

***Staurosira longwanensis* sp. nov., a new araphid diatom (Bacillariophyta) from Northeast China**

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Abstract: *Staurosira longwanensis* sp. nov. is described from Sihailongwan maar lake, northeast China. The morphology of this new species is documented with light and scanning electron micrographs and discussed in comparison with several small species of Fragilariaceae. *Staurosira longwanensis* belongs in the genus *Staurosira* since it shares many features with taxa currently ascribed to it such as the position and characteristics of the spines, the characteristics of the areolae and apical pore fields. Apart from the type locality, *S. longwanensis* also occurs in another volcanic lake in northeast China, with circumneutral and oligotrophic water. Diatom analysis of the long sedimentary record retrieved from Lake Sihailongwan indicates that *S. longwanensis* has been present at low relative abundances in this lake at least since the Late Pleistocene.

Key words: Araphid diatoms, Bacillariophyta, China, morphometric analysis, palaeolimnology, *Staurosira longwanensis* sp. nov.

INTRODUCTION

The taxonomy and circumscription of genera belonging to the Fragilariaceae have long been problematic despite major taxonomic revisions in the 1980s (WILLIAMS & ROUND 1987). Even the most commonly reported of these genera such as *Staurosira* EHRENB., *Staurosirella* (EHRENB.) D.M. WILLIAMS et ROUND and *Pseudostaurosira* D.M. WILLIAMS et ROUND continue to be discussed (MORALES 2001), as numerous species are emended, newly transferred (MORALES 2003; EDLUND et al. 2006; GARCIA 2006; HAMILTON & SIVER 2008; ÁCS et al. 2009) and new species are constantly being encountered and described (e.g. MORALES 2001, 2002, 2005, 2006; MANOYLOV et al. 2003; MORALES & EDLUND 2003; SCHMIDT et al. 2004; MORALES & MANOYLOV 2006; WILLIAMS et al. 2009; MORALES et al. 2010a, b, c, 2012, 2013a, b; CEJUDO-FIGUEIRAS et al. 2011). In this paper a new species, named here as *Staurosira longwanensis*, is described using light (LM) and scanning electron microscopy (SEM). This new species was found in Lake Sihailongwan, a volcanic lake of northeast China.

MATERIAL AND METHODS

Study site. Lake Sihailongwan (42°17'N, 126°36'E) is a small and deep maar lake with steep rocky slopes located in the Long Gang Quaternary volcanic field (Jilin Province, NE China, Fig. 1). Detailed information on the geography, recent climate and vegetation of this region and the characteristics of its volcanic lakes can be found in MINGRAM et al. (2004), STEBICH et al. (2009) and CHU et al. (2011). For ecological information, the pH, conductivity, and the concentrations for the main cations, anions and nutrients were measured on several occasions on water samples collected between 2005 and 2013. The detailed methods used for obtaining these environmental data are given in RIOUAL et al. (2013). A summary of Lake Sihailongwan morphometric features and surface water quality characteristics is given in Table 1.

Sampling. A large number of samples (including phytoplankton, epilithon, epiphyton, epipelon, off-shore surface sediments, monthly sediment traps and sediment core samples) were taken from Lake Sihailongwan between 2005 and 2013. Among all samples investigated, the new species of *Staurosira* was most abundant in a surface-sediment sample retrieved using a UWITEC corer from the littoral zone of the lake by 17 m water depth in November 2006

(Fig. 1). Preliminary observations under the light microscope revealed that numerous live diatom cells were present in this sample. It is therefore likely that the epipelagic community was sampled. Other specimens of *Staurosira* were investigated from a short gravity core retrieved in 1998 (SHL98–2) and a 38.8 m-long piston core retrieved in 2001. The short gravity core spans the last 400 years, while the long piston core spans the late Pleistocene and the entire Holocene. Details on the recovery and dating of the gravity and piston cores are given in Mingram et al. (2004) and Stebich et al. (2009), respectively.

Laboratory methods. Samples were prepared according to the standard techniques described in Battarbee et al. (2001) using hydrogen peroxide and acid cleaning. Slides for the light microscope (LM) were prepared by mounting the cleaned suspension in Naphrax®. LM observations were made at $\times 1000$ magnification using a Leica DM LB2 equipped with immersion objectives (N Plan, 1.25 numerical aperture) and differential interference contrast (DIC). Photomicrographs were captured using a machine vision camera (MVC3000 from UVTEC).

For scanning electron microscope analyses, aliquots of cleaned samples from two lakes were left to dry onto glass coverslips of 12 mm diameter. These were mounted onto aluminum stubs using Ted Pella® double-coated carbon conductive adhesive tape and coated with gold using a Polaron SC7640 sputter coater for viewing on a LEO 1530 VP at the State Key Laboratory of Paleobiology and Stratigraphy (Chinese Academy of Sciences, Nanjing). SEM images were captured directly in digital format.

Taxonomic metrics such as valve length, width at mid-valve and width near the apices (measured at 2 μm from the tip of the valve), and stria density were taken from the LM digitized images calibrated against a slide micrometer using the public domain ImageJ software (RASBAND 2013). The form factor of each valve was measured following SIVER & BASKETTE (2004). For SEM, areola density was measured in addition to the metrics already listed for LM. For areola density, the length of 5 five areolae was measured on 5 striae located in the middle of the valve. The mean values were converted to a number of areolae per 10 μm (CORTESE & GERSONDE 2007). In total 175 valves were measured, including 24 under the SEM.

Relative diatom abundance data in the different samples investigated were calculated from counts under LM of at least 200 valves, except for the surface sediment collected in November 2006 (holotype slide), for which over 1000 valves were counted.

Morphometric analysis. The 175 specimens investigated came from three populations: a “modern” population composed of specimens from the November 2006 epipelagic sample and from off-shore surface sediment samples (Figs 2–46); a population from the short gravity core SHL98–2 (AD 1615–1960); and a population from the long piston core (1263–13,070 yrs BP). To establish if the morphological characteristics of these three populations differ from each other, single characters analyses and bivariate analyses were performed. For single characters analyses, non-overlapping notches on boxplots were used to infer statistically significant dissimilarity at the 95% confidence interval (CHAMBERS et al. 1983; PAULL et al. 2008). Notched boxplots were also used to identify outlier specimens from each population (Figs 156–160). Bivariate analyses were based on scatter plots

Table 1. Environmental characteristics of Lake Sihailongwan, the type locality of *Staurosira longwanensis* sp. nov. The ranges for water chemistry values were obtained from the analyses of 55 samples collected from Apr–2005 to Nov–2012 (outlier values, as defined in box-and-whisker plots, were excluded).

Location	42°17'N, 126°36'E
Elevation (m a.s.l.)	790
Lake area (km ²)	0.50
Catchment area (km ²)	0.70
Maximum water depth (m)	50
pH	6.3–7.1
Conductivity 25° C ($\mu\text{S}\cdot\text{cm}^{-1}$)	51–78
Alkalinity ($\mu\text{eq}\cdot\text{l}^{-1}$)	420–601
Ca ($\text{mg}\cdot\text{l}^{-1}$)	4.7–15.2
Na ($\text{mg}\cdot\text{l}^{-1}$)	1.3–4.2
Mg ($\text{mg}\cdot\text{l}^{-1}$)	2.1–3.8
K ($\text{mg}\cdot\text{l}^{-1}$)	0.4–2.8
Cl ($\text{mg}\cdot\text{l}^{-1}$)	0.6–2.9
SO ₄ ($\text{mg}\cdot\text{l}^{-1}$)	4.2–6.8
NO ₃ ($\text{mg}\cdot\text{l}^{-1}$)	0.0–1.5
Total Phosphorus ($\mu\text{g}\cdot\text{l}^{-1}$)	0.4–21.3 (mean 9.1)
Total Nitrogen ($\mu\text{g}\cdot\text{l}^{-1}$)	113–558 (mean 363)
Dissolved silica ($\text{mg}\cdot\text{l}^{-1}$)	0.0–0.8
Dissolved Organic Carbon ($\text{mg}\cdot\text{l}^{-1}$)	0.1–2.6
Secchi disk depth (m)	4.0–7.6

(Fig. 161). Boxplots and scatterplots were drawn using R (R DEVELOPMENT CORE TEAM 2013).

RESULTS

Staurosira longwanensis RIOUAL, E. MORALES et ECTOR sp. nov. (Figs 2–155)

Description

In LM (Figs 2–143): Frustules rectangular in girdle view, forming chains (Figs 40–42). Valves isopolar with inflated central area, poles rostrate, less so in small valves which tend towards narrowly elliptical; length 4.6–17.8 (22.5) μm , width 2–3.9 μm . Sternum often very narrow but sometimes lanceolate. Striae alternate, parallel or slightly radial towards the apices; stria density 11.2–14.8 striae per 10 μm . Plastids not seen.

In SEM (Figs 144–155): Striae uniseriate, composed of up to 9 areolae, of which 2–4 located on mantle

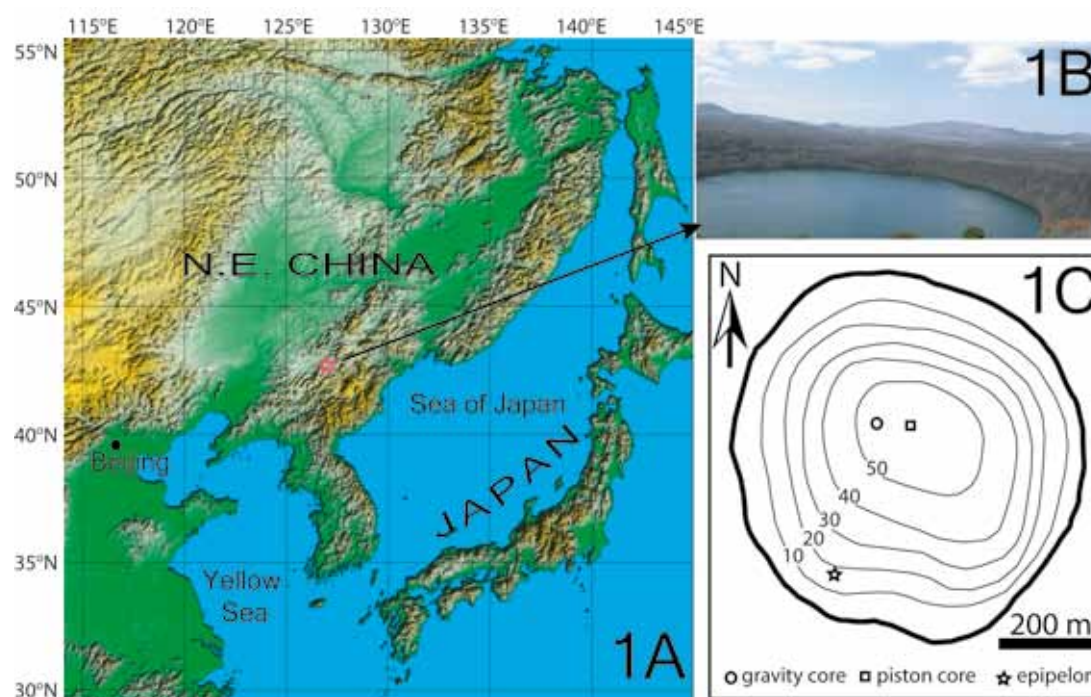


Fig. 1. (A) Location of Lake Sihailongwan in Northeast China; (B) view of the lake; (C) bathymetry of the lake and sampling sites (the interval of the isobathic curves is 10 m).

(Fig. 147). Areolae vary in shape from oval (Figs 146–149) to transapically extended (Figs 144, 145, 150, 151); areolae bear delicate volla, finely branched and extending inner periphery of areolae (Figs 152–154); density is 49–59 areolae per 10 μm . Spines solid, spatulate (Figs 146, 149) or poorly developed and conical (Fig. 148), located between the striae. Apical pore field located at transition between valve face and valve mantle, well developed even in small valves (Figs 144–151), composed of several rows (up to 5) of small rimmed poroids (Figs 148, 152, 153). Rimoportulae absent. Girdle bands bear one row of perforations (Fig. 155).

Etymology: The epithet of the new species refers to “long wan” the Chinese name for the crater lakes of the Long Gang volcanic field in Jilin Province and which means “Dragon bowl” in English.

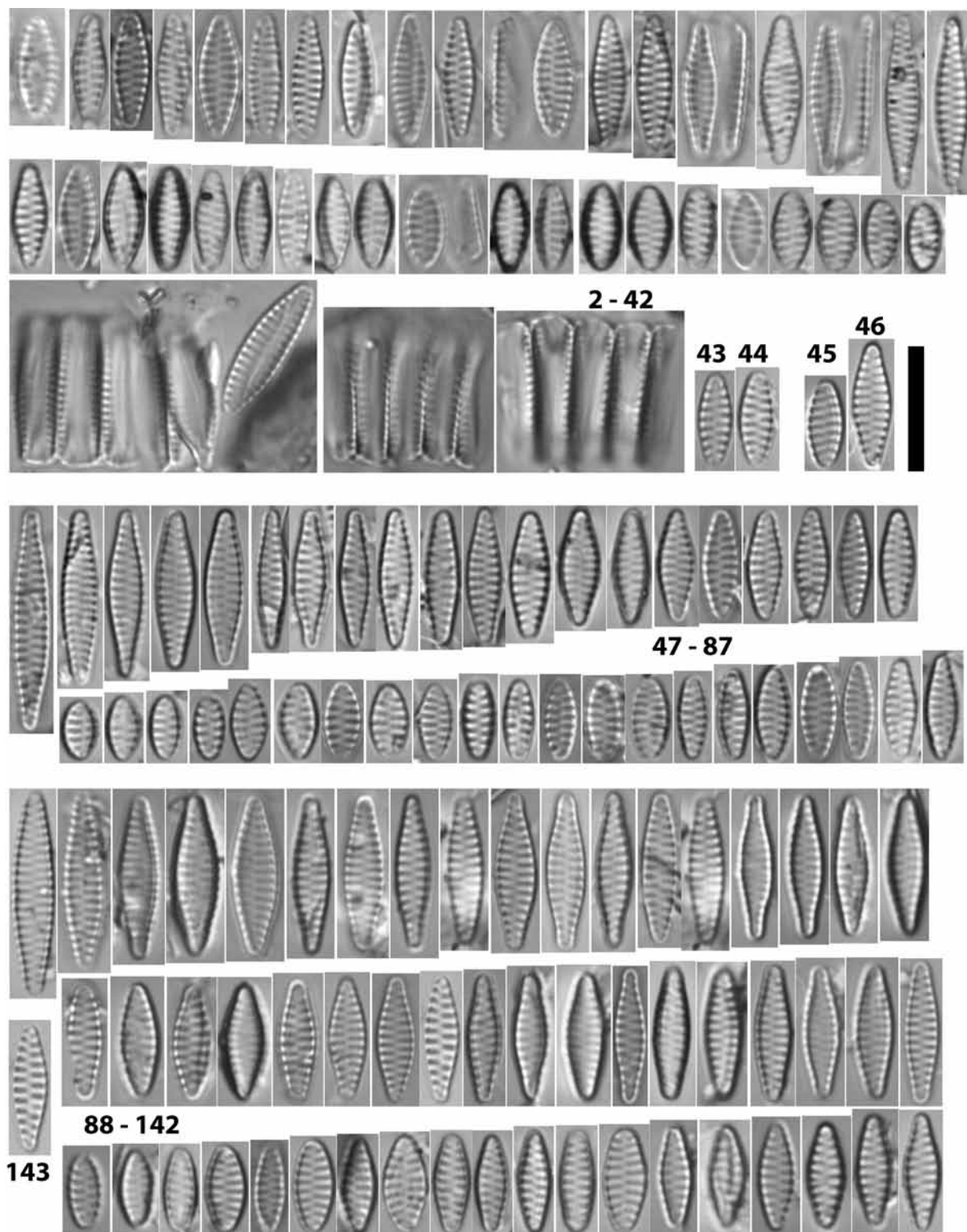
Holotype: Slide IGGDC–DB–SIHA–0611! (holotype specimen illustrated in Fig. 17, located using England Finder N48/2 or at coordinates 48.1 and 103.7 with a Leica DM LB2 light microscope).

Isotype: Slide BM 101 673, Natural History Museum, London, United Kingdom.

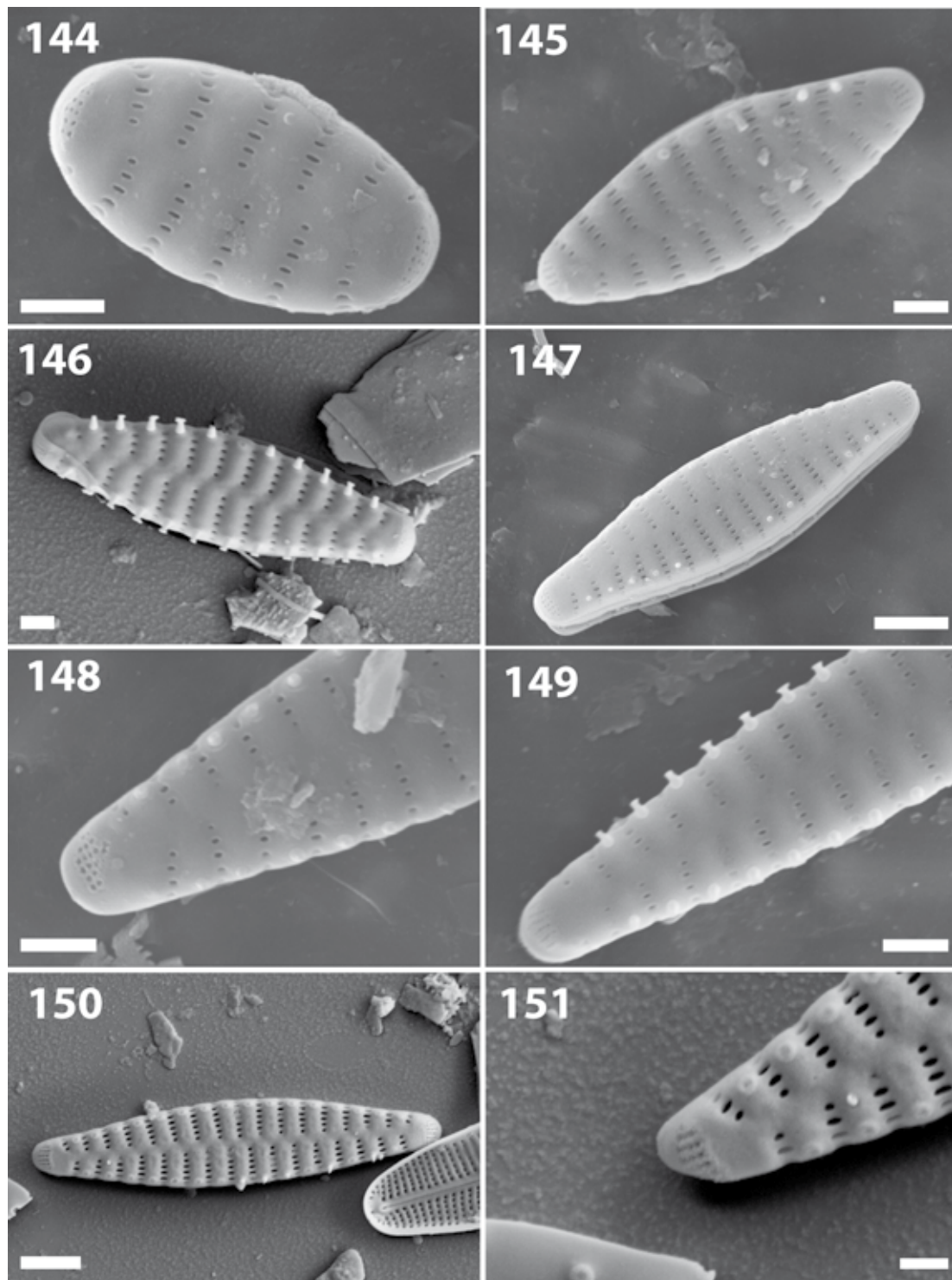
Type locality: CHINA, Jilin Province, Long Gang volcanic Field, Lake Sihailongwan, 790 m, 42° 17'01"N, 126° 36'07"E. Surface sediment, collected 19th November 2006 by Dr. Patrick Rioual, IGG–CAS, Beijing. Institute of Geology & Geophysics Diatom Collection, coded in the Index Herbariorum as IGGDC (<http://sciweb.nybg.org/science2/IndexHerbariorum.asp>).

Ecology and associated diatom species: Lake Sihailongwan has circumneutral and oligotrophic water (Table 1). Diatom analyses of many modern samples collected since 2005 in Lake Sihailongwan indicate that *S. longwanensis* is most abundant in the epipelon of the deep littoral zone (holotype collected at 17 m water depth). It is absent or only sporadically present in epilithon and epiphyton (on filamentous green algae) samples collected near the shore. It is also absent from the periphyton communities collected on the surface of sediment trap buoys.

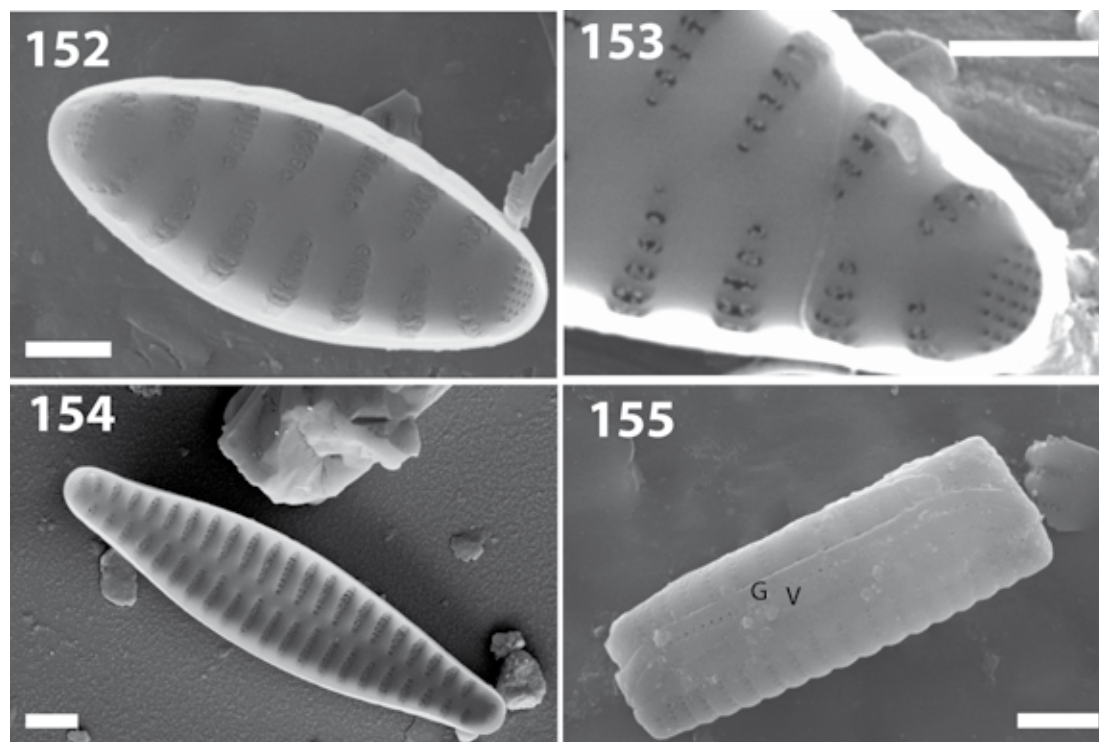
LM analysis of the surface–sediment sample used as holotype slide yielded 77 taxa. The sample was dominated by planktonic species including *Discostella stelligeroides* (HUSTEDT) HOUK et KLEE (32%), *Handmannia balatonis* (PANTOCSEK) KULIKOVSKIY et SOLAK (10.6%) and *Fragilaria nanoides* LANGE–BERTALOT (1.2%). Interestingly, the same three taxa dominate the assemblages of all sediment trap samples collected so far (over the period 2002–2012) as well as the late Holocene sedimentary record since ~3600 yrs BP. Among the benthic species found in the holotype slide (46.2%), the most abundant species were *Staurosirella pinnata* (EHRENBERG) D.M. WILLIAMS et ROUND *sensu* MORALES (2001) (15.4%), *Pseudostaurosira microstriata* (MARCINIAK) FLOWER (5.9%), *Eolimna raederae* (LANGE–BERTALOT) LANGE–BERTALOT et KULIKOVSKIY (4.1%), *Staurosira construens* var. *venter* (EHRENBERG) P.B. HAMILTON *sensu* MORALES (2001) (2.2%), *Pseudostaurosira trainorii* E. MORALES (2.1%), *Nupela vitiosa* (SCHIMANSKI) LANGE–BERTALOT (2.0%) and the new species described here (2.9%).



Figs 2–143. Light microscopy images of *Staurosira longwanensis* from Lake Sihailongwan: (2–42) population from an epipelonal sample (17 m water depth) collected in November 2006; (43–44) and (45–46) specimens found in surface sediment samples (50 m water depth) collected in October 2005 and August 2007, respectively; (47–87) specimens from the short gravity core SHL98–2 (AD 1615–1960); (88–142) late Holocene specimens from the long piston core (1263–3487 yrs BP). 143. Late Pleistocene specimen (13 070 yrs BP). Scale bar 10 μ m.



Figs 144–151. Scanning electron microscope images of *Staurosira longwanensis* showing external view of valves of various length and outline and the characteristics of axial area, striae and spines: (144) short elliptic valve; (145, 146, 149) detail of the spines; (148, 149, 151) detail of the valve apices showing the characteristics of the striae and apical pore fields; (151) detail of figure 150 showing broken spines that suggest a solid structure. Scales bars 2 µm (Figs 147, 150); 1 µm (Figs 144, 145, 146, 148, 149); 200 nm (Fig 151).



Figs 152–155. Scanning electron microscope images of *Staurosira longwanensis*: (152–154) internal view of valves of various length and outline; (153) characteristics of the apical pore field and the volae; (155) side view of a frustule showing detail of the cingulum with a valvocopula ‘V’ and a perforated girdle band ‘G’. Scales bars 2 μm (Figs 154, 155); 1 μm (Figs 152, 153).

Distribution: Until now *S. longwanensis* was only found in Lake Sihailongwan and in the surface-sediment of another maar lake in the Longang volcanic field (Longquan longwan maar lake), that has similar water chemistry.

Morphometric analysis: Notched boxplots show that there are no significant differences in morphometry between the valves of *S. longwanensis* found in modern and subfossil populations (Figs 156–160). Bivariate analyses (Fig. 161) also show that the three populations (modern, gravity core, piston core) cannot be clearly separated and occupy the same size trajectories. Form factor values are controlled by valve length, while valve width has low influence. Variation in stria density is not related to the other metrics. Bivariate analyses on the subset of valves observed under the SEM ($n=21$), indicate that areola density is not significantly correlated to the other morphometric variables (result not shown).

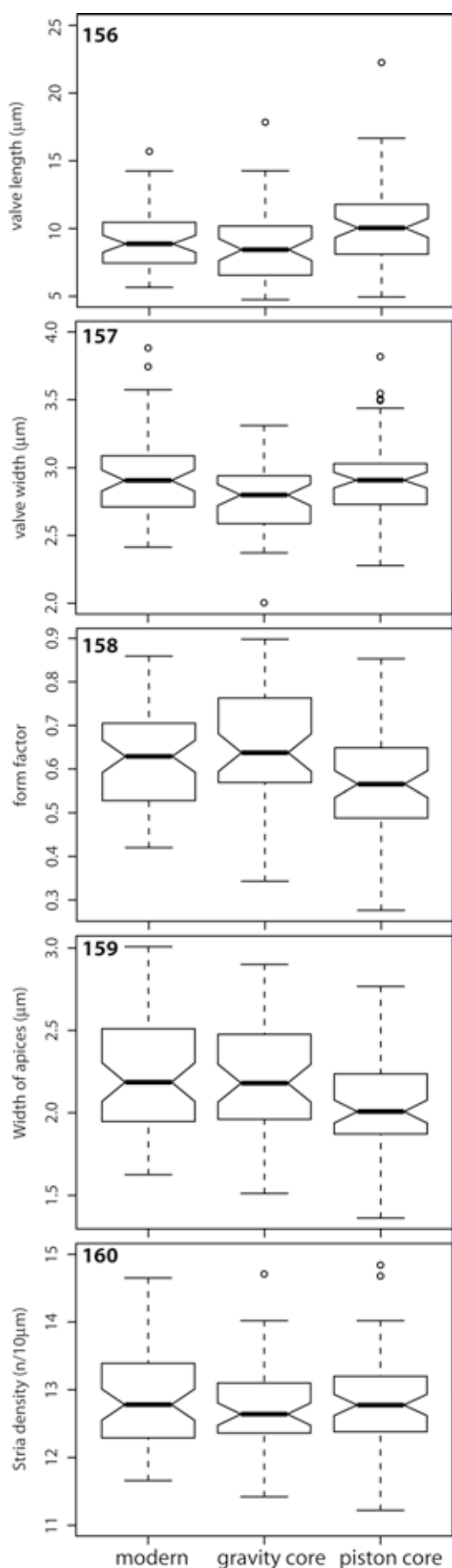
DISCUSSION

An extensive literature search was conducted and revealed a number of taxa that appear morphologically similar under the LM to *Staurosira longwanensis* and could therefore be confused with this new species.

By its valve outline *Staurosira longwanensis* resembles several other fragilarioid taxa such as *Staurosirella confusa* E. MORALES, *Staurosirella oldenburgiana* (HUSTEDT) E. MORALES, *Pseudostaurosira tenuis* E. MORALES et EDLUND and *Pseudostaurosira elliptica* (SCHUMANN) EDLUND, E. MORALES et S.A. SPAULDING. However, the combination of morphological features as presented above differs from taxa belonging to the genera *Staurosirella* D.M. WILLIAMS et ROUND and *Pseudostaurosira* (GRUNOW) D.M. WILLIAMS et ROUND. The genus *Staurosirella* has well-developed costae and striae composed of slit-like areolae while *Pseudostaurosira* has wide areolae and spines located between the interstriae, features that are not present in *Staurosira longwanensis*.

Staurosira longwanensis is a representative of *Staurosira* because it shares with taxa currently allocated to this genus several features such as the position and characteristics of the spines (on the costae), the characteristics of the areolae (small oval to lineate openings with and internal vola occlusion) and apical pore fields (composed of rimmed poroids). *Staurosira longwanensis* is different from other species of *Staurosira* because of its unique combination of characters which are its valve outline, well-developed apical pore fields, solid spines and the row of perforations on the girdle bands.

Small specimens of *S. longwanensis* may be confused with *Staurosira construens* var. *venter*. However, short valves of *S. construens* var. *venter* are



wider, frequently possess a wider sternum and have a more elliptical outline (MORALES 2010). The apical pore fields are also much more reduced in this taxon (MORALES 2001).

Another species with morphology comparable to that of *S. longwanensis* is *Staurosira oldenburgioides* (LANGE–BERTALOT) LANGE–BERTALOT, KULIKOVSKIY et WITKOWSKI, but it differs from *S. longwanensis* in having a higher stria density (15–17 instead of 12–15 in 10 μm), wider valve (3.3–4.2 instead of 2–3.9 μm) and more raised costae (LANGE–BERTALOT et METZELTIN 1996).

Fragilaria construens var. *javanica* HUSTEDT and *Fragilaria capensis* GRUNOW have a valve outline and a general appearance very similar to *S. longwanensis*. However, *F. construens* var. *javanica* has a wider central area and higher stria density (16–19 in 10 μm, HUSTEDT DIATOM STUDY CENTRE 2013) while *F. capensis* has much coarser areolae, girdle bands perforated by a double row of poroids and was described from a marine environment (WITKOWSKI et al. 1995). It remains unclear what the taxonomic position of *F. construens* var. *javanica* and *F. capensis* should be as these taxa have no rimoportula while species belonging to *Fragilaria sensu stricto* have one (TUJI & WILLIAMS 2006).

The variability in the shape of areolae observed in *S. longwanensis* is a character shared with other species of *Staurosira* such as *Staurosira stevensonii* MANOYLOV, E. MORALES et STOERMER (MANOYLOV et al. 2003), *Staurosira dimorpha* E. MORALES, EDLUND & S.A. SPAULDING and *Staurosira ambigua* E. MORALES, EDLUND et S.A. SPAULDING (MORALES et al. 2010a).

The apical pore fields of *S. longwanensis* are well-developed and similar to those of *Staurosira grigorszkyi* ÁCS, E. MORALES et ECTOR (ÁCS et al. 2009) and *Staurosira obtusa* (HUSTEDT) M. GARCIA (GARCIA 2006). The apical pore fields are located on the apex right to the edge between the valve face and the mantle, as in most *Staurosira* species (GARCIA 2006).

The spines of *S. longwanensis* are solid and spatulate and are surrounded at the base by a ring of silica with projections. By contrast, spines in *Staurosira* are often hollow as in *S. ambigua* (MORALES et al. 2010a), *Staurosira elliptica* (SCHUMANN) D.M. WILLIAMS et ROUND *sensu* HAWORTH (HAWORTH 1975;

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Figs 156–160. Box plots showing the quantile distributions of valve characters for *Staurosira longwanensis* observed in the 3 populations investigated: modern (n=57), gravity core (n=49) and piston core (n=67): (156) valve length; (157) valve width; (158) form factor; (159) width of apices; (160) stria density (= number of striae in 10 μm). The 25–75 percent quartiles (excluding outliers) are drawn using a box. The median is shown with a horizontal line inside the box. The whiskers represent the upper and lower “inner fence”, i.e. are drawn from the edge of the box up to the largest/lowest data point less than 1.5 times the box height. Outliers, i.e. values outside the inner fences, are shown as circles if they lie further from the edge of the box than 3 times the box height.

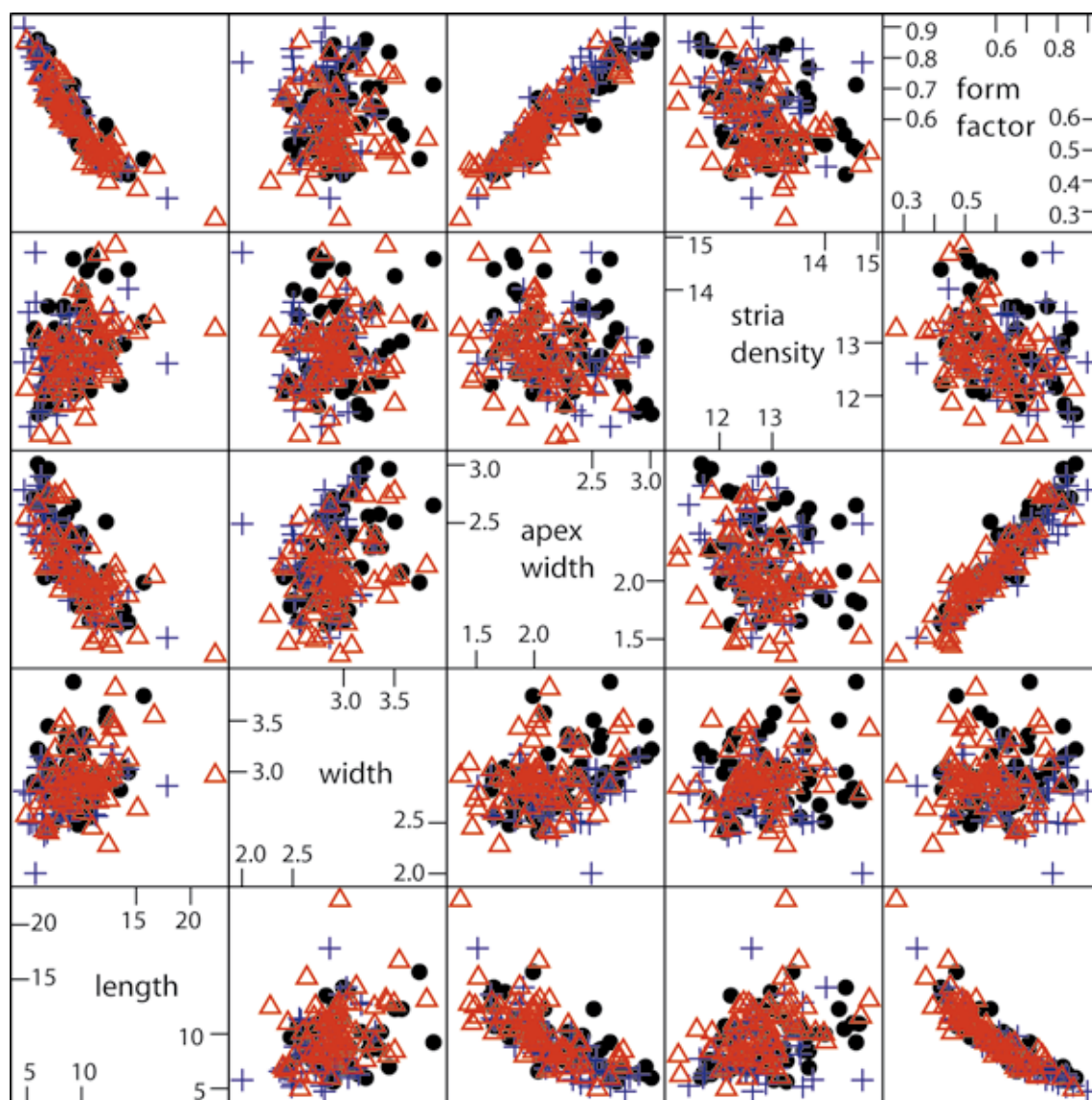


Fig. 161. Matrix of biplots of morphometric characters comparing populations of *Staurosira longwanensis* from the modern (black filled circles), gravity core (blue crosses) and piston core (red open triangles) populations. Measurements are in μm for valve length, width and width of apex. Stria density is in number of striae per 10 μm .

MORALES 2001) *Staurosira incerta* E. MORALES, *Staurosira construens* EHRENB. (MORALES 2006), *Staurosira construens* var. *binodis* (EHRENB.) P.B. HAMILTON (MORALES 2005) and *S. construens* var. *venter sensu* MORALES (MORALES 2001). The only other species of *Staurosira* reported to have solid spines is *Staurosira kjotsunarum* E. MORALES, NOVAIS et ECTOR (MORALES et al. 2012).

The presence of perforations on the girdle band is another peculiar feature of *S. longwanensis* as *Staurosira* species generally have copulae free of ornamentation (ROUND et al. 1990; GARCIA 2006). However, MORALES et al. (2010a) recently described *S. dimorpha* with a double row of poroids on the girdle bands.

Staurosira longwanensis appears to be most abundant in the deep littoral zone of the lake where it co-occurs with other small Fragilariaceae species.

Previous studies focusing on diatom distribution along a water–depth gradient also reported a mid–depth assemblage dominated by small Fragilariaceae in various lakes from Europe (CANTONATI et al. 2009) and North America (KINGSBURY et al. 2012). Light availability and water turbulence, which decrease as water depth increases, strongly influence diatom depth zonation (FLOWER et al. 2004). In Lake Baikal, numerous rare and endemic taxa have been reported from the deep littoral zone, stable but light limited, by contrast with the shallow littoral zone dominated by cosmopolitan species (FLOWER et al. 2004; KULIKOVSKIY et al. 2012).

In conclusion, we described a new species of fragilarioid diatom using LM and SEM. Our observations suggest that *S. longwanensis* is endemic to northeastern China and may be associated with the deep littoral zone. This study contributes to the

immediate need to document and illustrate diversity to facilitate research on diatom biogeography, ecology and palaeoecology in China.

ACKNOWLEDGEMENTS

Dr Yan Fang (State Key Laboratory of Paleobiology and Stratigraphy, Nanjing) is thanked for assistance with the SEM. This research was supported by the “Strategic Priority Research Program” of the Chinese Academy of Sciences, Climate Change: Carbon Budget and Relevant Issues (grant # XDA05120201–2) and the National Science Foundation of China (grants # 40502018 and # 40872113).

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Received October 16, 2013

Accepted December 13, 2013