

## A new freshwater diatom genus *Trialacinia* gen. nov. (Naviculaceae: Bacillariophyta) from Guangxi Autonomous Region, China

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**Abstract:** A new genus and species of freshwater diatoms from the Guangxi Autonomous region of southern China were studied with light and scanning electron microscopy. The newly proposed genus has several features that assign it to the Naviculaceae, including symmetry about the apical, transapical and perivalvar axis, slit-like areolae that are occluded internally by fine hymenes, hook-like external distal raphe ends and the presence of an internal auxillary rib bordering the raphe sternum. These features also suggest a strong morphological similarity with the genus *Navicula*. The new genus and species, however, have several external flaps of silica on the valve face and apex that give the raphe a bifurcate appearance, a feature not found in any *Navicula* species and more reminiscent of the genus *Neidium*. Also present is a hooded structure on the auxillary rib that covers the central nodule and expanded central area on both sides of the raphe, on one side resembling a forked expanded fascia. These features of the exterior and interior of the valve are not seen in other *Navicula* species, or in other genera within the family. The proposed new genus and species, *Trialacinia guangxiana* joins other diatom genera and species being endemic from the Guangxi Autonomous region of China.

**Keywords:** diatoms, taxonomy, Naviculales, SEM, morphology, new genus, new species

### INTRODUCTION

The diatom genus *Navicula* Bory (1822) at one time had the largest number of species assigned to it (ca. 10,000) among all groups governed by the International Code of Nomenclature for Algae, Fungi and Plants. Since the break-up of this large genus into many smaller groups in the 19<sup>th</sup> (e.g. PFITZER 1871), 20<sup>th</sup> (e.g. MERESCHKOWSKY 1902, 1903; COX 1988; ROUND et al. 1990; LANGE-BERTALOT & METZELTIN 1996) and 21<sup>st</sup> (e.g. LANGE-BERTALOT 2001; LOWE et al. 2014) centuries, the genus has become more modest in its composition in terms of species richness (ca. 300 according to LANGE-BERTALOT 2001). Some examples of other newly-described genera separated from *Navicula* in the late 20<sup>th</sup> century and into the 21<sup>st</sup> century include *Haslea* SIMONSEN (SIMONSEN 1974), *Luticola* MANN (ROUND et al. 1990), *Fallacia* STICKLE et MANN (ROUND et al. 1990), *Lyrella* KARAYEVA (KARAYEVA 1978), and *Aneumastus* MANN et STICKLE (ROUND et al. 1990) among others.

COX (1999) suggested that there was significant variation in a variety of valve and girdle features amongst species assigned to *Navicula*, and that separation of taxa out of *Navicula* should consider not only the type species

of the genus (*N. tripunctata* (O.F. Müller) Bory), but this wider range of variation contained in the genus. A more restrictive definition for *Navicula* was offered by LANGE-BERTALOT (2001). He suggested that species belong to *Navicula* if they have frustules symmetrical about three axes, raphe with a more or less developed silicified sternum (“additional rib”, called an “auxillary rib” herein), two plate-like chloroplasts and external proximal raphe ends more or less deflected to the secondary side (the same side as the placement of the Voigt faults). He noted in this more restricted diagnosis of *Navicula* that species in the genus lacked a conopeum, external distal raphe ends that are not hooked, biseriate striae, stauros (“distinct lateral areae”, p. 13), continuous raphe across the central area, or double T-shaped costae at the apices (as in *Hippodonta* Lange-Bertalot et al.). Based on this new, more restrictive definition of *Navicula*, there was the establishment of new genera for those taxa that did not fit the genus. LANGE-BERTALOT (2001, p. 9) commented on the establishment of these new genera: “...[now] a fashion prevails for which the name “generomania” doesn’t seem to be exaggerated, and even we can’t withdraw ourselves from this trend not to be named as adherents of a stubborn conservatism. The

search for new genera constantly brings about new name combinations for the same old taxa, with the result that torsos are left over which are definitely more paraphyletic than before.... We think that such a mode of operation is very questionable.” Ironically six of the 10 genera he considered as separated from *Navicula* in his 2001 work were described by him (e.g. LANGE–BERTALOT 1997) or with his colleagues (LANGE–BERTALOT & METZELTIN 1996; MOSER et al. 1998; LANGE–BERTALOT & GENKAL 1999), let alone the number of genera he created pre–2001 (27) and post–2001 (43; KOCIOLEK et al. 2022).

Of the taxa remaining within *Navicula*, Lange–Bertalot divided them into two groups. One group, Section *Navicula*, is composed of the majority of species in the genus and has external proximal raphe ends curved to the secondary side of the valve (the side bearing the Voigt fault). The second, much smaller group in terms of species richness, was described as Section *Alinea* (named for *Navicula alineae* Lange–Bertalot in NEVO & WASSER 2000). This group has species with the external proximal raphe ends curved towards the primary side of the valve (away from the side with the Voigt faults). Variation in these groups can be demonstrated in the development of the external proximal raphe ends, silica coverings running longitudinally the length of the valves and whether and how the external central sternum is developed (see LANGE–BERTALOT 2001, plates 67–73).

In terms of freshwater diatom genera, Asia has the highest number and the most endemic genera as compared to all other continents (KOCIOLEK 2019). Within Asia, aside from Lake Baikal, the largest number of endemic genera can be found in China and SE Asia (KOCIOLEK 2019). Many of these endemic genera in China and SE Asia are part of the Cymbellales (e.g. KULIKOVSKIY et al. 2022) but only one of them is part of the Naviculales (*Sichuanella* LI et al. 2013).

In the present report we describe a new genus of freshwater diatom from Guangxi, a region that also bears an endemic genus of eunotioid diatoms (LIU et al. 2018). The new genus has many features similar to *Navicula* but also has several features that distinguish it from *Navicula* as typified by *N. tripunctata* Bory. We present light and scanning electron microscopic observations to demonstrate the features of the new genus and compare it with *Navicula* and other members of the family Naviculaceae.

## MATERIALS AND METHODS

Diatom samples were collected from Guangxi Zhuang Autonomous Region, during the biodiversity investigation of freshwater diatom from islands in Southern China. Benthic diatom samples were collected from Yujiang island, Hepu county, south of Guangxi. It is a very small island separated from the mainland by the Nanliu River, which is the largest river flowing to the sea. This area near Guangxi north Bay be-

longs to subtropical monsoon marine climate zone, with high average temperature and rainfall annually. The sampling site is located at the place where the Nanliu river flows into the island, 109.054041° E, 21.609067° N. This area is heavily influenced by human activity, and it is a well-known breeding area for aquatic migratory waterfowl (ducks). Samples were collected in February 2021, environmental parameters were determined by YSIPro DSS multiparameter meter (YSI, Ohio, USA).

Samples were fixed with 4% formaldehyde in the field. Materials were boiled with HNO<sub>3</sub> for 2–3 hours in the lab, then washed using distilled water until the pH was neutral, cleaned material were preserved by ethyl alcohol. For light microscope (LM) observations, permanent slides were mounted by Naphrax, and examined with a Zeiss Imager A2 microscope equipped with a digital camera (AxioCam MRc 5) and observed with DIC (differential interference contrast) optics (Zeiss, Jena, Germany at Harbin Normal University). For scanning electron microscope (SEM) observations, cleaned material was air-dried and coated with gold–palladium, and observations made with a Hitachi S–4800 field emission SEM (Hitachi, Tokyo, Japan at Harbin Normal University) at an operating voltage of 15 kV. Diatom images were compiled with Photoshop 7.0. Samples are archived at the Key Laboratory of Biodiversity of Aquatic Organisms, Harbin Normal University.

## RESULTS

### *Trialacinia* Kociolek, Liu et Fan gen. nov. (Figs 1–27)

**Description:** Valves naviculoid, slightly asymmetrical along the apical axis, linear to elliptical. Externally, raphe undulate, with proximal raphe ends slightly expanded, slightly curved to the same side; angular, hooked distal raphe ends are hidden under flaps on the valve face and from the terminus, giving an image of bifurcate, anchor or T-shaped ends of the raphe externally. Striae uniseriate, radiate in the valve center, becoming parallel to convergent near the apices. Areolae slit-like externally and occluded by hymenes internally. Central sternum developed more to one side of the axial area than the other, forming the auxillary rib. The primary side developed with a large auxillary rib which covers the raphe slit and the central nodule.

**Type species:** *Trialacinia guangxiana* Kociolek, Liu et Fan sp. nov.

**Etymology:** The name refers to the three external flaps at the terminus of the valves.

### *Trialacinia guangxiana* Kociolek, Liu et Fan sp. nov. (Figs 1–27)

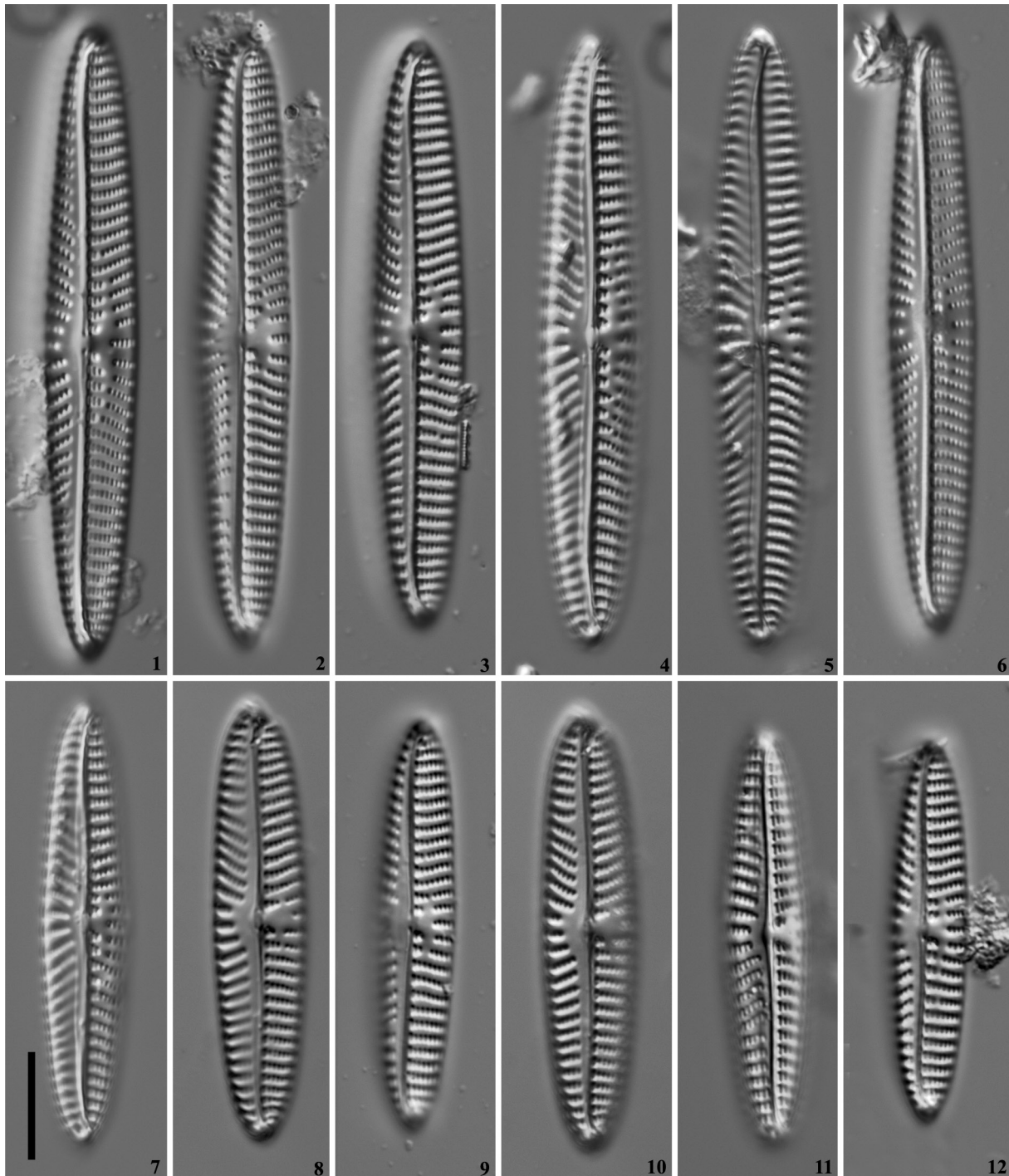
#### Description

**LM (Figs 1–12):** Valves naviculoid, with larger valves nearly linear and smaller valves nearly elliptical, with acute–rounded apices, length 34.0–60.0 μm, breadth 7.0–9.5 μm (n=30). Valves asymmetric about the central sternum. The secondary side of the valve with a wider axial area, and with the central area swollen in the valve center; primary side with a narrower axial area, in the center an elliptical area without areolae present.

Raphe almost straight in the valve center. Striae radiate in the center, parallel to convergent towards the apices, 5–6/10  $\mu\text{m}$  in the valve center and becoming denser towards apices, 7–9/10  $\mu\text{m}$ .

**SEM (Figs 13–27):** Externally, raphe undulate, with the primary raphe ends slightly expanded, and curved to the secondary side (Figs 13–14). The distal raphe ends covered by flaps and the raphe occurs underneath the flaps near the ends (Figs 15–16). One flap on the secondary side (Fig. 19 white arrow) and the other on the primary side (Fig. 19 wi-

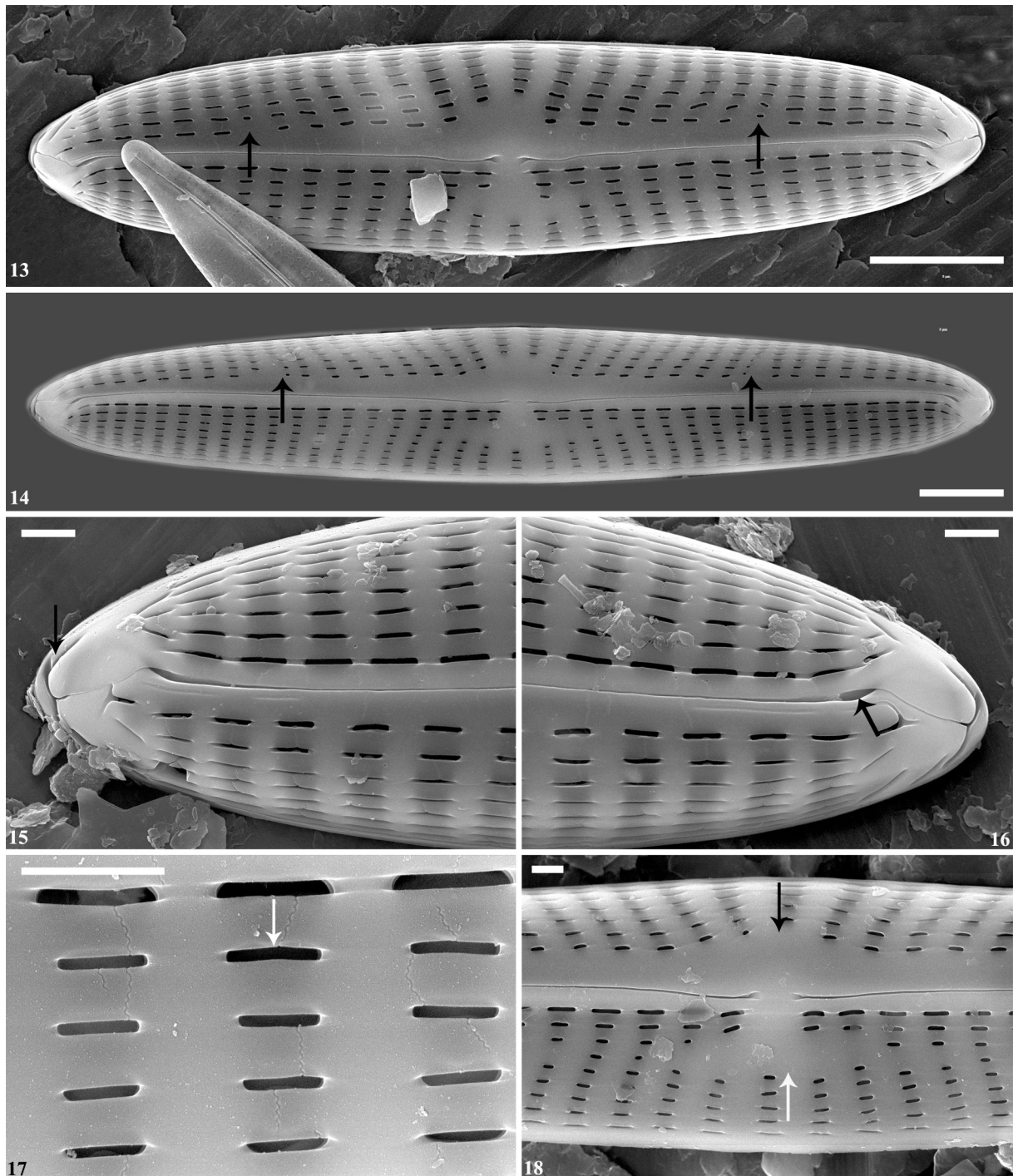
der black arrow). A small, nearly triangular cone-shaped flap present at the very end of the valve (Fig. 20 wider black arrow). The three flaps meet and cover the distal raphe ends (Figs 15–16; Figs 19–20), usually forming a wider slit near the distal raphe ends (Fig. 16). The resulting structure “Y”-shaped, being bifurcate on the primary side (Figs 15, 21–22, black arrow). One or two long slit-like areolae evident near the apices on the secondary side (Figs 19–20, narrower black arrow). Striae uniseriate, with slit-like shaped areolae openings (Fig. 17). Voigt faults present



Figs 1–12. *Trialacinia guangxiana* gen. et sp. nov.: valve views, LM; (3) holotype. Scale bar 10  $\mu\text{m}$ .

at the secondary side with abnormally-shaped areolae (Figs 13–14). Central area nearly triangular in shape on the secondary side (Fig. 18, black arrow). On the primary side in the center, 4–5 short striae present along the axial area, discontinued near the very center, and 3–4 short striae along the margin, form an elliptical shaped central area with a rectangular opening towards to the proximal raphe ends (Fig. 18, white arrow). Internally, the central sternum

with a prominent auxillary rib, with the primary side larger and covering the central nodule and raphe slit (Fig. 23). At the central area, a stauros-like structure present on the primary side (Fig. 27, black arrow). The areolae openings rectangular to round and occluded by hymenes (Fig. 26). The inner openings of the areolae larger than the external openings (Fig. 27, white arrow). Helictoglossae displaced to one side and prominent at both apices (Figs 24–25).



Figs 13–18. *Trialacinia guangxiana* gen. et sp. nov., SEM, external view: (13, 14) whole valves; (15) apex of the valve, black arrow showing the “anchor or T-shaped” distal raphe end; (16) apex of the valve, black arrow shows the raphe underneath the flaps; (17) striae composed by slit-like areolae, white arrow shows the areola; (18) asymmetrical central area of the valve, black arrow shows the secondary side. Scale bars 5  $\mu\text{m}$  (13–14), 1  $\mu\text{m}$  (15–18).

**Holotype:** HANU!. Slide no. THGX2021159. Key laboratory of Biodiversity of Aquatic Organisms, College of life science and technology, Harbin Normal University, China. Holotype specimen illustrated as Fig. 3.

**Isotype:** COLO! Slide number 652040 is deposited in the Kociolek Diatom Collection at University of Colorado, Boulder.

**Type locality:** Nanyu village, Hepu county in Guangxi Zhuang Autonomous Region, China, 109.054041° E, 21.609067° N. Collected on 28<sup>th</sup> February 2021 by Liu Y. and Lu X.X.

**Habitat:** Benthic.

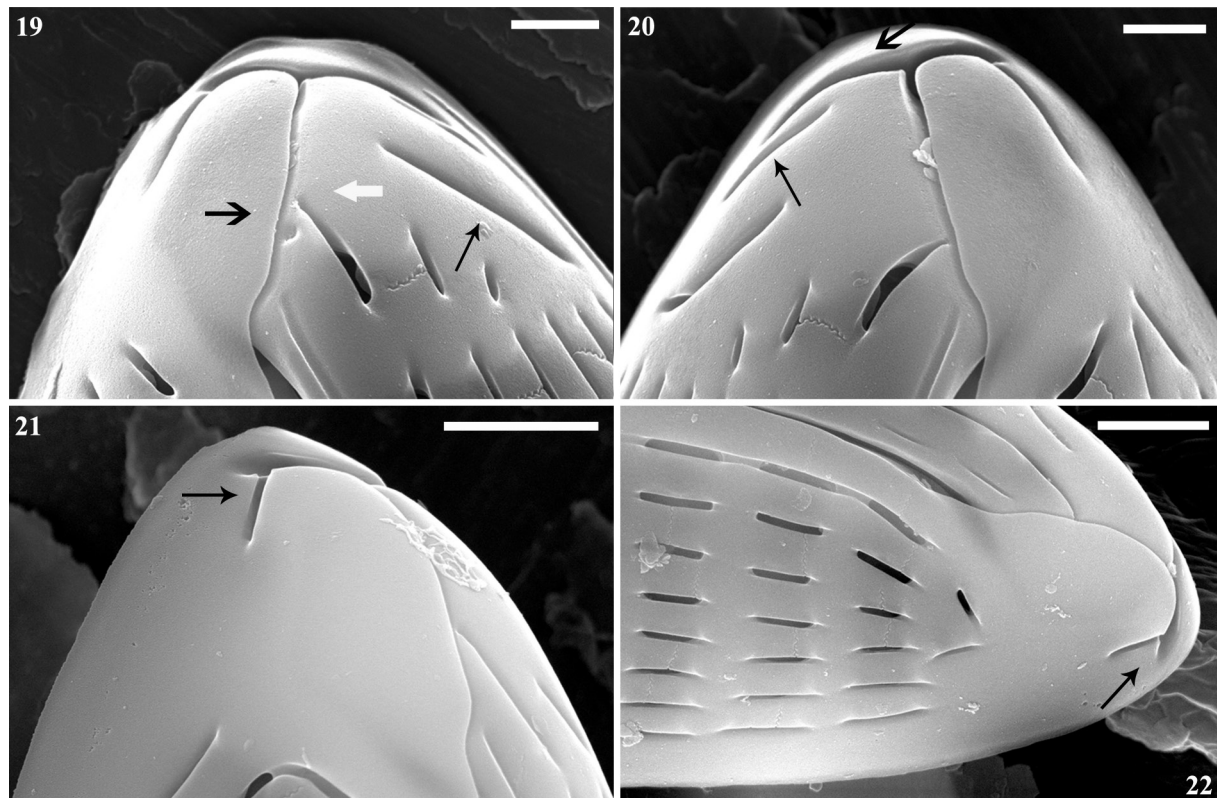
**Etymology:** Named for the Autonomous Region in China where it was found.

**Ecology:** During sampling, pH was measured as 6.93, water temperature 17.7 °C and conductivity 254  $\mu\text{S}\cdot\text{cm}^{-1}$ . This new species occurred with some cosmopolitan species such as *Cyclotella meneghiniana* Kützing, *Melosira varians* Agardh, *Aulacoseira granulata* (Ehrenberg) Simonsen, *Achnanthisdium minutissimum* (Kützing) Czarnecki, *Achnanthisdium catenatum* (Bily et Marvan) Lange–Bertalot etc., and species belonging to the genera *Nitzschia* Hassall (10 taxa), *Achnanthes* Bory (1 taxon), *Navicula* (2 taxa), *Cosmioneis* Mann et Stickle (1 taxon), *Halamphora* (Cleve) Levkov (2 taxa), *Psammodictyon* Mann (1 taxon) were also observed.

## DISCUSSION

Based on the structure of the areolae, hooked external distal raphe end and the presence of an auxillary rib internally running next to the raphe, *Trialacinia* gen. nov. resembles other genera of the Naviculaceae and especially the genus *Navicula*. These features align with the circumscription of *Navicula* as diagnosed by both COX (1999) and LANGE–BERTALOT (2001). There are a number of features that are found in *Trialacinia* gen. nov., however, that differentiate it from *Navicula*. In the center of the valve there is an expanded area on one side of the central area that splits into two and extends a short distance length–wise. This region is differentiated both externally (with the striae interrupted) and internally (with a raised, stauros–like area). LANGE–BERTALOT (2001) indicated stauros–like structures are not present in *Navicula*.

The auxillary rib is quite extensive in *Trialacinia* gen. nov. and in that regard, it covers the central node. Such a large auxillary rib is not seen in any other *Navicula* species. It produces an expanded area that runs nearly the length of the valve. In other *Navicula* species the auxillary rib is nearly non–existent (VAN DER VIJVER et al. 2010; POTAPOVA et al. 2019) to somewhat developed (e.g. FERNANDES & PROCOPIAK 2003; VAN DE VIJVER & LANGE–BERTALOT 2009; SABBE et al. 2019;



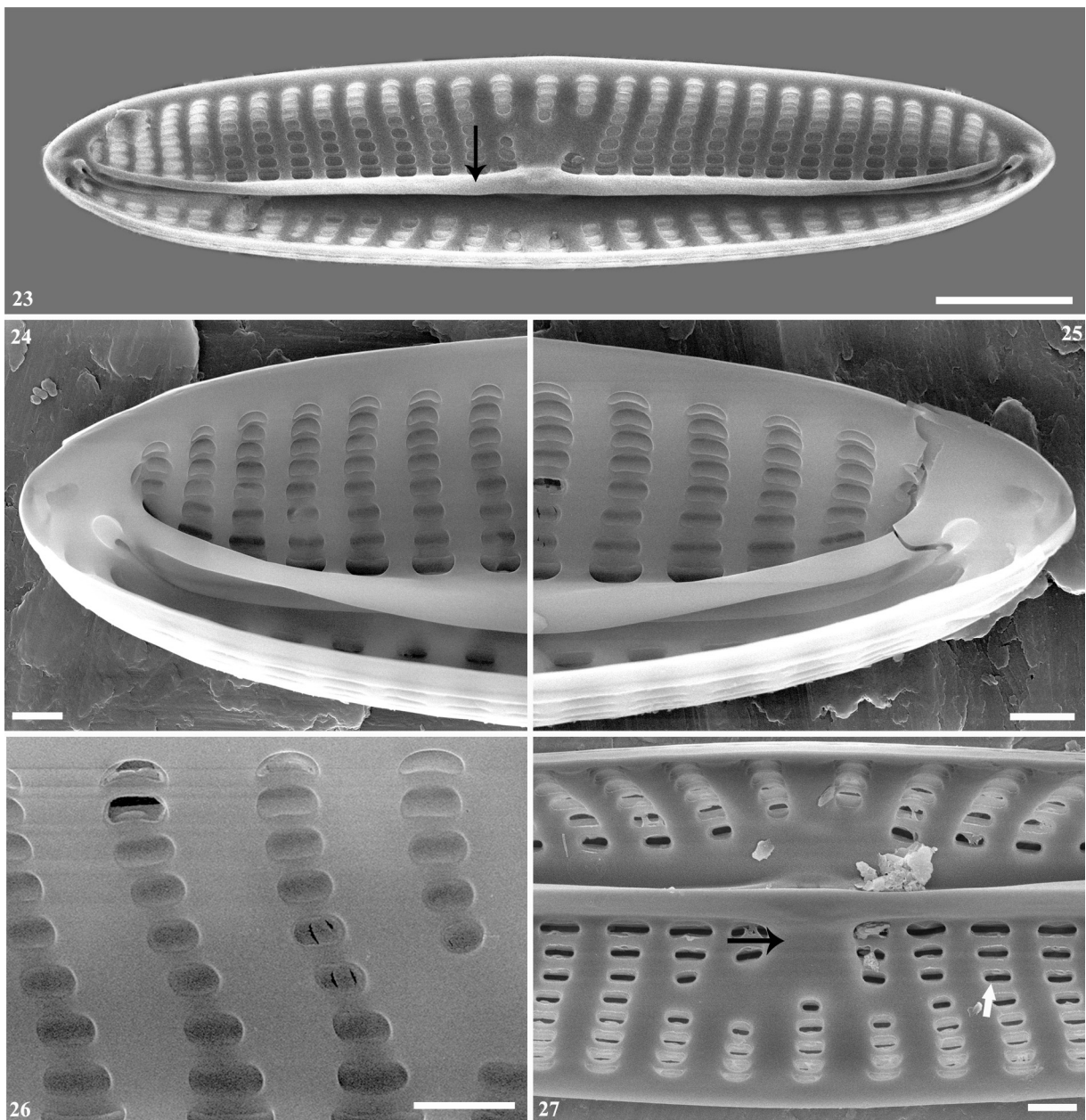
Figs 19–22. *Trialacinia guangxiana* gen. et sp. nov., SEM, external view of the apices from the same valve: (19) wider black arrow indicates the flap from primary side, white arrow shows flap from the secondary side, narrower arrow shows a large slit–like areola near the apex; (20) wider black arrow shows the flap from the mantle, narrower arrow indicates the large slit–like areola near the apex; (21–22) black arrow shows the anchor shaped ends are “Y” shaped, bifurcate on the primary side. Scale bars 500 nm (19–20) and 1  $\mu\text{m}$  (21–22).

CHUDAEV & JÜTTNER 2020), to strongly developed (TAYLOR et al. 2016). Even in taxa where the auxiliary rib is well-developed, like *N. amoyensis* Gao, Sun et Chen, the accessory rib only has additional thickening at the center (CHEN et al. 2017), while in others such as *N. nielsfogedii* TAYLOR et al. 2016, the central nodule is not covered as it is in *Trialacinia guangxiana*.

The raphe is undulate in *Trialacinia* gen. nov., which is quite different from the situation in other naviculacean genera. For example, in *Gyrosigma* Hassall and *Pleurosigma* W. Smith the raphe is sigmoid (PATRICK & REIMER 1966; KRAMMER & LANGE-BERTALOT 1986), and the same is true for another genus in the family, *Envekadea* Van de Vijver et al. (GLIGORA et al. 2009).

In nearly all *Navicula* species, the raphe is straight and filiform (e.g. LANGE-BERTALOT 2001; WITKOWSKI et al. 2010; BAHLS 2012).

Perhaps the most striking difference between *Trialacinia* gen. nov. and *Navicula*, however, is the presence of flaps on the valve face and mantle that cover the raphe slit, giving the impression of a forked external distal raphe end. The flap on the valve mantle at the apex is similar to the lacinia found in the genus *Neidium* Pfitzer (SIVER et al. 2003; HAMILTON et al. 2004; STACHURA-SUCHOPLES et al. 2010; LIU et al. 2014). The flap present on the valve face of *Trialacinia* gen. nov. is not known in any other genus of raphid diatoms. The flap on the valve face distinguishes this genus from all



Figs 23–27. *Trialacinia guangxiana* gen. et sp. nov., SEM, internal view of the valve: (23) whole valve, black arrow indicates prominent accessory rib formed by central sternum; (24–25) apices of the same valve; (26) areolae covered by hymenes; (27) central area of the valve, black arrow shows the central stauro, white arrow shows the areolae with larger internal openings. Scale bars 5  $\mu\text{m}$  (23), 1  $\mu\text{m}$  (24–27).

others, including those in the Naviculaceae. These features in *Trialacina* gen. nov. meet the criterion of COX (1999, p. 207) that, “clear discontinuities” between *Navicula* and any newly proposed genus must be present.

The discovery of this new genus of freshwater diatom in the Guangxi Zhuang Autonomous Region is perhaps not surprising. The region is a biological diversity hotspot for a wide range of organisms, including those that are terrestrial as well as aquatic. Of the aquatic diversity, there are unique taxa described from both marine and freshwater environments. Plants (KADOORIE & BOTANIC 2003), spiders (LI et al. 2018), amphibians and reptiles (ZHAO et al. 2000) and fishes (HE et al. 2022) among other groups all have new families, genera and species described from this region. A few diatoms, including the genus *Sinoperonia* Kociolek, Liu, Glushchenko et Kulikowsky (LIU et al. 2018) and several species (LIU et al. 2021; ZHANG et al. 2020; LIU et al. 2020; LI et al. 2019) have been described from the Guangxi Autonomous region. As the area has four major river drainages, and already has been demonstrated to harbor endemic taxa, this region of China warrants future research on endemic species and genera of freshwater diatoms.

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