. The other valve with shorter raphe slits and separated proximal endings. Proximal raphe ends externally simple and straight. Terminal raphe ends

curved to same side of the valve. Transapical striae radiate to slightly convergent at the apices, ca. 50 in 10 µm.

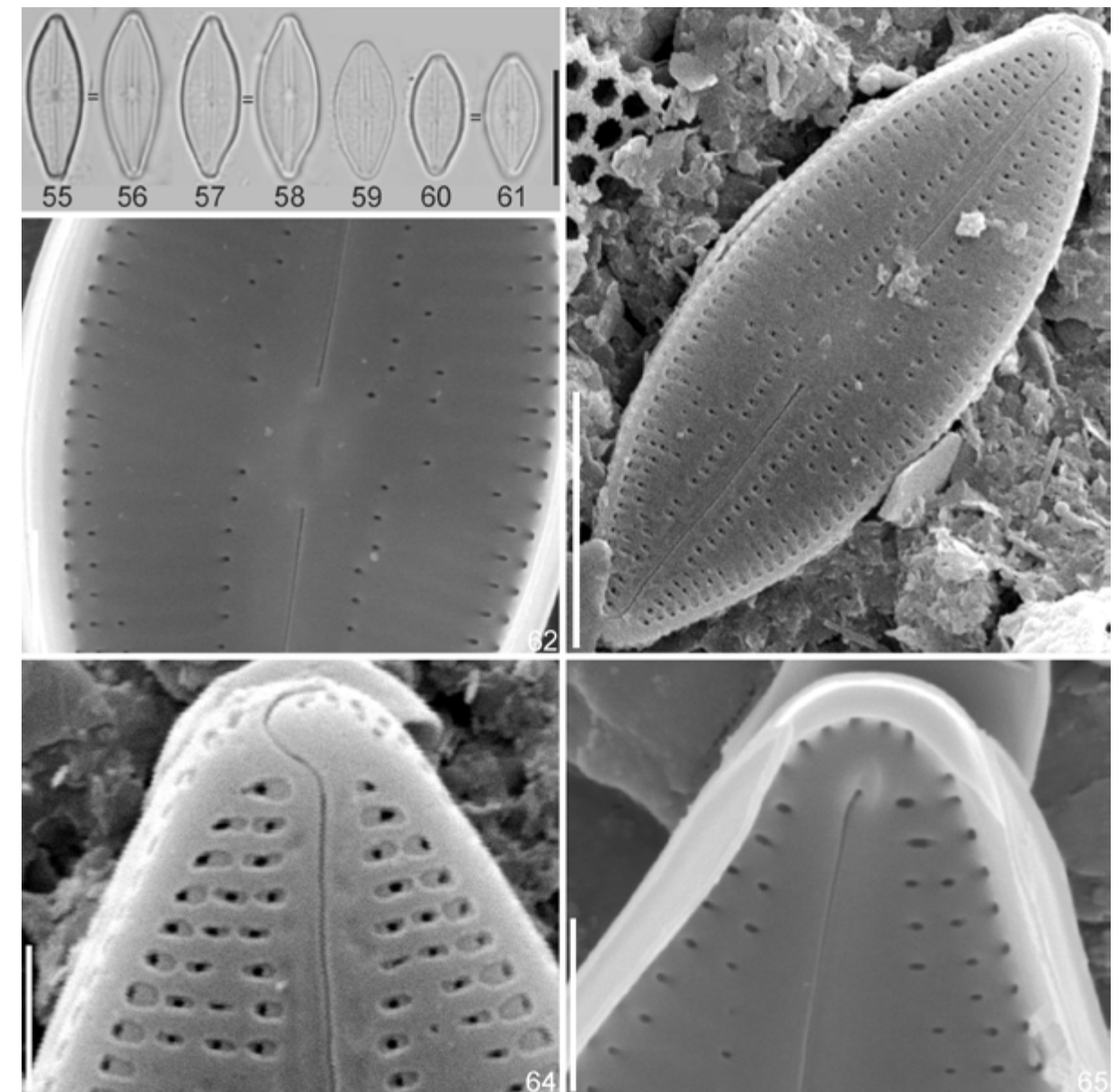
Etymology: This species is dedicated to the diatomist Ditmar METZELTIN from Germany.

Holotype: Slide UPCB 47494, Diatom collection of the Botany Department, Universidade Federal do Paraná, illustrated here in Figs 89–90.

Isotype: ANSP GC26822.

Type material: Guaraguaçu river, Pontal do Paraná, State of Paraná, Brazil, 25°43'5.77"S, 48°33'26.81"W, October 2003.

Taxonomical remarks: *Nupela metzeltinii* is similar in shape and dimensions of the valves, apices and number of striae to *N. neotropica* LANGE–BERTALOT, which differs by the unilateral central area (LANGE–BERTALOT & MOSER 1994). *Nupela marvanii* WOJTAŁ also have lanceolate valves and heterovalvar frustules as *N. metzeltinii*, however the former have wider valves



Figs 55–65. *Nupela exotica* MONNIER, LANGE–BERTALOT et BERTRAND, LM (55–61) and SEM (62–65): (62) Internal view of central region of valve with longer raphe; (63) External view of valve with shorter raphe; (64, 65) Distal end of raphe in external and internal view, respectively. Scale bars 10 µm (55–61); 5 µm (63); 1 µm (62, 64, 65).

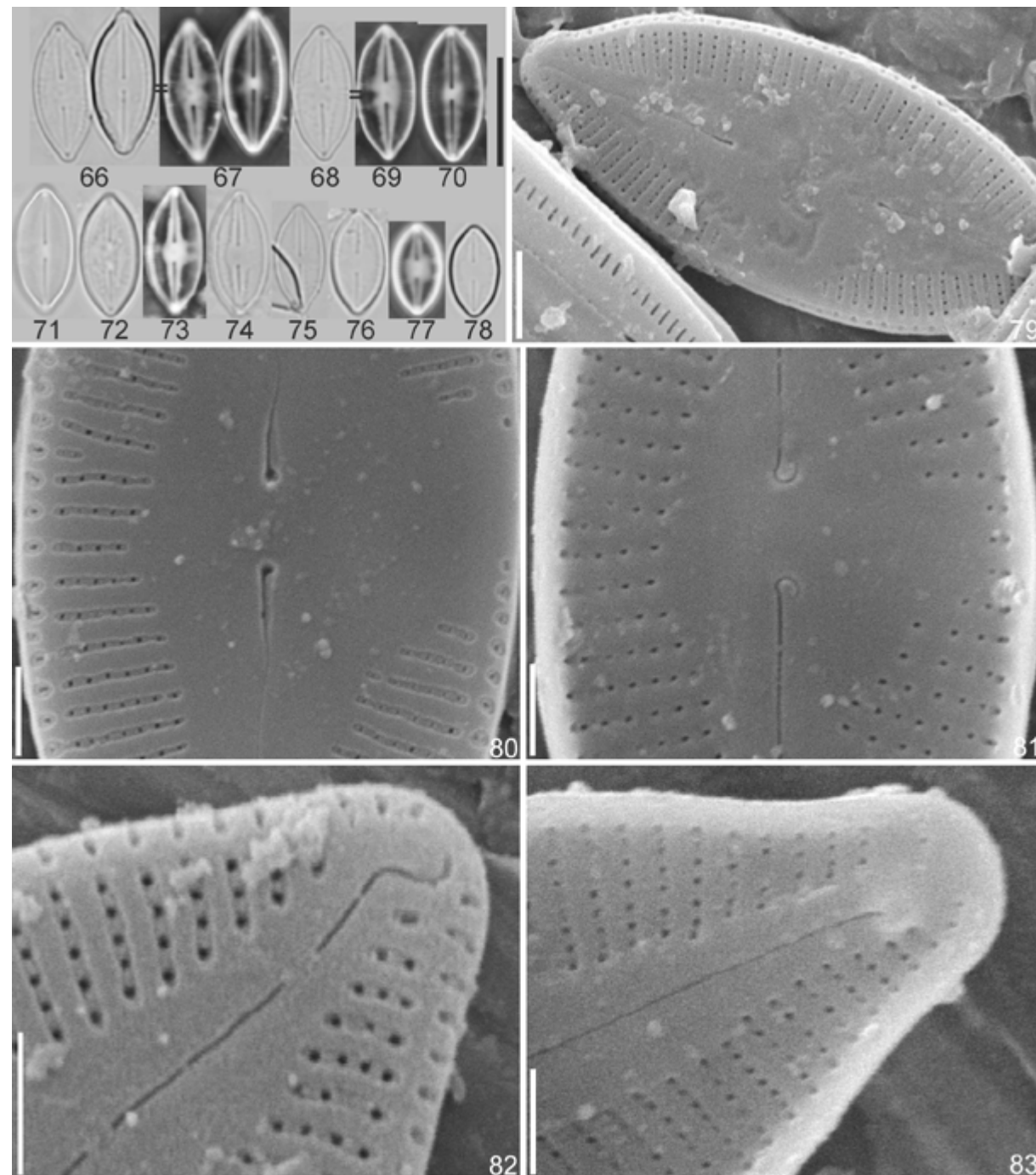
(5–6 µm), capitate apices and a very short raphe on one of the valves (WOJTAŁ 2009). *Nupela metzeltinii* was rare in the analyzed samples. Although it has not been possible a detailed description of the internal valve, no similar known species was found. The proposition of *Nupela metzeltinii* is justified by a set of characteristics, as heterovalvarity, lanceolate valves, subcapitate ends, raphe slits little shorter on one of the valve, and delicate depressions in the axial area.

***Nupela neglecta* PONADER, LOWE et POTAPOVA (Figs 93–96)**

Description: Frustules heterovalvar, slightly asymmetric about apical and transapical planes. Valves lanceolate with subrostrate apices, 10.4–10.7 µm long

and width 4.1 µm wide. Axial area linear and narrow. Central area small. One valve with long raphe slits. The other valve with very short raphe slits and widely separated proximal endings. Proximal raphe ends simple. Terminal raphe ends curved to same side of the valve and internally ending in a small helictoglossa. Transapical striae slightly radiate, 40 in 10 µm, composed by discontinuous lines of areolae, 2–4 per striae. Inner openings of areolae small, round or oval.

Taxonomical remarks: *Nupela lesiothensis* (SCHOEMAN) LANGE–BERTALOT and *N. jahniae-reginae* LANGE–BERTALOT are the most similar species to *N. neglecta* in relation to size and shape of the valves and development of raphe, with long raphe slits on one



Figs 66–83. *Nupela kociolekii* STRAUBE, TREMARIN et T. LUDWIG, LM (66–78) and SEM (79–83): (66–67) Holotype; (79) External view of valve with shortened raphe; (80, 81) Central region of valve showing the proximal ends of raphe in external and internal view, respectively; (82, 83) Distal end of raphe in external and internal view, respectively. Scale bars 10 μ m (66–78); 2 μ m (79); 1 μ m (80–83).

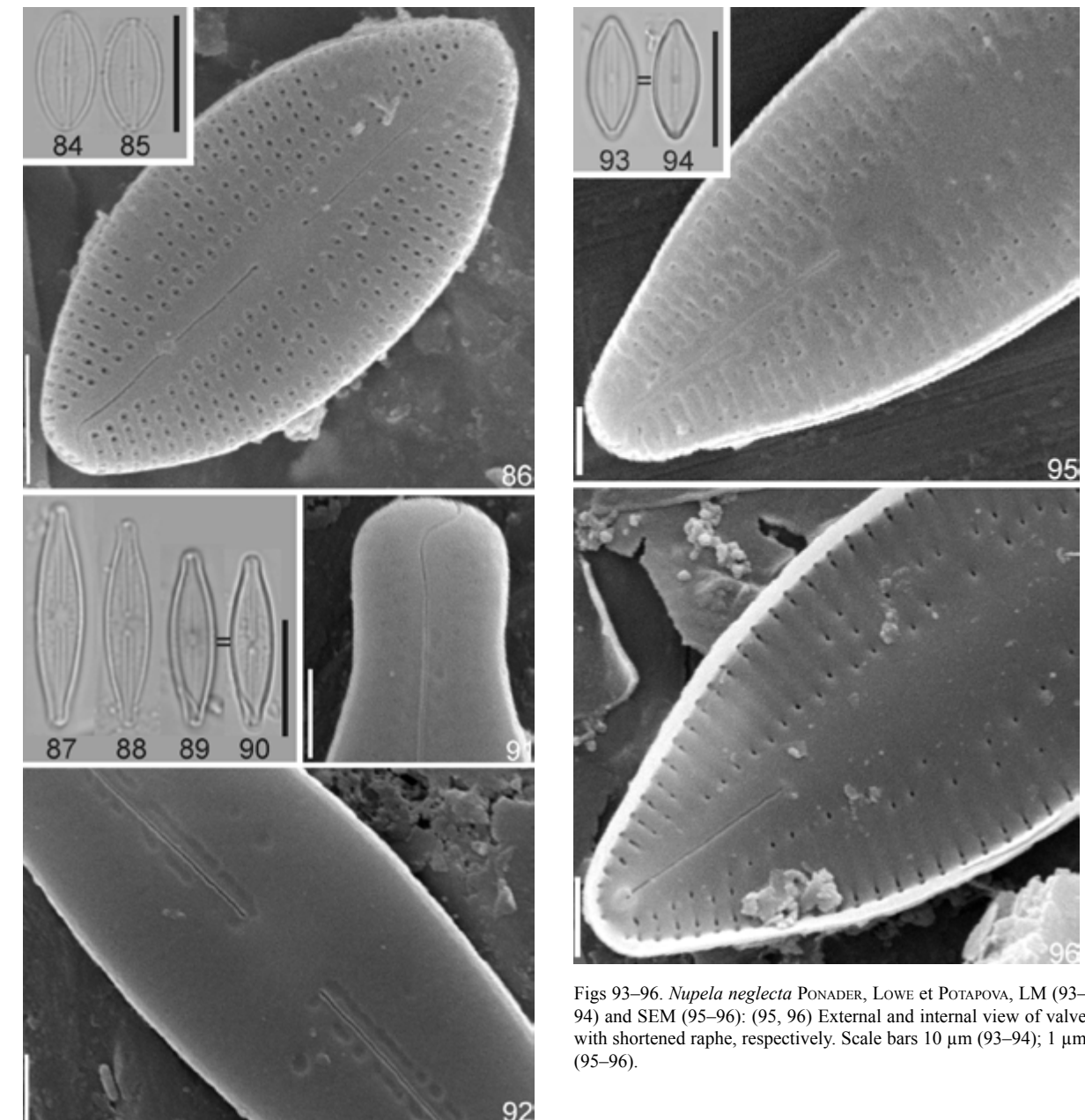
valve and shorter on the other. These species differs mainly by the shape of apices, disposition of the areolae in the stria and number of longitudinal rows of areolae on each side of the raphe. *Nupela neglecta* presents slightly protracted apices, discontinuous striae and 3–4 rows of areolae while the other two species have rounded obtuse apices, continuous striae and more than 4 rows of areolae (METZELTIN & LANGE–BERTALOT 1998; RUMRICH et al. 2000; POTAPOVA et al. 2003).

Nupela neglecta was described by epilithic

samples from New Jersey (USA). This is the first record of *N. neglecta* to South America.

***Nupela praecipuoides* TREMARIN et T. LUDWIG sp. nov. (Figs 97–111)**

Description: Frustules heterovalvar, slightly asymmetric about apical plane. Valves lanceolate with slightly protracted to subrostrate apices, 9.2–21.5 μ m long and 4.1–5.5 μ m wide. One valve with long raphe slits and the other valve araphid. Raphid valve with axial



Figs 84–86. *Nupela* cf. *matrioschka* KULIKOVSKIY, LANGE–BERTALOT et WITKOWSKI, LM (84–85), SEM (86): (86) Overview of valve. Figs 87–92. *Nupela metzeltinii* TREMARIN et T. LUDWIG, LM (87–90) and SEM (91–92): (89–90) Holotype; (91) Distal end of raphe in external view; (92) Detail of central region of valve showing the proximal ends of raphe in external view. Scale bars 10 μ m (84–85, 87–90); 2 μ m (86); 1 μ m (91, 92).

area linear and narrow, central area rounded. Proximal raphe ends externally expanded and internally simple. Terminal raphe ends curved to same side of the valve and internally ending in small helictoglossa. Araphid valve with lanceolate axial area, smooth or generally with irregular depressions visible or not under LM. Valve araphid sometimes with internal slits occluded by silica, near the apices. Transapical striae radiate to straight at the apices, 36–38 in 10 μ m, composed by continuous lines of areolae, 35–39 in 10 μ m.

Outer openings of areolae transapically elongate and occluded by delicate hymenate layer. Inner openings of areolae small, round to oval.

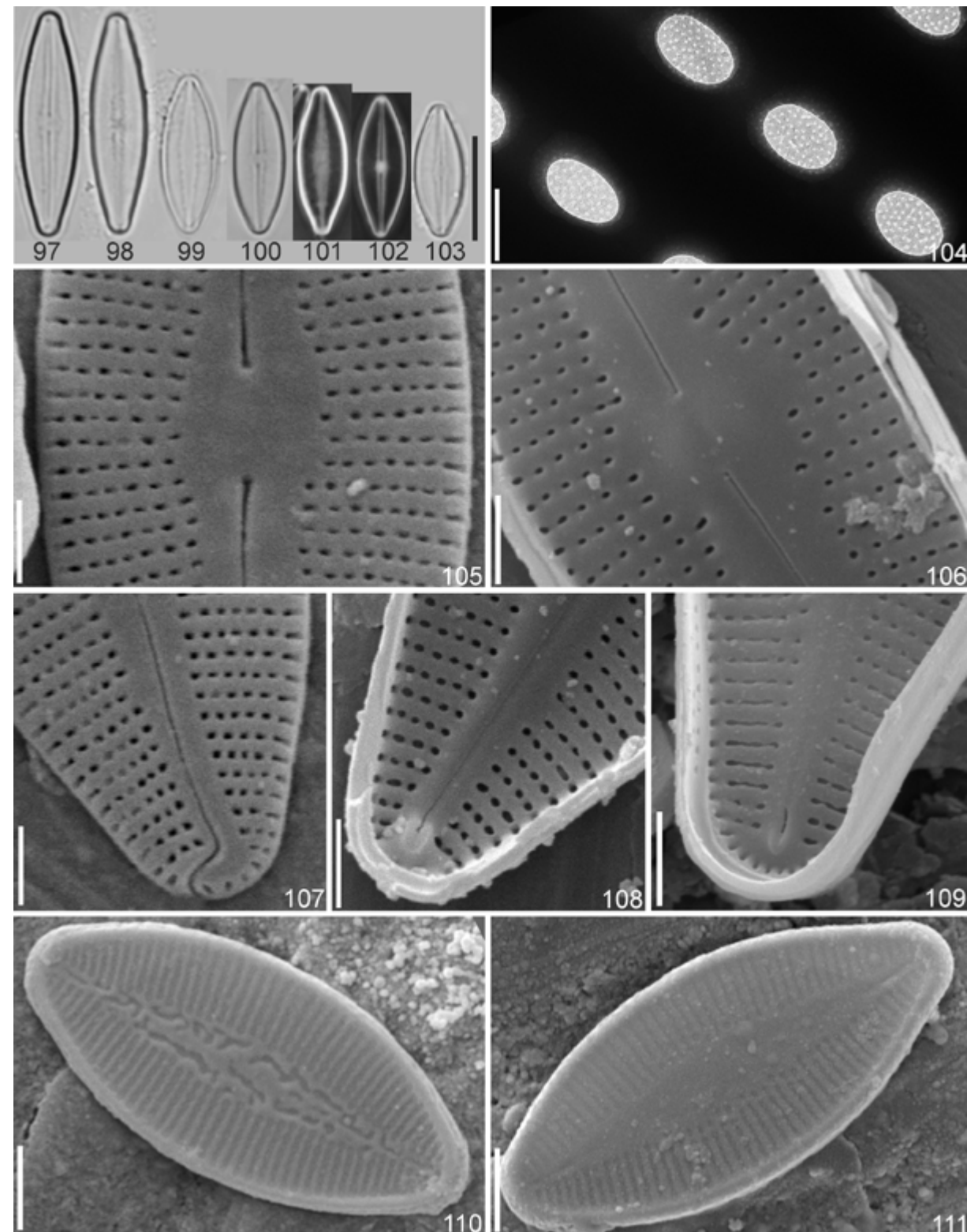
Etymology: This species name was given due to its morphological resemblance to *Nupela praecipua*.

Holotype: Slide UPCB 47494, Diatom collection of the Botany Department, Universidade Federal do Paraná, illustrated here in Fig. 103.

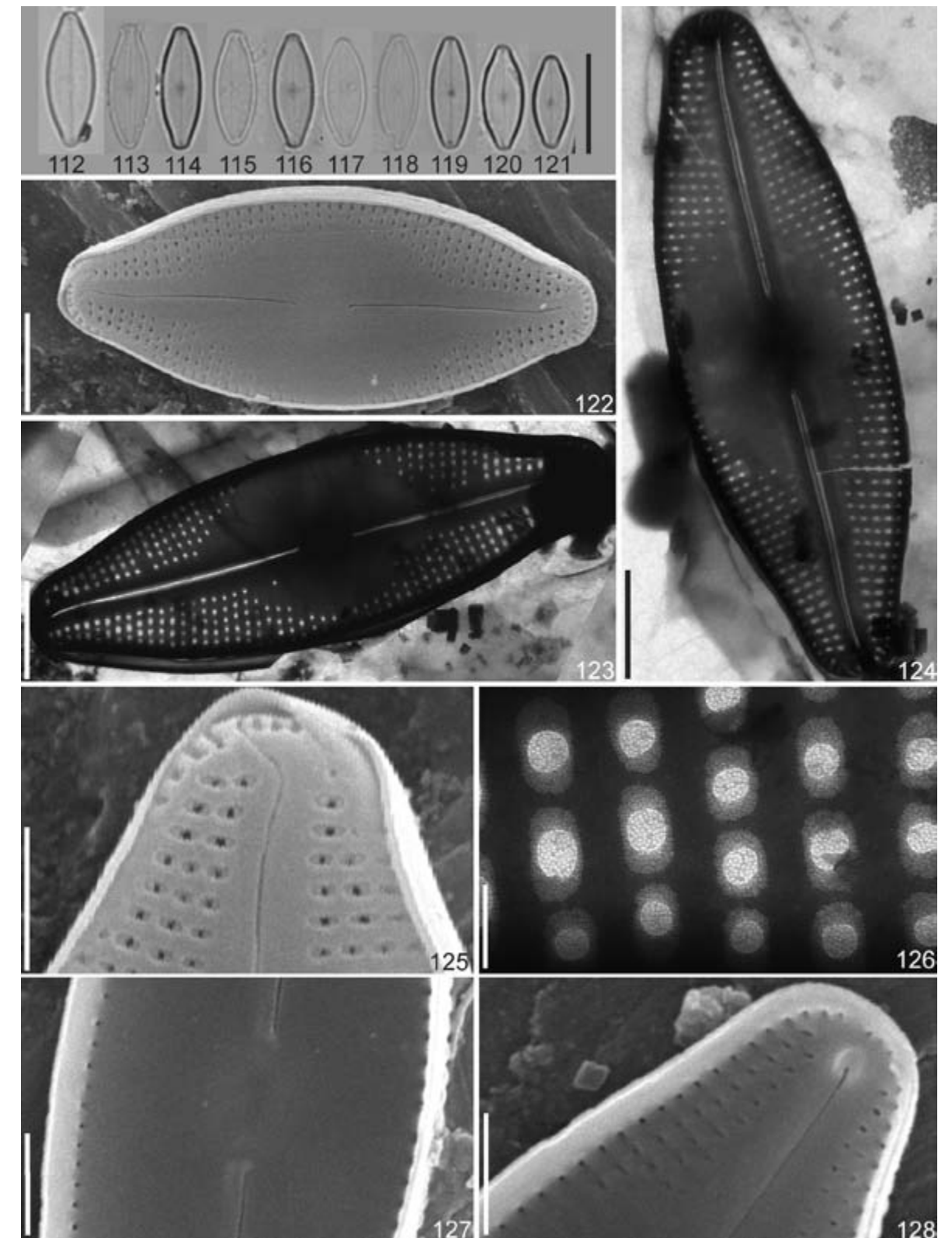
Isotype: ANSP GC26822.

Type material: Guaraguaçu river, Pontal do Paraná, State of Paraná, Brazil, 25°43'5.77"S, 48°33'26.81"W, October 2003.

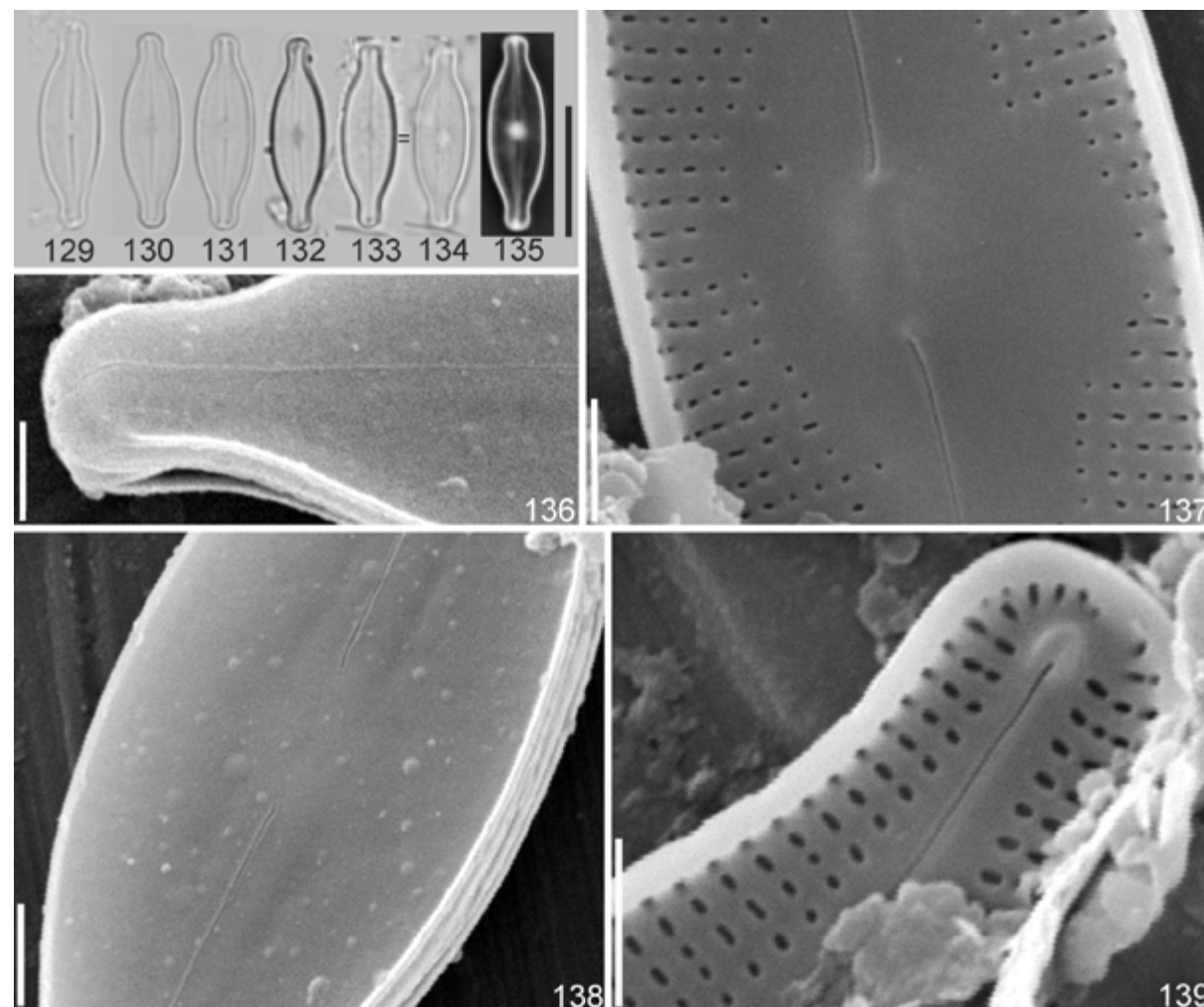
Taxonomical remarks: The exemplars of *Nupela praecipuoides* were similar to the type material of *N. praecipua* (REICHARDT) REICHARDT described to Mexico. However, striae and areolae of *N. praecipua*



Figs 97–111. *Nupela praecipuoides* TREMARIN et T. LUDWIG, LM (97–103); SEM (105–111) and TEM (104): (103) Holotype; (104) Detail of areolae with hymenate occlusions; (105, 106) Central region of valve showing the proximal ends of raphe in external and internal view, respectively; (107–108) Distal end of raphe in external and internal view, respectively; (109) Extremity of valve without raphe in internal view; (110–111) Valves without raphe in external and internal view, respectively. Scale bars 10 μ m (97–103); 2 μ m (110, 111); 1 μ m (105–109); 100 nm (104).



Figs 112–128. *Nupela torganiae* TREMARIN et T. LUDWIG, LM (112–121), SEM (122, 125, 127, 128) and TEM (123, 124, 126): (120) Holotype; (122) External view of valve with longer raphe; (123, 124) Overview of valve with longer and shortened raphe, respectively; (125) Distal end of raphe in external view; (126) Detail of areolae with hymenate occlusions; (127) Central region of valve showing the proximal ends of raphe in internal view; (128) Distal end of raphe in internal view. Scale bars 10 μ m (Figs 112–121); 2 μ m (122–124); 1 μ m (125, 127, 128); 0.2 μ m (126).



Figs 129–139. *Nupela wellneri* (LANGE–BERTALOT) LANGE–BERTALOT, LM (129–135) and SEM (136–139): (136) Distal end of raphe in external view; (137, 138) Central region of valve with longer raphe in internal and external view, respectively; (139) Distal end of raphe in internal view. Scale bars 10 μm (129–135); 1 μm (136–139).

are coarser (32–36 striae/10 μm , 30–35 areolae/10 μm) than those of *N. praecipuoides*, being very conspicuous under LM. Furthermore, *N. praecipua* presents deeper depressions in the axial area of araphid valves, and slightly convergent striae at the apices (REICHARDT 1988; RUMRICH et al. 2000).

Some similarity was observed between *N. praecipuoides* and *N. chilensis* (KRASSKE) LANGE–BERTALOT in relation to the valve outline and striation pattern. *Nupela chilensis* have wider central area, long raphe slits in both valves and lower density of striae (30–32 in 10 μm) than *N. praecipuoides* (LANGE–BERTALOT et al. 1996).

Exemplars similar to *N. praecipuoides* were recorded by RUMRICH et al. (2000) as *Nupela spec.* cf. *praecipua* to Ecuador, and in south Brazil as *N. praecipua* by SCHNECK et al. (2008), TREMARIN et al. (2009) and MORESCO et al. (2011).

***Nupela torganiae* TREMARIN et T. LUDWIG sp. nov. (Figs 112–128)**

Description: Frustules heterovalvar, slightly asymmetric about apical plane. Valves elliptical to elliptical–lanceolate with rostrate to subrostrate apices, 9.2–13.7 μm long and 3.3–4.8 μm wide. Axial area lanceolate. Central area asymmetric, broadly elliptical, unilaterally reaching the valve margin. One valve with long raphe slits and external proximal ends slightly curved to same side. The other valve with shorter raphe slits, with straight and slightly separated proximal endings. Raphe on both valves, slightly sinuous. Proximal raphe ends internally simple. Terminal raphe ends curved to the same side of the valve and internally ending in a small helictoglossa. Transapical striae radiate to convergent in the apices, 45–48 in 10 μm , composed by continuous lines of areolae, 40–48 in 10 μm . Outer openings of areolae transapically elongate and occluded by delicate hymenate layer. Inner openings of areolae small, round to oval.

Etymology: This species is dedicated to Dr. Lezilda Carvalho TORGAN, Natural Sciences Museum of Rio Grande do Sul, Brazil.

Holotype: Slide UPCB 47494, Diatom collection of the Botany Department, Universidade Federal do Paraná, illustrated here in Fig. 120.

Isotype: ANSP GC26822.

Type material: Guaraguaçu river, Pontal do Paraná, State of Paraná, Brazil, 25°43'5.77"S, 48°33'26.81"W, October 2003.

Taxonomical remarks: RUMRICH et al. (2000), based on Ecuadorian sample, found an exemplar that resembles *Nupela torganiae* and cited as '*Nupela spec.*' in plate 32, fig. 10. The lacks of SEM image of this taxon difficult the confirmation of its real identity. Some similarity was observed between *N. torganiae* and *N. carolina* in the raphe development, striation pattern, valve shape and dimensions, but the latter has central area elliptical, not expanded to the valve margin, and attenuate apices (POTAPOVA et al. 2003).

Nupela torganiae occurred with *N. amabilis* in Guaraguaçu river and presented similarities in the size of valves and shape of apices, but differed in the expansion of the axial area, shape of valves and raphe. We do not find other similar species to *Nupela torganiae*. The new species is characterized mainly by the wide central area and the presence of raphe in both valves, being one of this slightly shorter than the other.

***Nupela wellneri* (LANGE–BERTALOT) LANGE–BERTALOT (Figs 129–139)**

Basionym: *Navicula wellneri* LANGE–BERTALOT in LANGE–BERTALOT & KRAMMER, Biblioth. Diatomol. 15: 123–124, pl. 40, figs 28–31, 1987.

Description: Frustules heterovalvar, slightly asymmetric about apical and transapical planes. Valves lanceolate with subcapitate apices, 11.8–16.6 μm long and 3.7–4.4 μm wide. Axial area linear and narrow. Central area asymmetric, limited by short marginal striae. Raphe on both valves, straight. One valve with long raphe slits. The other valve with shorter raphe slits and separated proximal endings. Proximal raphe ends externally simple and internally deflected. Terminal raphe ends curved to same side of the valve and internally ending in small helictoglossa. Transapical striae slightly radiate to convergent in the apices, ca. 42 in 10 μm , composed by continuous lines of areolae, 40–50 in 10 μm . Inner openings of areolae small, round to oval.

Taxonomical remarks: *Nupela wellneri* can be easily confused with *N. deformis* LANGE–BERTALOT and *N. pallavicinii* (KRASSKE) LANGE–BERTALOT in LM, but the higher density of striae (ca. 60 in 10 μm), some small depressions in axial area and one valve with very shortened raphe slits distinguish *N. deformis* from *N. wellneri* (POTAPOVA et al. 2003; LANGE–BERTALOT & MOSER 1994). *Nupela pallavicinii* seems to have smaller

central area than *N. wellneri*, more pronounced apices and lower density of striae (30 in 10 μm), discernible in LM (LANGE–BERTALOT et al. 1996). *Nupela wellneri* and *N. bicapitata* are similar concerning valve outline, but the latter have larger valves, axial area more expanded and shorter raphe fissures.

The exemplars analyzed here agree with the protologue (length 12–14 μm , width 4.0–4.8 μm , 45 striae/10 μm and 50 areolae/10 μm) (LANGE–BERTALOT & KRAMMER 1987). *Nupela wellneri* was proposed to Germany, and recorded in Andes and United States (LANGE–BERTALOT & KRAMMER 1987; RUMRICH et al. 2000; POTAPOVA 2003).

Nupela amabilis, *N. bicapitata*, *N. decipiens*, *N. metzeltinii*, *N. neglecta*, *N. torganiae* and *N. wellneri* co-occurred only in the Guaraguaçu river. The high *Nupela* richness was especially found in this river, probably due to greater number of collected samples (six points and two different periods – autumn and spring). In those samples we found high richness *Eunotia* EHRENBURG and *Pinnularia* EHRENBURG (see TREMARIN et al. 2008, 2010), and also some estuarine taxa [e.g. *Cyclotella stylorum* BRIGHTWELL, *Staurosira obtusa* (HUSTEDT) GARCIA, *Catenula adhaerens* (MERESCHKOWSKY) MERESCHKOWSKY, *Delphineis surirella* (EHRENBURG) ANDREWS and *Seminavis strigosa* (HUSTEDT) DANIELIDIS et ECONOMOU–AMILLI]. Among all the rivers studied, only the Guaraguaçu river presented salinity zero to 14 along the sampling points, explaining the records of estuarine taxa (TREMARIN et al. 2010).

Nupela praecipuoides was the most common taxon, occurring in eight rivers. *Nupela cf. matrioschka*, *N. neglecta* and *N. metzeltinii* were considered rare in the samples, less than 10 valves were analyzed during the study. The *Nupela* taxa co-occurred with other periphytic freshwater diatoms, as *Melosira varians* C. AGARDH, *Fragilaria fragilarioides* (GRUNOW) CHOLNOKY, *Ulnaria ulna* (NITZSCH) P. COMPÈRE, *Eunotia* spp., *Gomphonema* spp., *Encyonema* spp. and *Cocconeis* spp.

Nupela species were found in all rivers located at Serra do Mar, but we have not detected them in four (da Onça, Sertãozinho, das Pombas and do Salto rivers) from the seven analyzed lowland rivers. Generally, fast rivers with clean waters, low conductivity and neutral pH seem to facilitate the development of these poorly known species.

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