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Aulacoseira limbata sp. nov. (Melosirophyceae, Melosiraceae), a new short-chained centric diatom from Tonle Sap Lake (Cambodia)

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The examination of the phytoplankton communities of the Tonle Sap Lake, particularly the diatom flora, has highlighted the unique microflora of the lake. One new genus (*Praecyclostephanos* Tudesque, Le Cohu & Van de Vijver) and several new species have recently been described (*Aulacoseira micropunctata* Tudesque, Le Cohu & Van de Vijver, *A. spiralorimoportula* Tudesque, Le Cohu & Van de Vijver, *Cyclotubicoalitus tonlesapensis* (Tudesque, Le Cohu & Van de Vijver) K.Schultz, Tudesque & Van de Vijver, and *Urosolenia anlongreangensis* Tudesque, Le Cohu & Van de Vijver), highlighting a high level of endemism. A recent survey conducted in 2022 investigated the taxonomy and distribution of the planktonic diatom flora and recorded the presence of a novel *Aulacoseira* species. Detailed light and scanning electron microscope observations show that this species possesses a unique combination of taxonomic characteristics not found in any other known taxon. In particular, this short-chained species, which lacks linking spines and has small and barely visible rimoportulae, exhibits a characteristic pronounced thickening on the mantle – also known as the Müller step on one of its valves. This article provides high-resolution micrographs and a diagnosis of the new species, examines its distinctiveness, and further underscores the taxonomic singularity of the diatom flora of the Tonle Sap Lake.

Keywords: *Aulacoseira, centrics, life form, lower Mekong basin, Müller step, taxonomy*

Introduction

The earliest taxonomic information on phytoplankton in the Tonle Sap Lake dates back to the 1950s, when Blache (1951) documented observations from a single site during the high-water phase (Campbell et al. 2006). Since the initial discovery of phytoplankton in the region, numerous studies – some specifically focusing on diatoms – have progressively improved our taxonomic understanding. Early work by Kyoto University researchers in the 1970s (Ohno et al. 1972, Yamagishi & Hirano 1973) was followed by broader surveys in the Mekong–Tonle Sap system (Nguyen & Nguyen 2001, in Lamberts 2001), and more recent studies have shown that phytoplankton dynamics are strongly driven by hydrological seasonality (Ohtaka et al. 2010, Tudesque et al. 2019, Yoshikawa et al. 2025). With regard to diatoms, previous studies consistently report that the genus *Aulacoseira* Thwaites and, more specifically, *A. granulata* (Ehrenberg) Simonsen, is the dominant component of the planktonic diatom flora. Nevertheless, the *Aulacoseira* community assemblage is more complex than it first appears. A detailed, comprehensive analysis of the Centrophycidae by Tudesque et al. (2021) shows that the flora is not only dominated by the genus *Aulacoseira*, but also that the genus is relatively diverse, with seven recorded *Aulacoseira* species, including two newly described taxa from the Tonle Sap Lake. These *Aulacoseira* taxa exhibit a broad geographical distribution, encompassing species that are globally widespread (*Aulacoseira granulata*, *A. ambigua* (Grunow) Simonsen, *A. ambigua* f. *japonica* Tuji & D.M.Williams and *A. pusilla* (F.Meister) A.Tuji & A.Houki), a tropical taxon (*A. herzogii* (Lemmermann) Simonsen), and two endemic species (*A. spiralorimoportula* Tudesque, Le Cohu & Van de Vijver, *A. micropunctata* Tudesque, Le Cohu & Van de Vijver). We note that the species *A. konstantinovii* Glushchenko, Genkal & Kulikovskiy described from a Tonle Sap sample by Glushchenko et al. (2016) has since been transferred to the genus *Praecyclostephanos* Tudesque, Le Cohu & Van de Vijver (Genkal et al. 2023).

A recent survey conducted in 2022 on the taxonomy and distribution of the planktonic diatom flora revealed a newly recorded species (the distributional results of this

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survey have not yet been published). Observations from detailed light and scanning electron microscopy demonstrate that this species possesses a unique combination of taxonomic characteristics not found in any other known taxon (among the 153 taxa belonging to this genus that are listed in AlgaeBase by Guiry & Guiry 2024). This article provides high-resolution micrographs and a diagnosis of this new species and discusses its taxonomic distinctiveness, underlining the ongoing discovery of diatom diversity in Tonle Sap Lake.

The identification and description of new species are particularly important, as they contribute to a more accurate understanding of local biodiversity and biogeographic patterns. The taxon described in this study adds to the growing evidence that this lake represents a significant center of endemism and highlights the need for detailed taxonomic investigations to document its unique flora.

Material and methods

Study sites and diatom sampling

The sampling campaign was carried out on Tonle Sap Lake at the end of November 2022, during the flood period when the permanent Great Lake expands across the entire floodplain. To cover the full range of environmental conditions and habitats, 12 sampling sites (Fig. 1) were selected around the lake across three habitat types: the open water, the lake edge or shoreline (often bordered by macrophytes), and flooded forest, which is accessible only during the high-water periods (for more details, see Tudesque et al. 2019, 2021). The sampling methodology for planktonic diatoms was designed to obtain both qualitative and semi-quantitative data (relative abundance) on their composition and distribution throughout the lake. The sampling protocol consisted of filtering subsurface water with a 10 µm mesh plankton net, allowing the collection of even the smallest known taxa. The filtration was stopped as soon as the net became clogged and no longer effectively filtered the water. In total, one phytoplankton sample was collected per habitat type at each site, resulting in between one and three samples per site, depending on the habitat availability. The material was then stored in a flask and fixed with 96% ethanol (v/v).

Sample preparation, typification and determining ecological traits

In the laboratory, benthic samples were processed following a standard methodology for diatom preparations. Samples were cleaned in boiling hydrogen peroxide and hydrochloric acid. All traces of hydrochloric acid were removed through successive rinse cycles with deionized water. To obtain permanent slides, oxidized samples were dried onto cover slips and fixed on microscope slides using a high-refractive index resin (Naphrax®, R.I. = 1.7). In addition, burn-mount preparations were carried out on several selected samples to preserve the morphological integrity of complete colonies.

Both standard and burn-mounted preparations were investigated, and images were captured with an Olympus BX51 light microscope (Nomarski optics, DIC) at 1000 × (N.A. 1.40), using an Olympus SC30 digital camera and Stream Essential software. For scanning electron microscopy, cleaned diatom material was dried onto a 5 µm isopore™ translucent membrane filter, affixed to aluminium mounts with carbon adhesive tabs, and coated with 5 nm of platinum. Observations and photo microscopy were performed with a FEG FEI Quanta 250 at an accelerating voltage of 5 kV and at a working distance of 10 mm. The plates were created using Microsoft PowerPoint and Adobe Photoshop CS5.

For typification purposes, and in accordance with Article 8.2 of the International Code of Nomenclature for Algae, Fungi, and Plants (Turland et al. 2019), we selected the entire microscope slide mounted with Naphrax resin. Both the holotype and isotype correspond to burn-mount preparations. The classification follows the updated classification proposed by Kociolek et al. (2026). Morphological terminology follows Crawford & Likhoshway (1999, 2002), Crawford et al. (2003), Houk et al. (2017), and Round et al. (1990). Morphometric data were obtained from measurements of 40 valves.

In parallel, to characterize the structure of the planktonic diatom community at each site, 400 valves were counted and identified to determine the relative abundance of each taxon. The estimated autecology of the new species was established following Carayon et al. (2019), who defined a new autecological trait matrix for French stream benthic diatoms. Although this matrix was developed for running waters rather than lentic systems, we used it as the default proxy.

Results

Class: Melosirophyceae Kociolek, Ashworth & A.J.Alverson

Subclass: Melosirophycidae Kociolek, Ashworth & A.J.Alverson

Order: Melosirales Crawford

Family: Melosiraceae Kützing

Genus: *Aulacoseira* Thwaites

***Aulacoseira limbata* Tudesque & Chrea, sp. nov.** (Figs 2–53)

Morphological description

LM observations (Figs 2–25): Cells cylindrical, rectangular in girdle view. Frustules are solitary (Figs 9, 10) or colonial, forming straight short chains composed of two, three, or four cells (Figs 2–8, 11–13, 19–25). Collum narrow. Linking spines absent. Mantle striae are straight to slightly curved, composed of

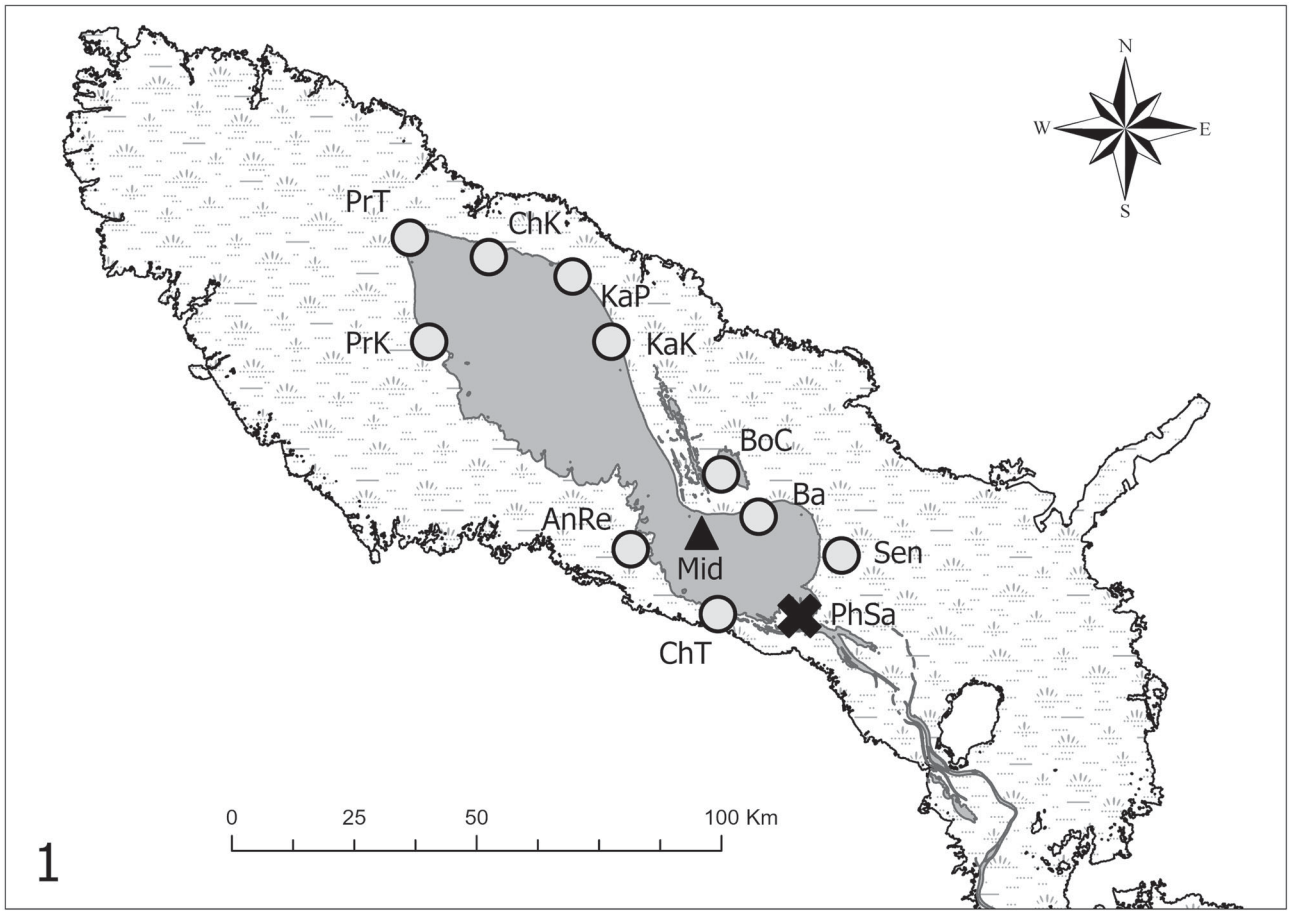


Fig. 1. Delineation of the Tonle Sap floodplain and location of the sampling sites in the permanent lake (grey). PrT: Praek Toal, ChK: Chong Khneas, KaP: Kampong Prahok, KaK: Kampong Khlaing, BoC: Boeng Chhmar, Ba: Balot, Sen: Stung Sen, PhSa: Phat Sanday (type sample), ChT: Chnok Tru, Mid: Middle lake – second population, AnRe: Anlong Reang, PrK: Praek Konteil.

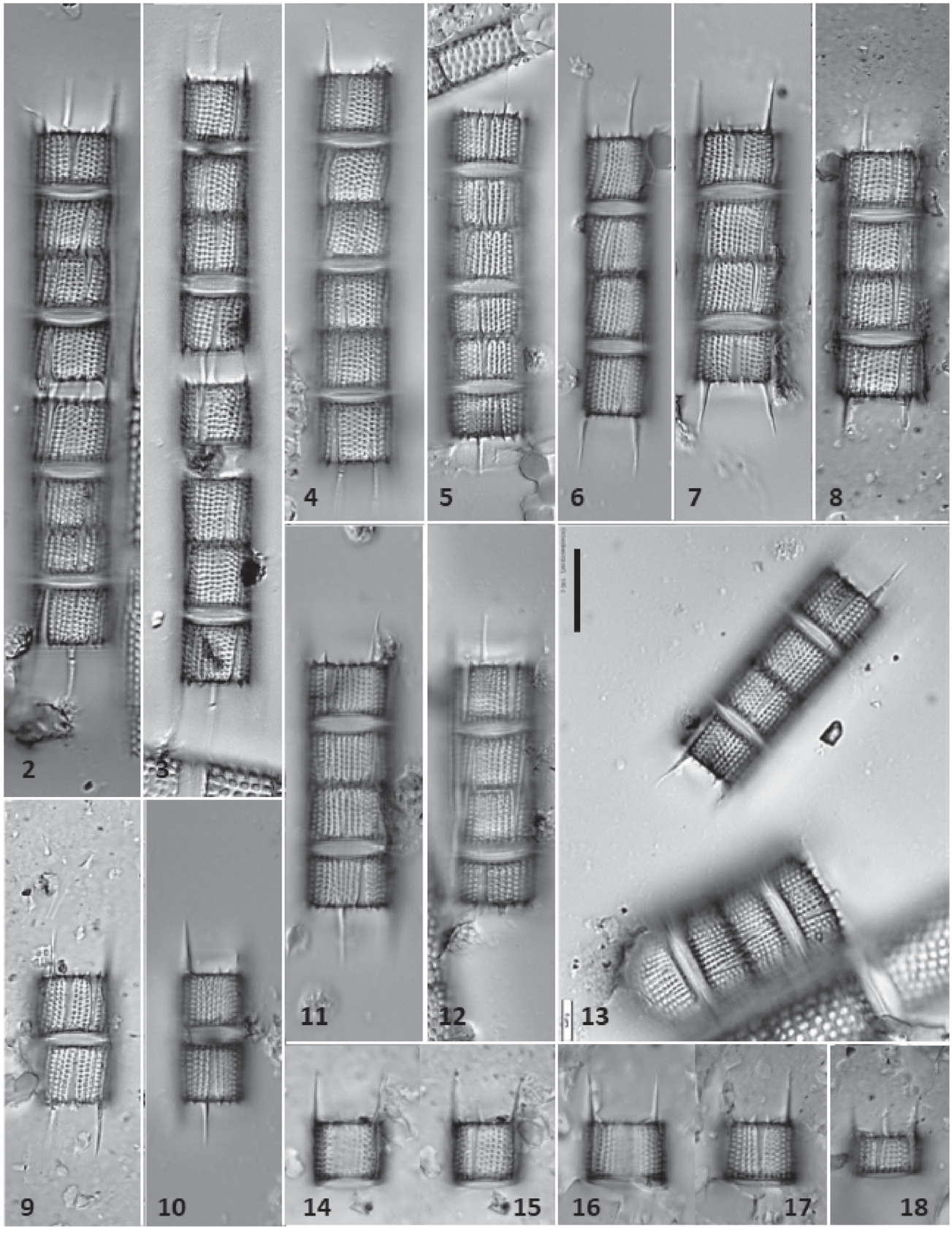
visible areolae. Valve face flat. Valve dimensions ($n = 40$): diameter 7.5–15.6 µm (mean = 10.4 µm), mantle height 5.5–9.0 µm (mean = 7.9 µm), mantle height to valve diameter ratio (MH/VD) 0.5–1.1 (mean = 0.8). Valves with four to six long, thin, straight to slightly curved separation spines, equal in length to the mantle height. Very short conical spines are positioned between the separation spines. Mantle with pronounced grooves up to the collum fitting the spines of adjacent valves. Rimoportulae indistinct.

SEM observations (Figs 26–53): Ornamentation of the valve face composed of the external openings of the areola, dot-like shaped, mainly located near the margin in two or three rows (Fig. 38). Narrow collum with granular ornamentation (Figs 30–35). Frustule composed of two valves whose mantle surface differs. Flat mantle in one valve (Figs 30–32) and distinct thickening on the other valve, making a Müller step (Figs 33–35). The prominent thickening, of variable height, extends from the collum, while a narrowing extends from the valve surface (Figs 36, 37). Mantle with straight to slightly curved striae, 14–17 in 10 µm; areola density 14–17 in 10 µm. Round to transaxial or axial rectangular areolae externally open (Figs 36, 37). Areolae externally open and internally covered by an individual rosette of the vela (Figs 40, 42). Internally, robust and broad ringleists separating the collum from the mantle areolae (Figs 43–46). Hollow ringleist having a narrow inner canal (Figs 39–42). Several rimoportulae, just above the ringleist, consisting of short internal labial projections (Figs 39, 40, 42, 45, 46, 50). Cingulum diaphanous composed of numerous open bands finely silicified and perforated by fine pores (Figs 27–29, 50–53). Copula with ligula (Figs 28, 29).

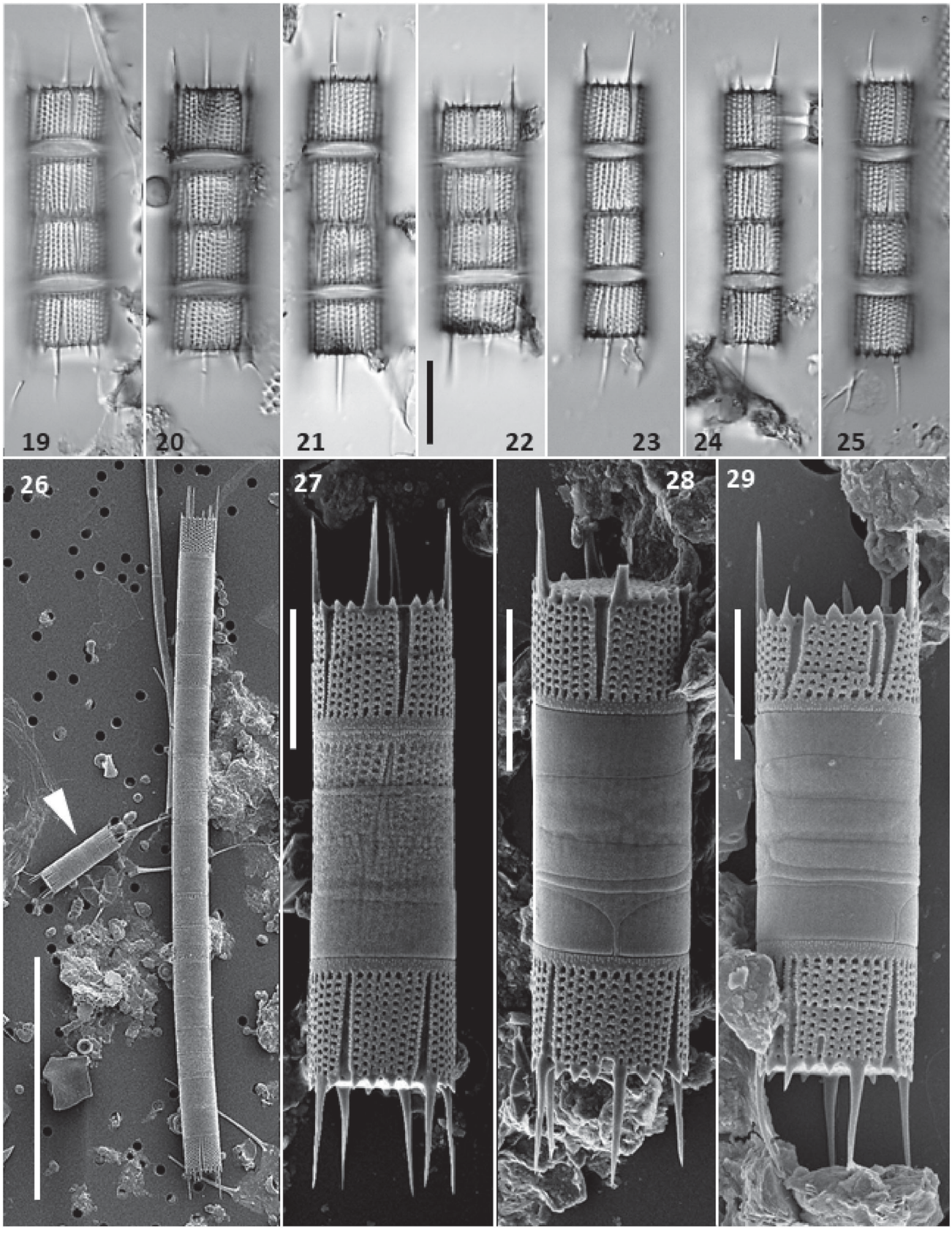
Holotype. BR! Naphrax slide BR-4926 (Meise Botanic Garden, Belgium).

Isotype. PC! Naphrax slide PC0671131 (Muséum National d'Histoire Naturelle de Paris, Paris, France).

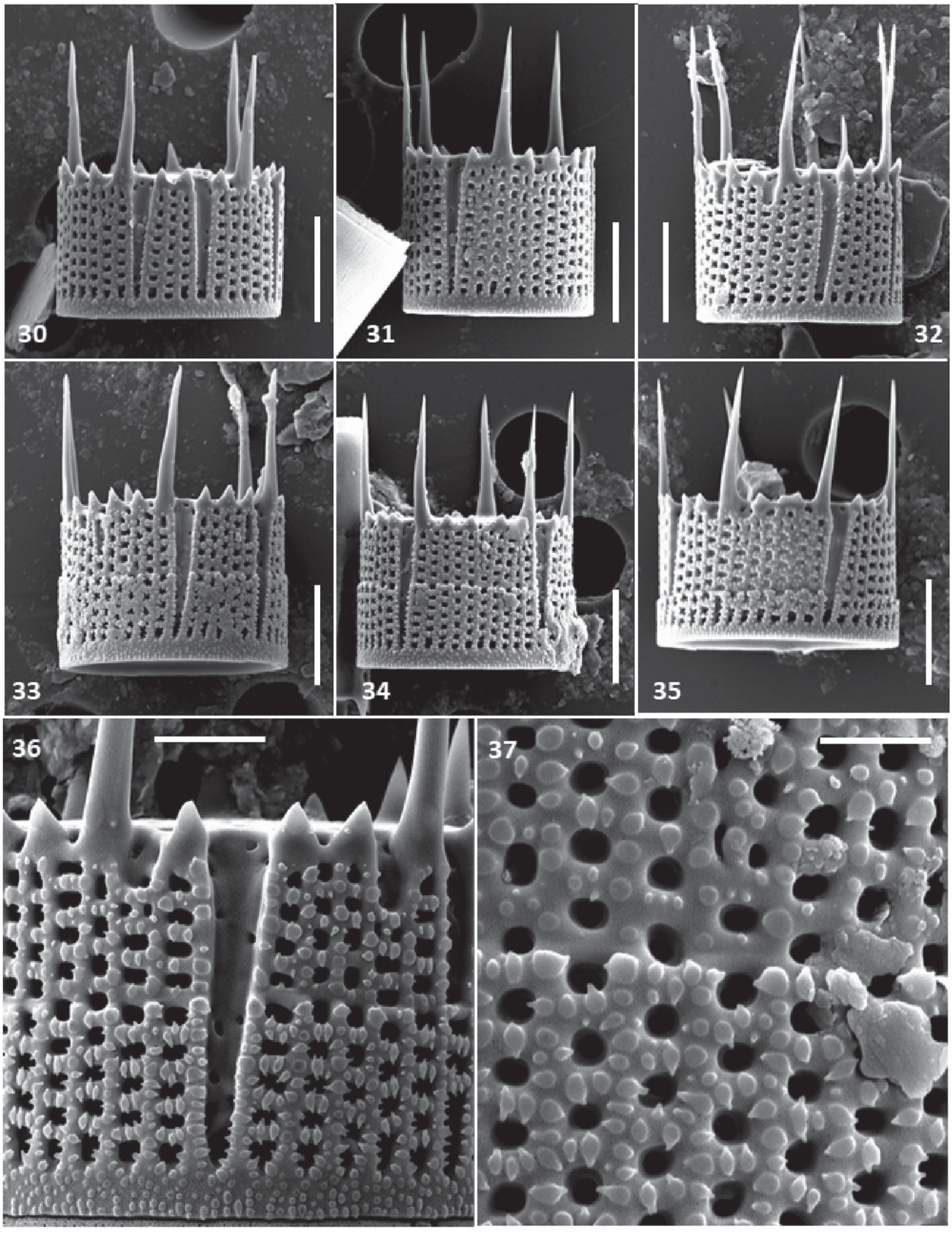
Type locality. Tonle Sap Lake, Phat Sanday, Kampong Thom province, Kampong Svay district, Cambodia (Lat. 12.54776; Long. 104,46339)., leg. S. Chrea, Coll. Date: 27 November, 2022.



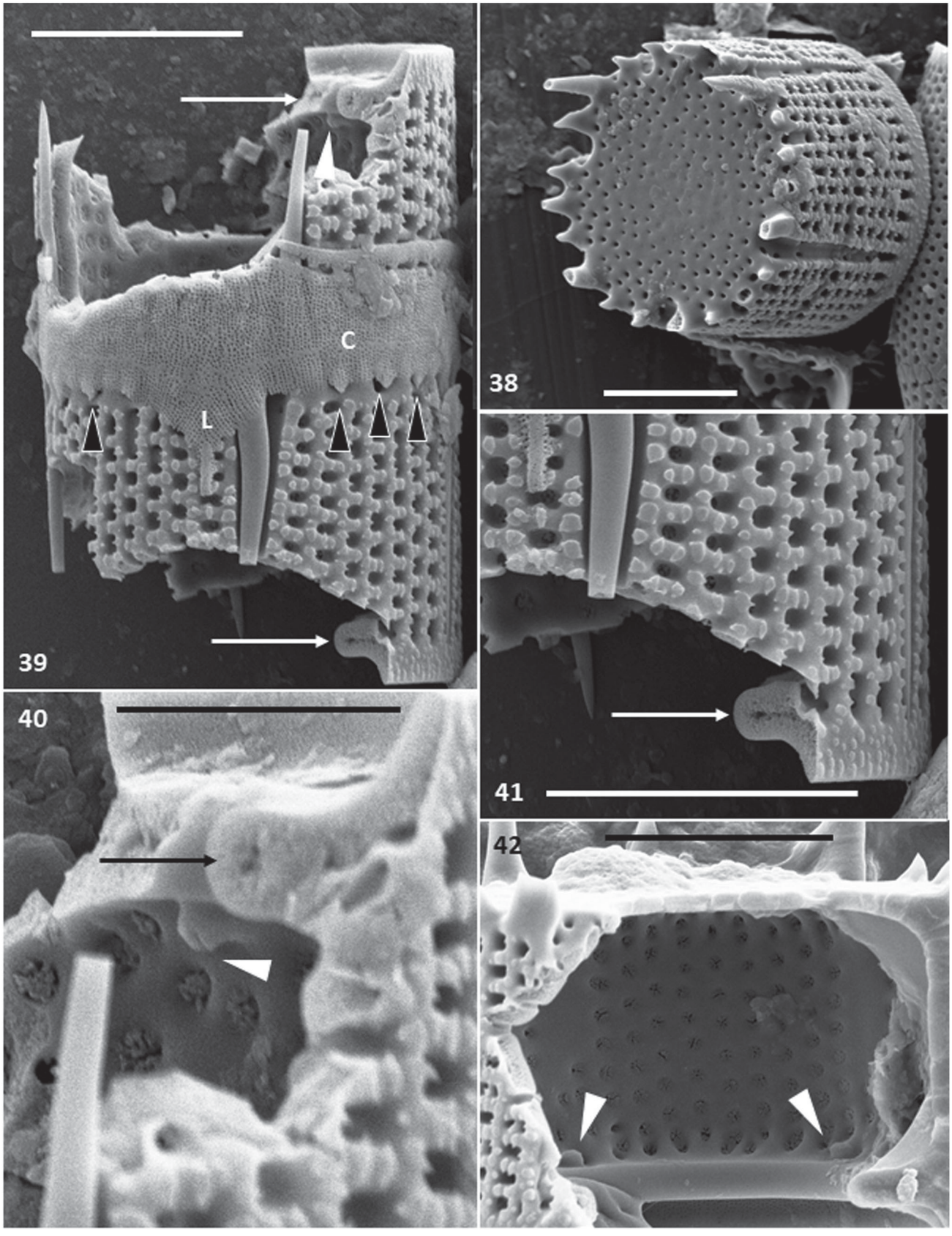
Figs 2-18. *Aulacoseira limbata* Tudesque & Chrea sp. nov. LM pictures taken from the holotype slide (BR-4926, Tonle Sap Lake, Phat Sanday). Figs 2–13. Size range of complete cells and colonies. In Fig. 13, part of a chain with a hemispherical initial valve. Figs 14–18. Size range of isolated valves. Scale bar = 10 µm.



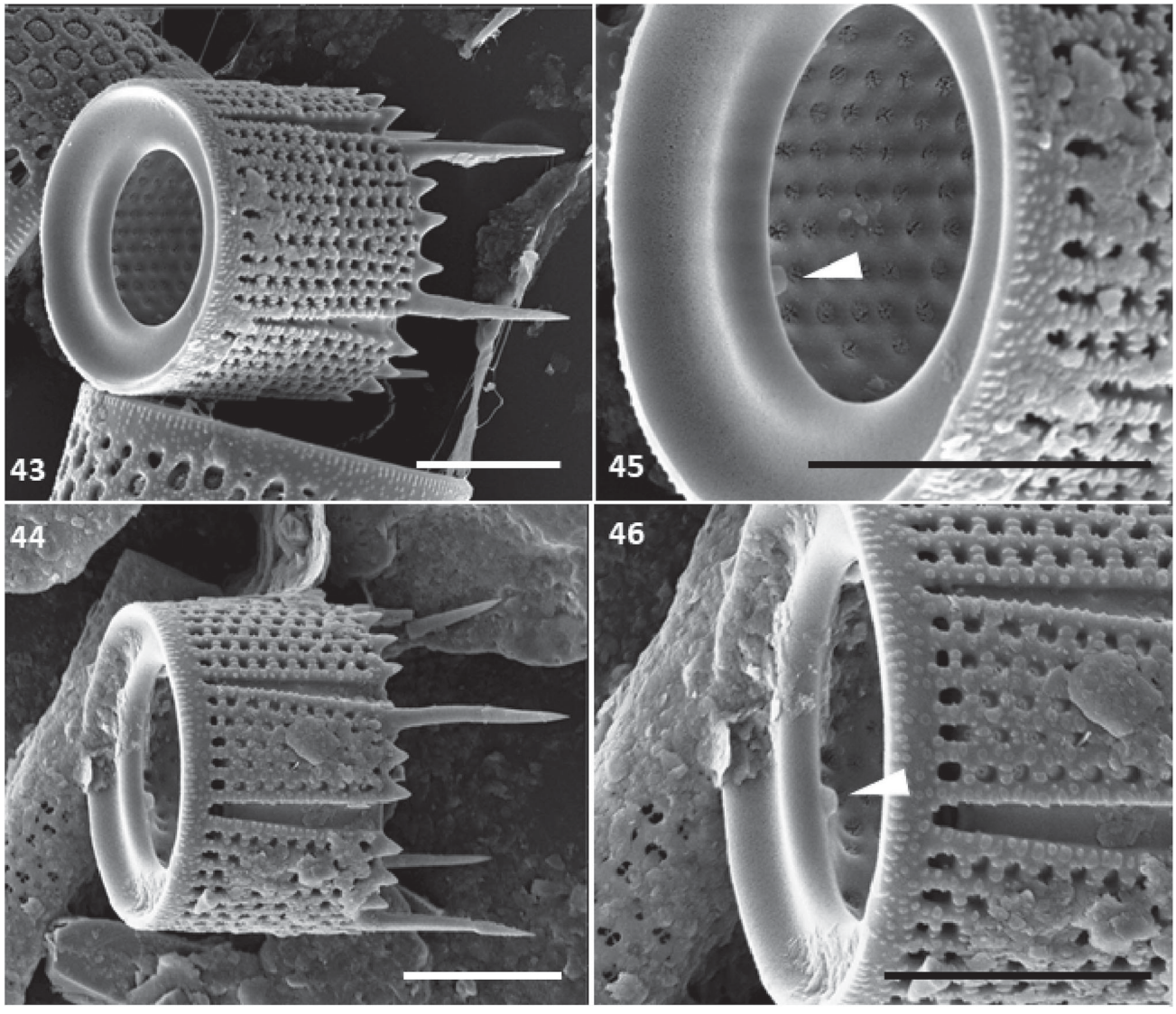
Figs 19-29. *Aulacoseira limbata* Tudesque & Chrea sp. nov. LM and SEM pictures taken from the holotype slide (BR-4926, Tonle Sap Lake, Phat Sanday). Figs 19–25. LM. The size range of complete chains composed of two cells. Figs 26–29. SEM. Fig. 26. Complete colony of *Aulacoseira spiralorimoportula* Tudesque, Le Cohu & Van de Vijver with a complete short chain of *A. limbata* sp. nov. (arrowhead). Figs 27–29. Girdle views of complete two-celled chains showing a cingulum composed of open bands with ligulae. Note the different degrees of band silicification. In Figure 27, the Müller step is present only in one terminal valve. In Fig. 28, neither terminal valve shows a Müller step, whereas it is present in both valves in Fig. 29. Scale bars = 10 µm (Figs 19–25), 100 µm (Fig. 26), and 10 µm (Figs 27–29).



Figs 30-37. *Aulacoseira limbata* Tudesque & Chrea sp. nov. SEM pictures taken from the holotype slide (BR-4926, Tonle Sap Lake, Phat Sanday). Figs 30–32. Terminal valves without a Müller step. Figs 33–35. Terminal valves with a Müller step of various sizes. Fig. 36. Detail focusing on the adjacent groove, the Müller step, very short conical spines positioned between the separation spines, and the granular collar. Fig. 37. Focus on the Müller step showing its offset on the mantle and the features of the areolae. Scale bars = 5 µm (Figs 30–35), 2 µm (Fig. 36), and 1 µm (Fig. 37).



Figs 38-42. *Aulacoseira limbata* Tudesque & Chrea sp. nov. SEM pictures taken from the holotype slide (BR-4926, Tonle Sap Lake, Phat Sanday). Fig. 38. External valve face showing dispersed areolae. Fig. 39. Broken valves connected by lace-like girdle bands. Fractures show the cross-section of the ringleists (white arrows) and the small internal projection of the rimoportula next to the ringleist (white arrowhead). Note the early stage of girdle band production, with the copula (C), the emergence of the ligula (L), and small triangular projections covering the space between the conical separation spines (black arrowheads). Fig. 40. Detail of a cross-section of the ringleist showing its slightly hollow structure (black arrow), the labium of a short rimoportula (white arrowhead), and the inner surface with individual rosettes of the vela. Fig. 41. Detail of a cross-section of the ringleist (white arrow). Fig. 42. Cross-section of a broken valve showing the internal vela and two rimoportulae (arrowheads). Scale bars = 5 µm (Figs 38, 39, 41, 42) and 2 µm (Fig. 40).



Figs 43-46. *Aulacoseira limbata* Tudesque & Chrea sp. nov. SEM pictures taken from the holotype slide (BR-4926, Tonle Sap Lake, Phat Sanday). Figs 43, 44. Tilted valves showing partial internal views with a well-developed ringleist. Figs 45, 46. Focus on the ringleist, with the small projection of a rimoportula barely visible (arrowheads). Scale bars = 5 µm.

Etymology. The specific epithet '*limbata*', meaning 'bordered', refers to the mantle thickening, the characteristic feature of this species.

Ecology and associated diatom flora

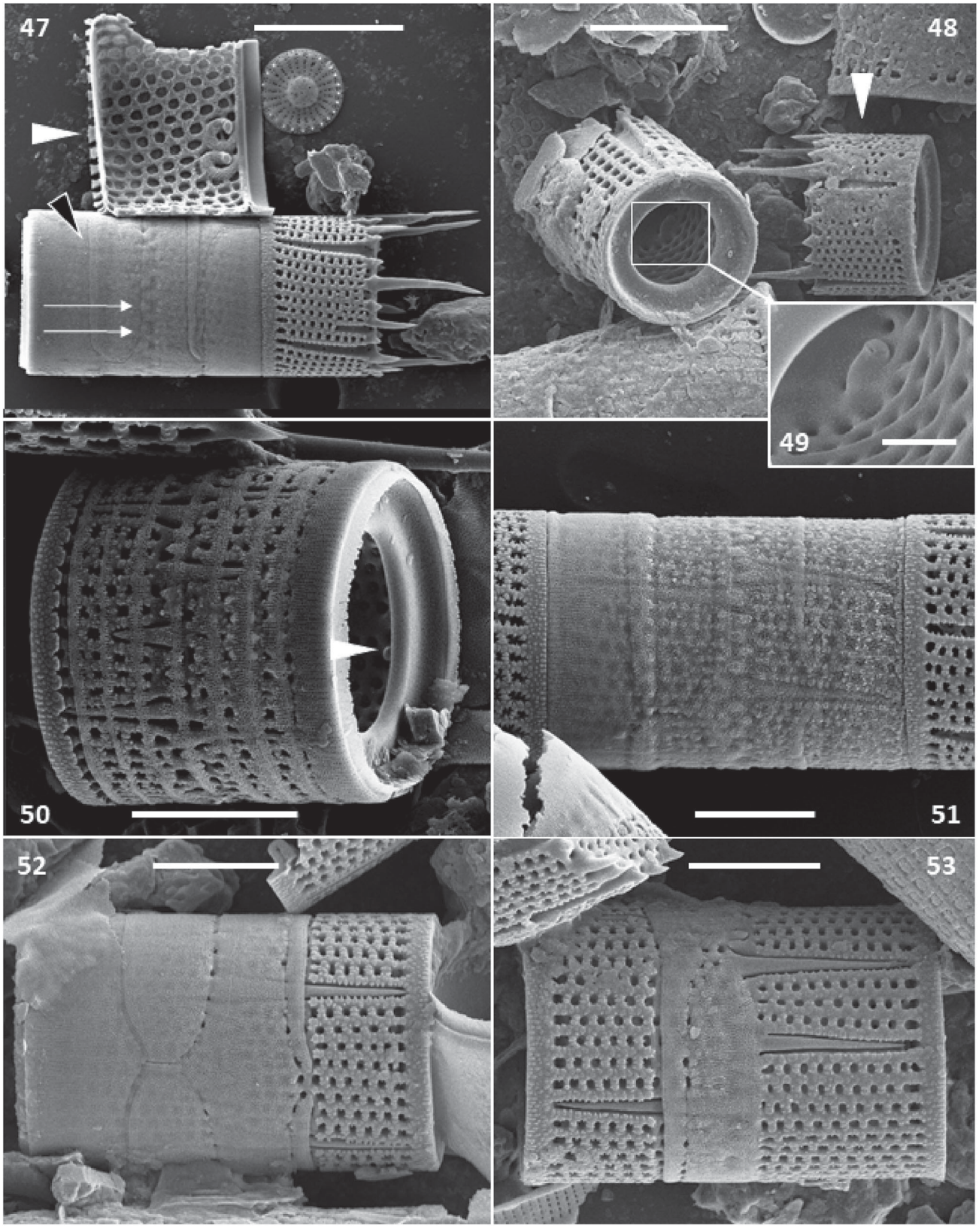
The type population occurs in standing water in the flooded forest. The physicochemistry in November reveals a warm water temperature (29.1 °C), a circumneutral pH (7.1), a low conductivity (89 µS/cm), a moderate oxygen saturation (89%), and a low nutrient load (undetectable levels for both nitrate and orthophosphate, total phosphorus 47.4 µg/l). The planktonic diatom community at the type locality is largely dominated by sympatric populations of *Aulacoseira spiralorimoportula* Tudesque, Le Cohu & Van de Vijver (65%) and *A. granulata* (20%). The new species ranks third with a relative abundance of 4%. Three other taxa of *Aulacoseira* have been recorded: *Aulacoseira ambigua* (Grunow) Simonsen, *A. ambigua* f. *japonica* Tuji & Williams, and *A. pusilla* (Meister) Tuji & Houki.

Additional populations. Only observed in the Tonle Sap Lake. *Aulacoseira limbata,* recorded in 2022, spread throughout the lake in low abundance (< 1%). A relatively more abundant population was recorded at the 'Mid-lake' station (6%).

Registration. http://phycobank.org/106274

Differential diagnosis and discussion

At first glance under LM, when colonies are dismantled during the standard slide preparation, this species may easily be misidentified as *A. granulata*, due to its clearly visible long separation spines (Figs 14–18, 30–35). More specifically, it resembles *A. granulata* morphs with a valve



Figs 47-53. *Aulacoseira limbata* Tudesque & Chrea sp. nov. SEM pictures taken from the holotype slide (BR-4926, Tonle Sap Lake, Phat Sanday). Fig. 47. Part of a chain of *A. limbata* showing barely visible long (black arrow) and conical linking spines (white arrows) covered by cingulum bands. Broken valve of *A. spiralorimoportula* Tudesque, Le Cohu & Van de Vijver, showing typical spiraling rimoportulae and spatulate linking spines (white arrowhead). Fig. 48. Valve of *A. limbata* (arrowhead) with an oblique view of *A. granulata*. Fig. 49. Close-up of the internal view of *A. granulata* showing a tubular rimoportula located on the mantle near the valve face junction. Figs 50–53. Variable degrees of silicification and stages of cingulum-band production covering adjacent valves. The rimoportula is barely visible in Fig. 50 (arrowhead). Scale bars = 10 µm (Figs 47, 48), 5 µm (Figs 50–53), and 2 µm (Fig. 49).

height-to-diameter ratio close to 1 (i.e. square rather than rectangular girdle view, see Figs 4, 14, plate 157, Fig.11, plate 158 in Houk et al. 2017). This is particularly likely given that the species occurs in the planktonic community of the Tonle Sap Lake, where *A. granulata* is abundant and dominant. Several species related to the *A. granulata* complex, characterized by long spines interspersed with smaller spines, overlap with the new species in terms of their morphometric and morphological parameters (Table 1). Among these species are *Aulacoseira sumatrana* var. *rectangularis* Ham et al., which shares a similar rectangular to square shape; *A. brasiliensis* Tremarin, Torgan & Ludwig, *A. gessneri* (Hustedt) Simonsen, *A. muzzanensis* (F. Meister) Krammer, and *A. pseudomuzzanensis* Olszynski & Zelazna-Wieczorek. Striation density is an important distinguishing feature that sets *A. limbata* sp. nov. apart from most of the species listed above. Only *A. pseudomuzzanensis* and *A. brasiliensis* overlap, having more than 14 striae per 10 µm.

Aulacoseira limbata sp. nov. differs from many other *Aulacoseira* species by its life form, lacking linking spines and consequently forming short chains (Figs 2–8, 11–13, 26–29). Short chains consist of a few cells that remain loosely connected by the cingulum (the connecting zone of Wallich), with chain length determined by the ratio of linking to separation valves (Crawford 1981). We note that the absence of linking spines in *A. limbata* can be inferred under LM, as only valves with straight striation have been observed. No valves with spiralled striations were recorded. The observation of preserved chain structure is therefore a key factor in identifying this species, as its morphological and morphometric variability overlaps with that of several other *Aulacoseira* species. *Aulacoseira agassizii* var. *malayensis* (Hustedt) Simonsen, described from Indonesian lakes, is a morphologically similar taxon. It shares the same life form, in that only short chains are observed, as well as a broadly similar shape, but it can be distinguished by a lower density of areola rows (8–10 per 10 µm). In addition, this taxon covers a much wider size range, reaching up to 60 µm in diameter, whereas the maximum size observed for *A. limbata* is 15.6 µm (colonies generated from the auxospore). *Aulacoseira brasiliensis* is another similar short-chain taxon, characterized by a narrower mantle, a lower mantle-to-diameter ratio (0.2–0.88), smaller separation spines, and the absence of mantle grooves.

In the SEM, a striking feature of the frustule of *A. limbata* is the pronounced thickening of the mantle in one valve, starting from the collum (Figs 27–29, 33–37, 43). Each frustule consists of one valve with a flat mantle surface and a second valve exhibiting a thickening, referred to as the Müller step, whose height varies among cells and may account for 30–50% of the mantle height. Such a structure is known to play an important role in size reduction in diatom cell walls during asexual reproduction. Otto Müller (1883, 1884) observed vegetative divisions in *Ellerbeckia arenaria* (D.Moore ex Ralfs) Dorofeyuk & Kulikovskiy and demonstrated that the presence or absence of valve thickening affects the kinetics of cell division, inducing, among other effects, latency periods between valves during vegetative division phases (Crawford 1981, Harbich 2025). Among centric diatoms, several genera exhibit a Müller step, including *Angusticopula* Houk, Klee & H. Tanaka, *Aulacoseira* Thwaites, *Ellerbeckia* R.M.Crawford, *Melosira* C. Agardh, and *Ferocia* Van de Vijver & Houk. This structure is relatively common in many *Aulacoseira* species, which occur across a broad range of environmental conditions. Species of *Aulacoseira* exhibiting a Müller step are found in both fossil taxa (e.g. *A. californica* (Ehrenberg) Houk, *A. procera* (Ehrenberg) Houk & Klee, *A. scalaris* (Grunow) Houk, Klee & Passauer) and recent taxa (e.g. *A. italica* (Ehrenberg) Simonsen, *A. longispina* (Hustedt) Simonsen). Most living species are planktonic and occur in lentic environments such as ponds, lakes, or large rivers; however, they can also be found in benthic habitats (e.g. *A. crenulata* (Ehrenberg) Thwaites) or in high-elevation streams (e.g. *A. kruegeriana* E. Morales, S. F. Riviera & Houk). Some species are restricted to tropical regions (e.g. *A. brasiliensis*, *A. multistriata* (R. M. Patrick) Houk, Klee & H.Tanaka), whereas others are cosmopolitan (e.g. *A. ambigua*, *A. italica*), occurring in oligotrophic waters (e.g. *A. crenulata*) or in mesotrophic to eutrophic conditions (e.g. *A. ambigua*). Thus, although the presence of a Müller step on the mantle of *Aulacoseira* represents a characteristic feature associated with vegetative cell division strategies, it does not appear, at first glance, to be linked to basic environmental parameters such as life form, geographic distribution, trophic status, or whether taxa are fossil or recent. However, we note that the new species differs from most other taxa exhibiting a Müller step in that this structure is particularly pronounced. In most species, the thickening is weaker, resembling a fold, and requires careful examination in the SEM to be observed. In *A. limbata*, the Müller step is deep and well developed, a feature otherwise observed in relatively few species such as *A. longispina*, *A. procera*, and *A. scalaris*. We hypothesize that this pronounced Müller step contributes to the relatively narrow size range observed in this species, which remains small throughout its life cycle. On the other hand, Geitler (1932, in Crawford 1981) noted that the formation of thin cingulum bands might also delay size reduction in several genera of biraphid diatoms. However, our SEM observations of untreated raw samples, preserving complete colonies, reveal highly variable levels of silicification and development of the connecting bands (Figs 27–29, 39, 50–53). Here, we propose a second hypothesis, namely that the strategy of delaying size reduction in this species may combine both a differential kinetics of large versus small mother valves and the production of a cingulum composed

Table 1. Key morphometric and ultrastructural features of species closely resembling *Aulacoseira limbata* Tudesque & Chrea sp. nov.

	A. limbata sp. nov.	*A. micropunctata* Tudesque et al.	*A. spiralorimoportula* Tudesque et al.	*A. granulata* (Ehrenberg) Simonsen	*A. granulata* (Ehrenberg) Simonsen	*A. pseudomuzzanensis* Olszyński & Żelazna-Wieczorek	*A. muzzanensis* (F.Meister) Krammer	*A. gessneri* (Hustedt) Simonsen	*A. gessneri* (Hustedt) Simonsen	*A. brasiliensis* Tremarin et al.	*A. agassizii* var. *malayensis* (Hustedt) Simonsen	*A. sumatrana* var. *rectangularis* Ham et al.
	Data from populations of Tonle Sap Lake				Data from the literature							
Valve diameter	7.5–15.6	3.0–6.5	4.5–12.5	5.0–24.0	3.0–30.0	10.0–20.0	8.0–25.0	10.0–23.0	8.0–21.0	8.0–24.0	16.0–58.0	14.5–36.5
Valve height	5.5–9.0	7.5–12.0	9.0–15.0	12.0–22.0	14.0–20.0	5.0–9.0	8.0–16.0	4.0–8.0	4.0–11.0	4.0–10.0	7.0–10.0	21.0–52.5
MH/VD	0.5–1.1	1.7–3.1	0.75–2.8	0.8–3.4	> 0.8	0.37–0.86	0.4–0.5*	–	0.25–1.12	0.20–0.88	–	0.6–3.6
Striae/10µm	14–17	16–23	8–10	6–14	7–15	12–16.1	11–13	10–14	10–14	10–16	8–10	5–7
Areolae/10µm	14–17	19–24	8–10, coarse, rectangular to oval	4–10/9–15, square to rectangular	–	12–15 rectangular	16–18, rectangular, often doubled	rectangular, irregularly round	14–20	10–15	10	5–6, coarse, large, round-to-elliptical
Separation spines	4–6 long, curved spines, with conical spines in between	2 long, thin, straight spines, with short conical spines in between	2–3 long spines with small to medium-sized spines in between	1–7 elongated spines with cone-shaped spines	short cone-shaped spines with one or few longer straight spines	short, long as mantle height	several long among shorter conical	long, lanceolate	elongated	attenuated, protruding	long thorns among shorter conical ones	2–4 long spines, 2–4 smaller in between
Linking spines	absent	absent	bi-lobed, triangular	triangular, slightly bi-lobed spines	short, spatulate or flared	bifurcated	short, spatulate or flared	spatulate	spatulate, sometimes tridentate	absent	absent	forked
Rimoportulae	several, short labial projections above the ringleist	at the valve face junction	long, spiralling, visible in LM, above or connected to the ringleist	short-curved tube with labia	several long curved tubes with labia	1–3, short, near the ringleist	long tube with labia	numerous, up to 12, elliptical, stalked, below the ringleist	small, at least 4, sessile, close to the ringleiste	inconspicuous, sessile, 2 rows in quincunx	sessil, 1 irregular row	tightly coiled,located on either ends of the mantle or closer to valve centre
Ringleist	robust, broad	–	robust, broad	barely developed	almost undeveloped, low, wide v-shaped	narrow	narrow	–	solid and shallow	undeveloped, narrow	highly developed, broad	broad
Müller step	present	absent	absent	absent	absent	absent	absent	absent	absent	present	absent	absent
Chain length	short	short	Long	Long	long	–	Long	long	long	short	short	–
Reference	present study	Tudesque et al. 2021	Tudesque et al. 2021	Tudesque et al. 2021	Houk et al. 2017	OlszyŃski & Żelazna-Wieczorek 2018	Houk et al. 2017	Wetzel et al. 2014	Tremarin et al. 2011	Tremarin et al. 2012	Tremarin et al. 2014	Ageli et al. 2024

*measurement based on Houk et al.'s plates (Plates 171–172)

of very thin bands, likely involving a distinct production kinetics.

Rimoportulae, which are key ultrastructural features for the taxonomy of *Aulacoseira* species, are not visible under LM and are relatively difficult to observe in the SEM. When the valves are intact, SEM observation requires a slight inclination of the valves in order for the rimoportula labium to be visible (Figs 43–46, 50). Detailed observation of the rimoportulae requires the valves to be fractured (Fig. 39). Small rimoportulae may also be observed attached above the ringleist. The external openings were not visible, and their number could not be determined (at least two). Thus, the characteristics of the rimoportulae distinguish *A. limbata* sp. nov. from species whose rimoportulae are easily visible under the LM and in the SEM, or whose rimoportulae are located on the mantle (e.g. *A. granulata*) or close to the mantle/valve face junction.

The combination of morphometric and ultrastructural features, multiple long separation spines, the deep mantle grooves, absence of linking spines, squared girdle views, very small rimoportulae just above the ringleist, the slightly hollow structure of the ringleist and pronounced thickening of the mantle in one valve of the frustule constitutes a unique set of taxonomic characters not reported in any other *Aulacoseira* species described to date. This species belongs to a group of taxa characterized by a relatively low mantle height and, due to the absence of linking spines, a short-chained taxon. Taxa of *Aulacoseira* resembling the new species are rare. It therefore joins *Aulacoseira herzogii, A. micropunctata,* and *A. pusilla*, which share the same life form and have been recorded in the Tonle Sap Lake. Ecologically, this species is neutrophilous and sensitive to pollution, occurring in oligonitrophilous to oligotrophic waters that are very clear, with moderate oxygen levels and low mineralization.

Taken together, taxonomic and ecological observations presented here highlight the unique features of the Tonle Sap Lake diatom flora and the importance of accurate species identification. In this context, the Tonle Sap Lake is marked by a high degree of endemism, reflecting its long-term environmental stability and geographic isolation. To date, one-third of the recorded centric diatom taxa were originally described from the lake, further supporting its status as a center of endemism in the lower Mekong basin, and emphasizing the importance of continued taxonomic work in this ecosystem. In the context of the increasing use of diatom-based tools to assess and monitor freshwater ecosystems, particularly for the implementation of such methods in the Tonle Sap Lake catchment (Chrea et al. 2024, Chea et al. 2025), accurate identification of diatom taxa is a prerequisite for ensuring the reliability and relevance of ecological diagnoses based on diatom taxonomy. Consequently, as previously suggested by Tremarin et al. (2012), we strongly recommend preparing burn-mounted slides for chain observation, as this facilitates easier and more accurate identification of *Aulacoseira* species.

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