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CYMBELLA LOESCHERAE SP. NOV. (BACILLARIOPHYCEAE) FROM FIRST-ORDER STREAMS OF SOUTHERN BRAZIL

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Abstract. This paper describes a new species of *Cymbella* C. Agardh from low-elevation countryside streams of the Serra do Sudeste, São Lourenço do Sul city, in southern Brazil. *Cymbella loescherae* M. Garcia & D. B. Dutra *sp. nov*. has as distinctive features the elliptical shape of stigmata, elliptical internal areolae openings, and the presence of areolae of diverse shapes (X, I, Y, zig-zag) located mainly at the valve center on the dorsal and ventral sides. It resembles *C. subhimalaspera* Jüttner & Van de Vijver which presents about the same metric data set, but differs from it primarily in the number and form of stigmata, and the form of areola internal openings.

Key words: *Cymbella aspera* group, diatom, freshwater, lotic habitat, new species, Rio Grande do Sul State, South America, taxonomy

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Introduction

Cymbella Agardh *s.l.* contains only freshwater species, distributed worldwide; the genus, especially the *C. aspera* group (Jüttner *et al*. 2010), is found living mainly in oligotrophic environments with bryophytes, or on moist sediments adjacent to streams. It includes more than 500 different species, not counting the different varieties (Fourtanier & Kociolek 2011).

Large dorsiventral lanceolate *Cymbella* species with an apical axis ranging from 90 to 320 µm, width of 19 µm or higher, with several small stigmata, and coarsely-punctate striae (5.5–14.0 areolae in 10 µm) are assembled in the *C. aspera* (Ehrenb.) H. Perag. group (Krammer 2002).

Van De Vijver and Lange-Bertalot (2008) and Jüttner *et al*. (2010) recognized other critical features in the *C. aspera* group, such as the morphology of external proximal raphe endings and the presence of areolae with distinct shapes such as X, Y, and I along the entire valve face, besides those with a round outline. This group includes, for example, *C. himalaspera* Jüttner & Van de Vijver, *C. subhimalaspera* Jüttner & Van de Vijver, *C. amelieana* Van de Vijver & Lange-Bert., *C. peraspera* Krammer, *C. peraspera* var. *colombiana* Krammer & Lange-Bert., *C. halophila* Krammer and *C. neogena* (Grunow) Krammer (Jüttner *et al*. 2010).

The *Cymbella aspera* group is rarely recorded to Brazil; only Metzeltin and Lange-Bertalot (1998) and Schuster *et al.* (2015) have cited it. Schuster *et al.* (2015) found it in a first-order stream with a small strip of riparian vegetation and adjacent areas with corn crop in Santa Catatina State. Here we describe and illustrate a new *Cymbella* species found in first- and second-order oligotrophic streams from Rio Grande do Sul State (Brazil) in areas that have not previously been the subject of diatom studies.

Material and methods

The first-, second- and third-order streams studied are situated in southern Brazil on a hillside of the Serra do Sudeste, São Lourenço do Sul city, in Rio Grande do Sul State (Fig. 1). The locality is a small village called Picada das Antas with dozens of farms. The stream drained into a forested watershed where angiosperms including *Campomanesia xanthocarpa* O. Berg, *Trichilia clausenii* C.DC., *Casearia sylvestris* Sw. and

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Fig 1. Study area in southern Brazil.

Cupania vernalis Cambess., and the gymnosperm *Podocarpus lambertii* Klotzsch *ex* Endl. along with the fern *Dicksonia sellowiana* Hook. are common (Figs 2 & 3). The surrounding area is 50–70 m above sea level and formed by hills and valleys. Pelotas presents a humid subtropical climate (Cfa in Köppen-Geiger classification) with four distinctive seasons. The average temperature is 18°C and annual precipitation averages 1378 mm (http://en.climate-data.org/region/187).

On six separate dates we sampled at five sites located 2 km apart (Table 1), described as follows:

1 – 31°39′42″S, 52°13′34.5″W – located in a first-order stream in a farm, next to its head and forested watershed with *Campomanesia xanthocarpa*, *Podocarpus lambertii*, *Trichilia clausenii*, and *Dicksonia sellowiana*.

2 – 31°19′33.9″S, 52°13′57.9″W – located in a second-order stream next to the main house of the farm and a small henhouse. Part of the native forest had been removed and fruit trees including orange, persimmon and lemon trees grew there.

3 – 31°19′47.5″S, 52°14′07.1″W – located in the same second-order stream as sampling site 2, at a neighboring farm, where it is a third-order stream. Cattle breeding takes place there. Very few trees (e.g., *Salix humboldtiana* Willd.) occur along the stream margin.

4 – 31°20′01.1″S, 52°14′02.6″W – located in another first-order stream 1.5 km away from the first site. The vegetation observed contained *Trichilia clausenii*, *Casearia sylvestris* and *Cupania vernalis*.

5 – 31°20′29.0″S, 52°14′12.6″W – located in a third-order stream next to a bridge. The vegetation was mainly *Salix humboldtiana.*

The stream beds are composed of sand, cobbles and pebbles, of sandstone and granite origin. The cobbles and small boulders were scraped with a toothbrush, and surface sediment from the bottom was sampled with a pipette. We collected two samples from each sampling site between January 2010 and May 2014. One of the two samples was kept alive and the other was preserved in flasks with 4% formalin. In total, 23 sediment samples were collected during the studied period.

Fresh material of all samples was observed by LM to check for the presence of plastids in the diatoms. An aliquot of 1 mL was removed from the bottles and the organic matter in the cells was removed using equal amounts of $KMnO_4$ and HCl in a water bath at *ca* 70°C until the sample became clear. The sample was then rinsed five times with bidistilled water until it was acid-free (Simonsen 1974). Part of the material was mounted in Naphrax® (Brunel Microscopes Ltd., Chippenham, Wiltshire, UK) and examined with a light microscope (LM-BX40, Olympus, Tokyo) equipped with a digital camera (OPT14000, Plustek, Taipei).

Table 1. Sampling sites and their respective environmental data during the studied period.

Location/ Environmental variable	(1)* First-order stream 1	(2) Second-order stream 1	(3) Second-order stream 2	(4) First-order stream 2	(5) Third-order stream
pH	7.00–7.93	7.41–7.84	7.44–7.84	7.26–7.76	7.26–7.70
Temperature	19–21.5	20–26.3	20–26.3	19–24.6	21–27
Conductivity ($\mu S.cm^{-1}$)	78–95	74–80	80–104	73–94	90–105
Width (m)	0.2–0.4	0.8–1.2	2.0–5.0	0.2–0.5	4.0–5.0
Altitude (m)	*ca* 62	*ca* 56	*ca* 52	*ca* 55	*ca* 40

* Parenthesized numbers match the sequence of sites given in Material and Methods.

Figs 2 & **3**. Images from type locality. 3 – *Dicksonia sellowiana* Hook. present at type locality. Picada das Antas, São Lourenço do Sul, Rio Grande do Sul State, Brazil.

At least two permanent slides were prepared for each collected sample. At 1000× with oil immersion, 400 valves per slide were examined and counted. After this, the whole coverslip area was scanned at 200× in order to check for the presence of rare species, including *Cymbella* species.

For scanning electron microscopy (SEM), cleaned specimens were dried on a stub and then coated with platinum at 40 mA for 100 seconds using a Denton Vacuum Desk V sputter coater. The stubs were observed using a JEOL (JSM 6610LV, JEOL, Tokyo) microscope at accelerating voltage of 15–20kV. The working distance was 9–11 mm.

Water temperature, pH and conductivity were measured at the sampling sites with a LUTRON pH-206 and a Hanna DiST Condutivimeter.

Raw samples are deposited in PEL (Pelotas) under accession numbers 24213 to 24219. Terminology follows Van De Vijver and Lange-Bertalot (2008) and Jüttner *et al*. (2010).

Results

We studied 33 valves by SEM and 55 by LM in order to describe *Cymbella loescherae* M. Garcia & D. B. Dutra as a new species. *Cymbella loescherae* was observed alive in all samples of fresh material studied.

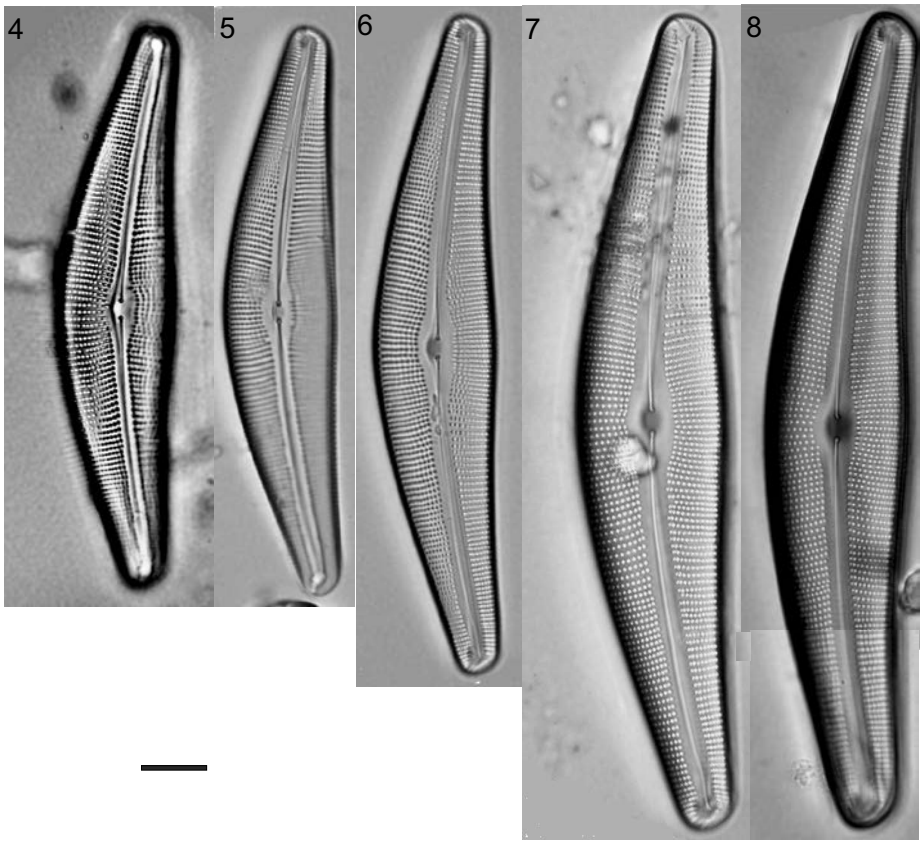
Cymbella loescherae M. Garcia & D. B. Dutra, ***sp. nov.*** Figs 4–21

Type locality: BRAZIL, Rio Grande do Sul State, São Lourenço do Sul, Picada das Antas, 31°39′42″S, 52°13′34.5″W, elev. *ca* 62 m a.s.l., *leg. D.B. Dutra & M. Garcia*, 13 September 2013.

Holotype: ICN 187445 in ICN, Herbário do Instituto de Ciências Naturais, Departamento de Botânica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil (Figs 4–9).

Paratypes: The raw sample PEL 24.213 in PEL, the herbariam of Universidade Federal de Pelotas, Pelotas (Brazil) and KRAM A-18 in the Department of Phycology, W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków (Poland).

LM description. Valves dorsiventral with convex dorsal margin gradually curved towards broadly rounded poles (Figs 4–8). Ventral margin almost straight, slightly inflated in central part. Length 117–170, width 24.2–28.0 µm, length/width ratio 4.2–5.6. Axial area linear and parallel to valve margin, expanded to ventral side of valve in elliptical, asymmetrical central area. Raphe moderately lateral. Proximal raphe endings widened (Fig. 4). Distal raphe endings deflected



Figs 4–8. Whole valves of *Cymbella loescherae* M. Garcia & D. B. Dutra, *sp. nov*. in LM (holotype slide). Scale bar = 10 µm.

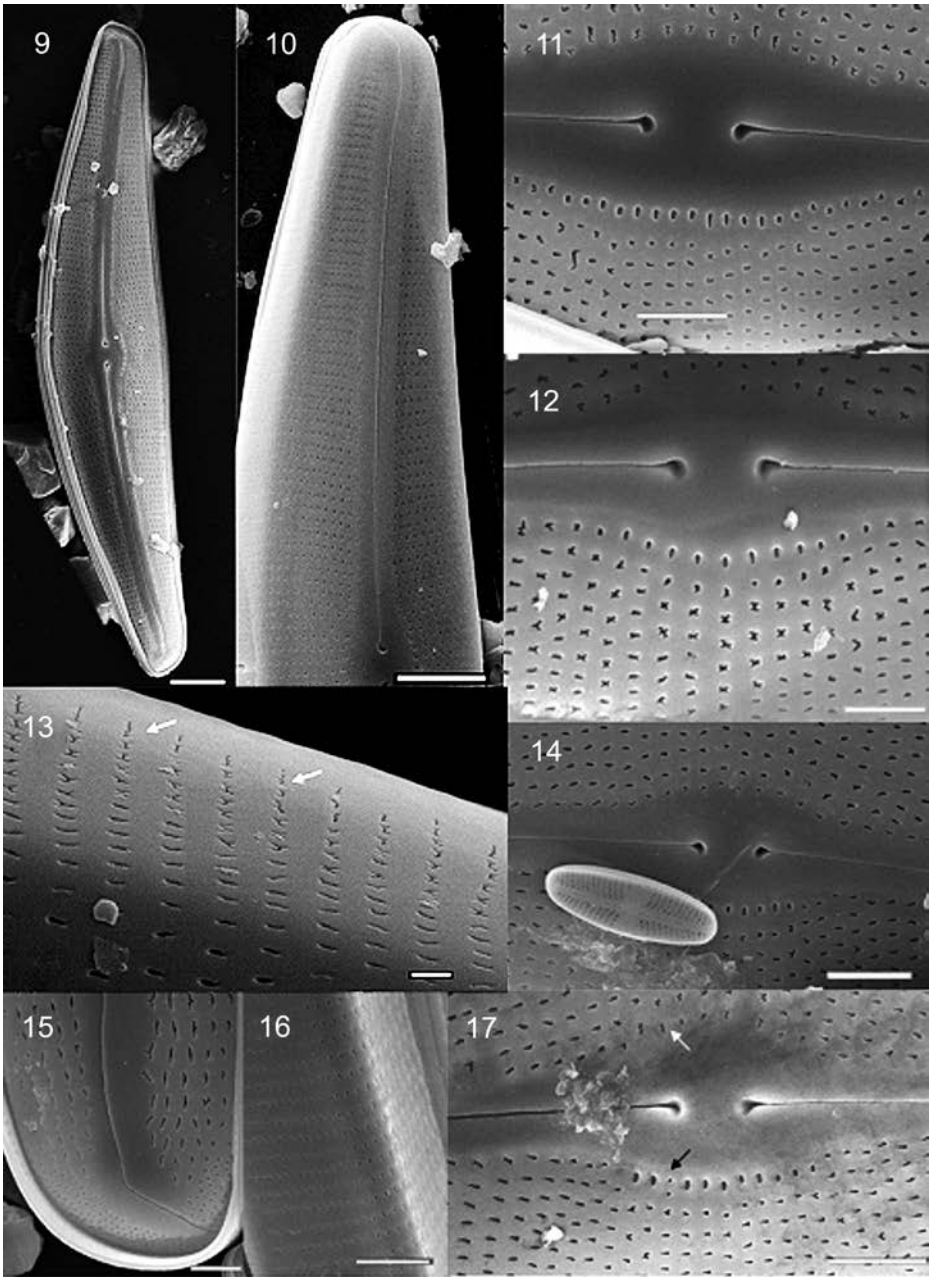
to dorsal side of valve. Dorsal striae radiate and slightly curved, 8–10 in 10 µm, areolae visible, 10–12 in 10 µm. Stigmata in line and formed by large pores on ventral side next to expansion of central area, and almost indistinct from areolae.

SEM description. External raphe slit rectilinear and sometimes undulated at poles and located on slightly elevated rib formed by thickened part of axial and central areas (Fig. 10). Proximal raphe endings form short drop-like shape and are

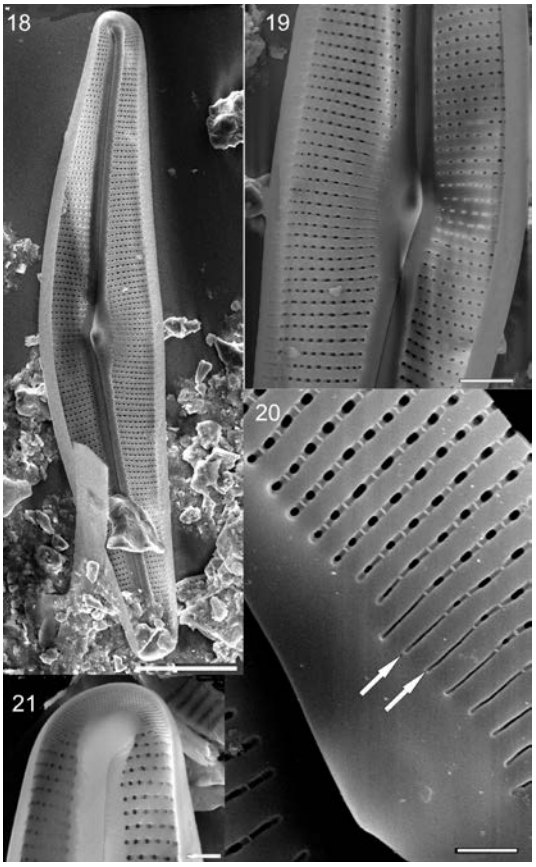
Table 2. Number of valves of *Cymbella loescherae* M. Garcia & D. B. Dutra, *sp. nov*. counted with whole coverslip scanning.

Locations/ Sampling date	(1) First-order stream 1	(2) Second-order stream 1	(3) Third-order stream 1	(4) First-order stream 2	(5) Third-order stream 2
January 2010	1	*	*	*	*
August 2012	18	2	*	*	*
November 2012	2	28	4	20	Not observed
March 2013	6	8	Not observed	2	Not observed
September 2013	36	10	Not observed	Not observed	Not observed
May 2014	1	1	2	1	2

* Samples were not collected on those dates.



Figs 9–17. External views of *Cymbella loescherae* M. Garcia & D. B. Dutra, *sp. nov*. in SEM 9 – External general view of frustule. 7 – Part of valve showing distal raphe bent dorsally. 11 & 12 – Central area with areolae in X, Y, I and zig-zag shapes on both dorsal and ventral valve sides. Note also the elliptical and round shape of stigmata. 13 – Detail of mantle showing the presence of areolae with X, Y and zig-zag shapes (indicated) next to mantle margin. 14 – Detail of another central area with areolae in X, Y and I shapes. Note raphe proximal ends slightly deflected to ventral side. 15 – Apex in detail showing large apical pore field and I- and flying-bird-shaped areolae. 16 – Mantle and valve face step with I-shaped areolae only. 17 – Detail of another valve center with areolae in X, Y, I and zig-zag shapes (white arrow) and one stigma subdivided in two (black arrow). Scales bars: 9 & 10 = 10 µm; 11, 12, 14, 16 & 17 = 5 µm; 15 = 2 µm; 13 = 1 µm.



Figs 18–21. SEM of internal views of *Cymbella loescherae* M. Garcia & D. B. Dutra, *sp. nov*. 18 – General internal view of valve. 19 – Central part of valve in internal view. 20 – Detail of apex. 21 – Central area showing long internal opening of stigmata (white arrows) and alveoli delimited by two struts. Note areolae internal opening in elliptical shape. Scales bars: 18 & 19 = 5 μm; 20 & 21 = 2 μm.

slightly deflected ventrally (Figs 11, 12, 14 & 17); distal raphe endings abruptly deflect dorsally (Figs 10 & 15). Large apical pore fields formed by small pores organized in rows (Fig. 21). Areolae apically oriented and stigmata transapically oriented (Figs 15 & 20). Areolae I-, X- and Y-shaped on central part of valve face (Figs 11, 12, 14 & 17), in I, X, Y and zig-zag shapes on mantle (Figs 13 & 16), and I and flying-bird shapes next to valve face apices (Fig. 15). Stigmata round, mainly slightly elongated and transapically oriented, numbering 9–14 on ventral side of valve next to central nodule. Very rarely subdivided into 2 small round stigmata (Fig. 17). Internally, between striae, large costae limiting rectangular alveoli separated by silica struts (Figs 18 & 21). Elliptical internal areolae opening inside alveoli (Figs 19 & 21). Stigmata alveoli found in narrow furrow (Fig. 21).

Etymology. Named to honor Waldemar Loescher (19 August 1935 – 11 November 2013) and his family, for their commitment to native forest preservation in Picada das Antas, São Lourenço do Sul city, Rio Grande do Sul State, Brazil.

Distribution. The distribution of *Cymbella loescherae* in the study area indicates that it is rather more frequent in first- and second-order streams, where the natural vegetation is better preserved, water pH ranges from 7.0 to 7.9 and conductivity between 73 and 95 $\mu S \cdot cm^{-1}$ (Tables 1 & 2). The distribution pattern observed at the studied sites may indicate it is becoming rarer in places subject to higher anthropopression, but more studies are needed to assess its ecological preferences.

Ecology. *Cymbella loescherae* was observed alive in all unprepared samples. It was never found during 400-valve counts, but while scanning whole coverslips, several valves were observed and enumerated as presented in Table 2.

Cymbella loescherae was the largest *Cymbella* found in the samples. It was frequently found in samples from bottom sediment, cobbles and pebbles. In the same samples, common diatoms were *Adlafia drouetiana* (R. Patrick) Metzeltin & Lange-Bert., *Navicula asymmetrica* Cleve, *Nupela praecipua* (E. Reichardt) E. Reichardt (abundant only at site 1) and *Achnanthidium minutissimum* (Kütz.) Czarn. The two first species listed were described in the first part of the 20th century by Patrick (1944) from streams in the northeast of Rio Grande do Sul State.

Discussion

Stigma morphology presents interesting and significant differences in the *Cymbella aspera* group. In *C. subhimalaspera* the stigmata are 1–3 times longer than the areolae, and in *C. peraspera* var. *colombiana* the stigmata are fine undulated slits about the same size as the areolae. *Cymbella*

Table 3. Comparative table of main features of *Cymbella loescherae* M. Garcia & D. B. Dutra, *sp. nov.* and similar related species.

Species/ Feature	*C. loescherae*	*C. aspera*	*C. amelieana*	*C. peraspera* var. *colombiana*	*C. himalaspera*	*C. subhimalaspera*
Valve length (µm)	117–170	110–200	160–270	163–228	150–280	104–133
Valve width (µm)	24.2–28.0	26–35	40–46	38–41	35–49	21–25
L/W ratio	4.2–5.6	5.7 (max.)	5.5–6.0	6 (max.)	4.1–5.6	4.9–5.8
Striae (10 µm)	8–10	6.5–8	7–8	5–8	7–9	8–11
Areolae (10 µm)	10–12	8–10	10–11	7–10	10–12	10–12
Areolae shape on valve face next to stigmata (externally)	X-, I-, Y-shaped	I-shaped	X-, I-shaped	X-shaped	X-, Y-, star-, tree-shaped	X-, I-shaped
Areolae shape on valve face next to stigmata (internally)	elliptical	elliptical	elliptical	–	round	round
Areolae shape on valve face next to apices	I-, flying-bird-shaped	I-shaped, round	slit-like, flying-bird-shaped	I-shaped	X-,Y-, star-, tree-shaped	I-shape, oval, flying-bird-shaped
Areolae shape on mantle at valve center	X-, I-, Y- shaped, zig-zag	–	–	–	–	–
Stigmata number and shape (relative size of stigmata in relation to areolae)	9–14, round, mainly slightly elongated (stigmata about same size as areolae)	7–10, slightly elongated (stigmata larger than areolae)	large number, small round (stigmata larger than areolae)	large number, fine undulated slits (stigmata larger than areolae)	large number, mostly round, slightly elongated (stigmata about same size as areolae)	8–11, elongate (stigmata larger than areolae)
Apical pore field	large number of small poroids	–	large, many small round pores	large number of small poroids	large number of small poroids	large number of small poroids
Proximal raphe ends	drop-like shape (short), slightly ventrally deflected	drop-like shape (short), slightly ventrally deflected	crook-shaped, almost entirely curved backwards	drop-like shape (elongated), slightly ventrally deflected	drop-like shape (elongated), slightly ventrally deflected	drop-like shape (short), slightly ventrally deflected
References	This paper	Krammer 2002; Van de Vijver & Lange-Bertalot 2008	Van de Vijver & Lange-Bertalot 2008	Krammer 2002	Jüttner *et al*. 2010	Jüttner *et al*. 2010

aspera and *C. amelieana* have stigmata larger than the areolae; *C. lanceolata* (C. Agardh) C. Agardh, on the other hand, has areolae larger than the stigmata (Van De Vijver & Lange-Bertalot 2008). In *C. subhimalaspera* they are larger than the areolae, while in *C. himalaspera* they appear to have areolae and stigmata of about the same size, as does *C. loescherae* (Table 3).

The shape of the areolae at the valve center, especially on the ventral side next to the stigmata, is an important character to distinguish species in the *C. aspera* group. *Cymbella himalaspera* is the species with higher diversity of areola shapes at the valve center.

Regardless of the form of the external openings of areolae, all present about the same morphology internally, and the internal areolae openings are distinct in the species of the *C. aspera* group. *Cymbella himalaspera* and *C. subhimalaspera* present the smallest ones (Jüttner *et al.* 2010), mainly round in shape, while in other species the internal openings are elliptical, occupying a larger area in the alveolus, as in *C. amelieana*, *C. aspera* and *C. loescherae.*

The species most similar to *C. loescherae* is *C. subhimalaspera*: both present about the same metric data set and have drop-like external proximal raphe endings which are slightly curved to the ventral side, although they are short drop-like in the former and elongated drop-like in the latter, and their external openings of the areolae show a distinct morphology. The most critical differences are in the shape of the stigmata, which are longer in *C. subhimalaspera* than in *C. loescherae*; this difference is associated with the high number of stigmata (9–14) in the latter. The number of subdivided stigmata also seems to be higher in *C. subhimalaspera*. Only one specimen illustrated in our Figure 19 presented such a feature. Furthermore, the areolae internal openings are elliptical in *C. loescherae* and round in *C. subhimalaspera*.

Conclusions

The new species belongs to the *C. aspera* group described by Krammer (2002) and Jüttner *et al.* (2010), characterized by areolae with a distinct morphology and a large apical pore field formed by very small round pores externally, and internally by robust costae, alveoli with struts, and 6 or more stigmata alveoli. The stigmata are slightly elongated, the areolae are round and small, and the proximal raphe endings are in long drop-like form.

Cymbella loescherae differs from the remaining species assembled in the *C. aspera* group by a group of distinctive features including the short elliptical shape of the stigmata, the elliptical internal areolae openings, and the diverse shapes of the areolae (X, I, Y, zig-zag), which are mainly located at the valve center on the dorsal and ventral sides.

The distribution in the study area suggests that *Cymbella loescherae* is rather more frequent at sites where the natural vegetation is better preserved (Tables 1 & 2). These results point to the need for more studies in such countryside areas. Its presence, even when rare, can serve as an indicator.

Cymbella loescherae is a peculiar species in well-preserved areas in the south of Brazil, found mainly in some first- and second-order streams (5 collecting stations) during four years of study. Its distribution is in accord with findings from other authors such as Van De Vijver and Lange-Bertalot (2008) and Jüttner *et al*. (2010), who have described large new *Cymbella* species from well-preserved areas of the world.

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