

Fine structure of two *Hygropetra* species, *Hygropetra gelasina* sp. nov. and *Hygropetra balfouriana* (Bacillariophyceae), and the taxonomic position of the genus with special reference to *Frankophila*

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SUMMARY

A small diatom, *Hygropetra gelasina* sp. nov., obtained from wet moss is described. This diatom is similar to *Hygropetra balfouriana* (Grunow ex Cleve) Krammer & Lange-Bertalot, which was found in the same moss sample. Fine structural observations revealed that *H. gelasina* has a reduced raphe slit and depressions along the margin of the axial area, at the proximal ends of the striae. Both species are characterized by multiple rows of areolae in each stria and a hexagonal pattern of sub-pores similar to that in *Pinnularia*, but differing in the position of the areola occlusions or hymenes, which are internal in *Hygropetra*. Comparison with *Frankophila*, which shares the characteristics of reduced raphe slits and areola structure with *Hygropetra*, provides a reference for future taxonomic study of these related genera.

Key words: *Frankophila*, *Hygropetra balfouriana*, *Hygropetra gelasina*, linking spine, moss diatom, reduced raphe.

INTRODUCTION

Frankophila Lange-Bertalot (1997) is an unusual genus characterized by a vestigial raphe slit with or without linking spines at the margin of the valve face. Among the various developments of the spines, the most elaborate bifurcate at the tips to form a chain of cells. Lange-Bertalot (1997) considered this genus a member of the Fragilariaceae; however, Lowe *et al.* (2006) stated that it should be transferred to a raphid order because of the presence of a raphe slit as well as the presence of loculate areolae.

Hygropetra Krammer & Lange-Bertalot (in Krammer 2000) is a small genus comprising two species; namely, *H. balfouriana* and *H. elongata* Krammer & Lange-Bertalot. It is similar to *Pinnularia* but differs in the position of the areola occlusions and the presence of

serrated valvocopula extensions. Krammer & Lange-Bertalot (in Krammer 2000) stated that the genus *Hygropetra* possesses some similarities to *Frankophila* but considered the longer raphe branches of *Hygropetra* a diagnostic characteristic.

We found *H. balfouriana* and a closely related species in a sample of Japanese moss growing on a rock sprayed by fall water and report the latter as a new species. We also discuss the taxonomical relationship between *Hygropetra* and *Frankophila*.

MATERIALS AND METHODS

Moss was collected from the Ryugaeshi Falls, Karuizawa, Nagano Prefecture, Japan, on 22 May 1987, leg. S. Mayama: TNS-AL-56392c (cleaned material), RM-003720 (= K-6496) (fixed material). Diatoms were detached from the surface of the moss by shaking in water (Mayama 1993) and boiled in sulfuric acid with potassium permanganate, followed by repeated washes in distilled water. Preparation methods of specimens for light and electron microscopy were the same as those described by Idei and Mayama (2001). Hitachi S-4000, JEOL JSM-5800 and ABT-32 microscopes were used for scanning electron microscopy (SEM), and a JEOL 200CX-II was used for transmission electron microscopy (TEM).

Additional SEM observations were carried out with samples of *Eunotia* and *Pinnularia* collected from Kori-dono Pond, Niigata Prefecture, Japan and Shimosu-eyosi Formation, Kanagawa Prefecture, Japan (Satake & Kobayasi 1991), respectively.

The terminology used here is compliant with Anonymous (1975) and Ross *et al.* (1979), except for 'virga' (Cox & Ross 1981), 'hymen' (Mann 1981; Cox 2004)

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and 'valve shoulder' (Kobayasi *et al.* 2006) instead of 'interstria', 'rica' and 'juncture of valve face and mantle', respectively.

RESULTS

The two *Hygropetra* species were found together in a sample with *Achnantheidium pusillum* (Grunow) Czarnecki, *Adlafia bryophila* (J. B. Petersen) G. Moser, Lange-Bertalot & Metzeltin, *Diademsis biceps* Arnott, *Diademsis contenta* (Grunow) D.G. Mann, *Diademsis gallica* W. Smith, *Navicula oppugnata* Hustedt, *Nupela lapidosa* (Krasske) Lange-Bertalot, *Orthoseira epidendron* (Ehrenberg) H. Kobayasi, and *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot, many of which prefer subaerial environments.

Hygropetra gelasina Mayama & M. Idei, sp. nov. (Figs 1–14, 23–26)

Descriptio: Frustula rectangulata in aspectu cingulari. Valvae ellipticae vel late lanceolatae, apicibus obtuse rotundatis, 5–8.5 µm longae, 3–5 µm latae. Raphe filiiformis rudimentalis. Area axialis anguste lanceolata, depressionibus externis ad basem striarum, area centralis non separata. Striae transapicales radiantes per valvae, circiter 10 in 10 µm.

Holotypus: TNS-AL-56392sa (National Museum of Nature and Science).

Isotypi: TNS-AL-56392sb and TNS-AL-56392sc.

Type locality: Ryugaeshi Falls, Karuizawa, Nagano Prefecture, Japan.

Etymology: The epithet *gelasina* was chosen because of the peculiar depressions on the valve face.

Frustules rectangular in girdle view (Fig. 25). Valves elliptic to widely lanceolate with bluntly round apices, 5.0–8.5 µm long, 3–5 µm wide (Figs 1–13). Raphe filiiform and rudimentary. Axial area narrowly lanceolate with external depressions at the proximal ends of the striae. Striae radiate through the valve, approximately 10 in 10 µm.

SEM observations

Externally, the valve face is flat, bearing several clearly defined depressions, which vary in shape and size (Figs 9–11). They are distributed at the margin of the lanceolate axial area; one for each stria. The bottom of the depression is somewhat granulate or rugose, with neither an inner opening nor a velum-like occlusion. The length of each raphe branch is almost one-third to one-fifth of the valve length (Figs 6–13). The measurements of the actual branch lengths show a tendency to become longer in smaller valves. Raphe branches are straight and subpolar in both halves of the valve with slightly deflected terminal fissures. Externally, both

central and terminal endings are often somewhat expanded to form a slightly pore-like structure (Figs 9–11). Internally, the raphe branches are also simple; the central endings are straight or slightly hooked, and the terminal endings are straight (Figs 12, 13, 26). The inner raphe fissure is not accompanied by an axial rib, central nodules, or helictoglossae (Fig. 26). Each stria is bounded by short robust virgae (Figs 12, 13, 24), comprising two to four rows of areolae, which extend from the axial area to the valve mantle over a somewhat rounded valve shoulder (Figs 23, 25). The outer openings of the areolae are circular and slightly smaller than the inner openings (compare Figs 23, 24), which are occluded internally by a scarcely domed hymenes (Fig. 24) with regularly arranged perforations (Fig. 14). The striae end in the middle of the mantle (Fig. 25). The mature cingulum is composed of at least two split bands; namely a valvocopula and a pleura (Figs 25, 26). The valvocopula is wide and has a single row of slit-like foramina along the advalvar edge of the pars exterior, whereas the pleura is very narrow and plain. Semicircular serrations extend from the pars interior of the valve copula, lying over the robust virgae for about half their length (Fig. 26).

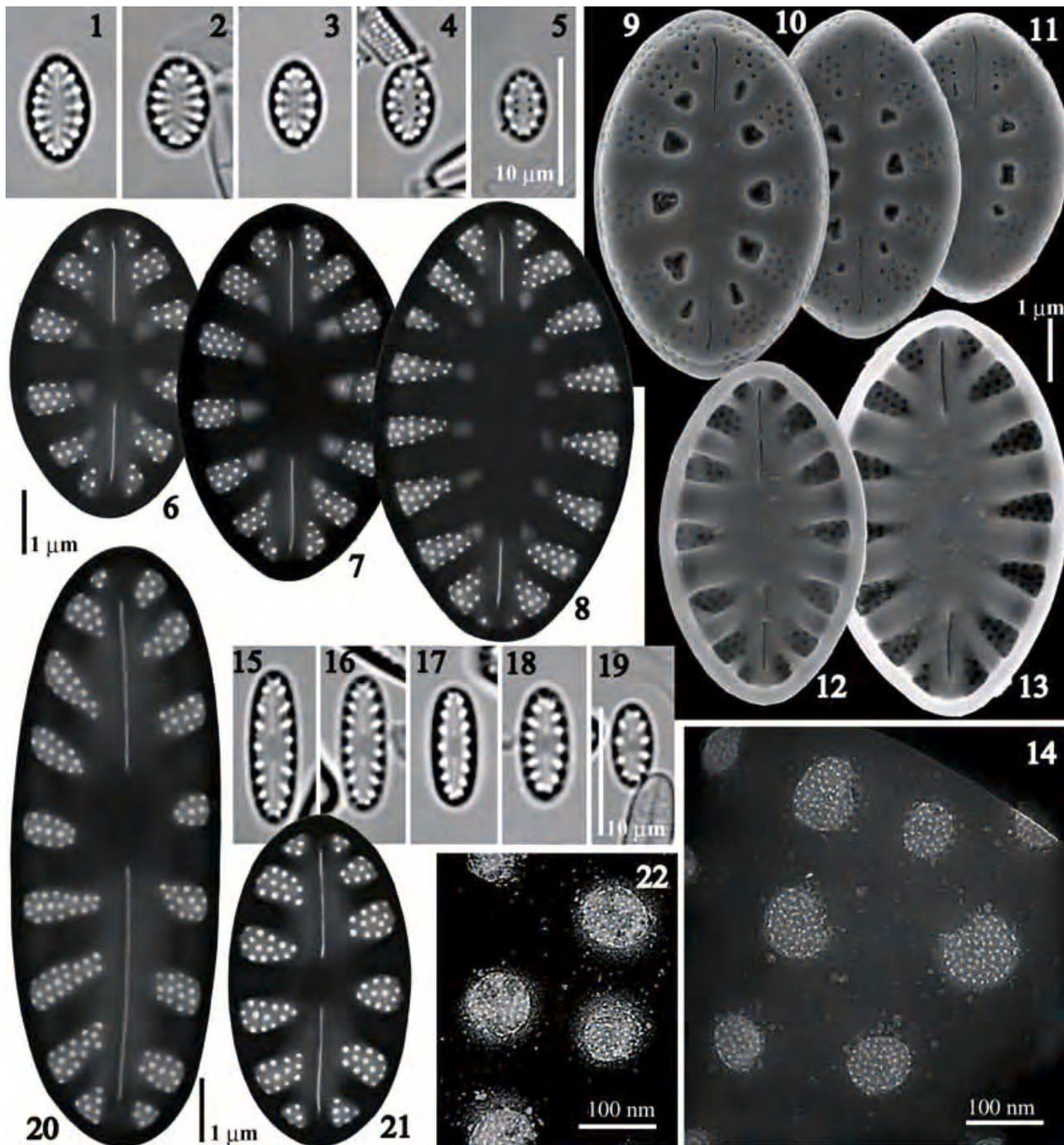
Hygropetra balfouriana (Grunow ex Cleve) Krammer & Lange-Bertalot (Figs 15–22, 27–34)

Basionym: *Pinnularia balfouriana* Grunow ex Cleve, P. T. 1895. K. Svenska Vet.-Akad. Handl. **27**(3): 80. pl. 1. f. 18.

Frustules rectangular in girdle view (Fig. 31). Valves elliptic to narrow elliptic, 6.5–13.0 µm long, 3.0–3.5 µm wide (Figs 15–21, 27–30). Raphe filiiform with slightly expanded central pores. Axial area narrow with a scarcely swollen central area. Striae radiate through the valve, approximately 10 in 10 µm.

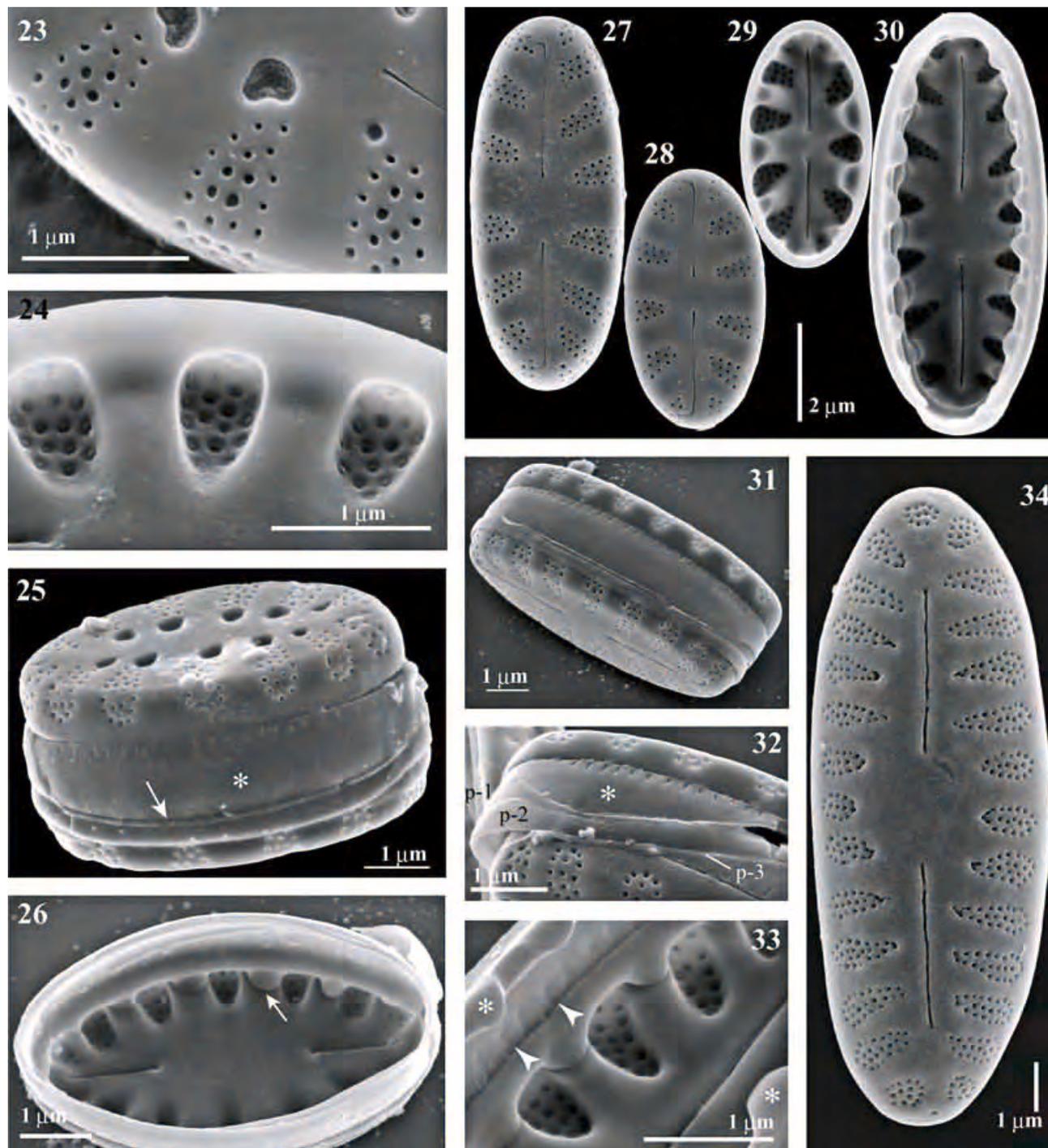
SEM and TEM observations

Externally, the valve face is flat with a narrow axial area, continuing smoothly to the mantle over a rounded valve shoulder (Fig. 31). The raphe branches have slightly expanded central endings, and bend toward the same side at both poles (Figs 27, 28). Internally the raphe fissures are straight with deflected (Fig. 30) or almost straight central endings (Fig. 29). The helictoglossa is slight or barely discernible (Figs 29, 30). Striae consist of two to four rows of areolae (Figs 27, 28, 31, 32) placed between thickened virgae (Figs 29, 30, 33). Each stria narrows toward the axial area but is broadly rounded where it ends on the valve mantle (Figs 31, 32). Hymenes with regularly arranged perforations (Fig. 22) are located near the inner openings of the areolae (Fig. 33). The epicingulum is composed of four bands,



Figs. 1–14. *Hygopetra gelasina*. 1–5. Light micrographs. 6–8. Transmission electron microscopy (TEM) photographs showing valves with short raphe branches. Note gray spots arranged along the margin of axial area, which reflect depressions on the valve face. 9–11. External view of the valve face with depressions at the proximal ends of the striae. Scanning electron microscopy (SEM). A step is often seen in the valve mantle (9). 12,13. Internal view of the valve face showing simple and short raphe branches and longitudinally elongated central area. 14. Detail of hymens showing regularly scattered perforations.

Figs. 15–22. *Hygopetra balfouriana*. 15–19. Light micrographs. 20,21. Transmission electron microscopy (TEM) photographs showing narrow axial area. 22. Hymens with perforations scattered regularly.



Figs. 23–26. Scanning electron microscopy (SEM) photographs of *Hygropetra gelasina*. 23. Enlarged valve surface showing indeterminate forms of the depressions and external openings of areolae. 24. Internal oblique view of the valve showing areolae occluded by hymens. 25. Oblique view of the frustule. Note the wide valvocopula (*) with a single row of slits and the narrow pleura (arrow). Hypovalve shows the prominent step in the valve mantle. 26. Internal oblique view of the theca showing the serrated pars interior of the valvocopula (arrow).

Figs. 27–34. *Hygropetra balfouriana*. Scanning electron microscopy (SEM). 27,28. External view of the valve face. 29. Internal view of the valve face. 30. Internal view of the frustule without the upside valve showing the serrated valvocopula. 31. Oblique view of the frustule. 32. Detail of the epicingulum composed of the wide valvocopula (*) and three narrow pleurae (p1–3). 33. Internal oblique view of the hypotheca accompanied by the epivalvocopula (*). Serrations of pars interior of the hypovalvocopula partly lying over the robust virgae. Note the internally occluded openings of the slits in the valvocopula (arrowheads). 34. External view of the initial cell showing the domed valve with the simple and straight raphe slits.

namely, a broad valvocopula with a single row of short slits and three narrow pleurae without any marking (Fig. 32). The inner openings of the slits are somewhat wider and occluded by hymenes (Fig. 33 arrowheads). The pars interior of the valvocopula has serrations that overlap part of the inner faces of the virgae (Figs 30,33). The initial valve is elongate and domed, without any distinction between the valve face and mantle, bearing short raphe branches (Fig. 34). The raphe slits terminate a short distance from the poles.

DISCUSSION

The vegetative valves and copulae of *H. balfouriana* that we observed were generally quite similar in fine structure to those shown by Krammer & Lange-Bertalot (in Krammer 2000). We found that the velum of *Hygropetra* was a hymen with regularly scattered perforations, as seen in some *Pinnularia* species (Idei & Mayama 2001). We also noted the hexagonal or quincunx areolation, which is typical in *Pinnularia* and caused by a half pitch shift of areolation between adjacent rows. Krammer & Lange-Bertalot (in Krammer 2000) said that *Hygropetra* was 'distinguished from *Pinnularia* by not honeycomb-like arranged areolae', but their SEM photographs also show a roughly hexagonal areola arrangement. These characteristics are shared by both genera; however, the genera can still be differentiated by the obscure terminal fissure, the position of the hymenes and the presence of 'septate' valvocopula, as mentioned by Krammer & Lange-Bertalot (in Krammer 2000), although the character of the band should not be called septa in the definition of the term (Anonymous 1975). Some *Pinnularia* species have a single row of slit-like or elongated poroids in the valvocopula, which is similar to that of *Hygropetra*. However, the location is different in *Pinnularia*, in which the row is arranged along the middle of the pars exterior (Round *et al.* 1990, Krammer 2000, Idei & Mayama 2001).

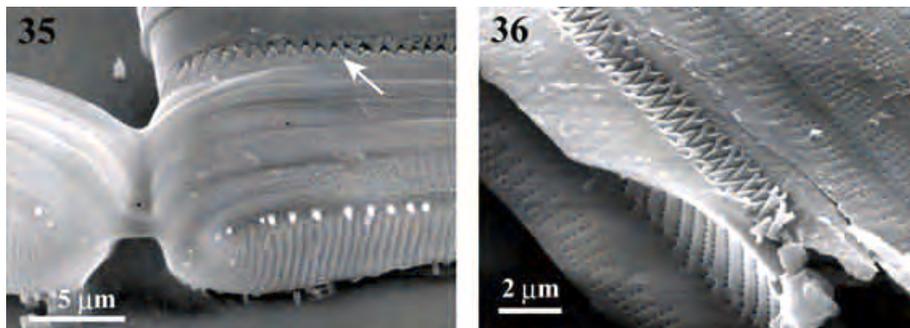
The fine structure of *H. gelasina* resembled that of *H. balfouriana*, except for the depressions in the external valve face and shorter raphe branches. The presence of the depressions aligned along the margin of the axial area are easily recognizable in light microscopy as white or black dot images (Figs 1–5) and distinguish this taxon from *H. balfouriana*. Similar depressions are found in some *Chamaepinnularia* species, that is, *Chamaepinnularia* sp. (Siver *et al.* 2005), *C. vyvernianii* Lange-Bertalot & Krammer, and *C. rhombelliptica* Lange-Bertalot (Werum & Lange-Bertalot 2004), in which each depression is located adjacent to the start of a stria. *Chamaepinnularia* have vela (hymenes) at the external openings of the areoli, the bottom of the depression is not velate but a solid siliceous layer, which is the same as in *H. gelasina*. However, each stria

in *Chamaepinnularia* is occluded by a single transversely elongated velum, and *Chamaepinnularia* species have evident terminal fissures; therefore, they are distinct from *Hygropetra*. Similar depressions are also seen in *Eolimna silvahercynia* (Lange-Bertalot) Lange-Bertalot (Werum & Lange-Bertalot 2004) and *Lacunicula sardiniensis* Lange-Bertalot *et al.* (Lange-Bertalot *et al.* 2003), although Morales and Le (2005) proposed the transfer of the latter to the genus *Craticula*. The depressions in *E. silvahercynia* are located in the axial area as in *Hygropetra*, although it is difficult to determine from figures in Werum and Lange-Bertalot (2004) whether the bottom is solid or hymenate. In *Eolimna*, occlusions also lie at the internal openings of the areolae, but, the striae are composed of single rows of areolae, and the raphe slits have distinct terminal fissures, allowing our species to be placed in a separate genus. *Lacunicula* also have prominently characteristic holes and/or depressions on the external valve face beside the axial area. However, they seem to be a modified conopeum. In addition, the fossil genus *Rouxia* has a primitive or 'degenerate' raphe with external depressions along the axial area but differs by the presence of large elongate areolae excluding *R. antarctica sensu* Schlader (Jordan 2001).

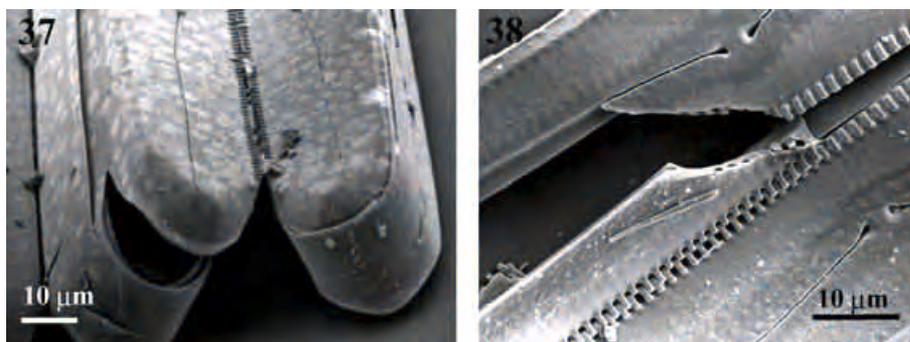
The depressions of *Hygropetra* are located at the proximal ends of each stria. Because virga formation in pennate genera precedes stria development (Chappino & Volcani 1977, Mayama & Kobayasi 1989, Cox 1999), the depressions cannot be part of the sternum but are presumably spaces between virgae in the early stage of valve ontogeny that are subsequently filled in. The space seems to be filled only internally as the axial area develops, as seen in the valve formation of some other biraphid diatoms (Cox 2001).

Except for the depression, the whole valve of *H. gelasina* is quite similar to the spine-less valves of *Frankophila similoides* Lange-Bertalot & Rumrich (the type of the genus) and *Frankophila loetschertii* (Lange-Bertalot) Lange-Bertalot (1997). These species have relatively longer vestigial raphe branches than other *Frankophila* species, although the length varies depending on the specimen. A hexagonal array of areolae, which is observed in *Hygropetra*, is also seen in *F. similoides* and *F. loetschertii*, sometimes with triple rows of areolae (Lange-Bertalot & Le Cohu 1985; Lange-Bertalot 1997). In pennate diatoms, this areola arrangement is only seen in raphid diatoms. In some araphid diatoms, the siliceous mesh sheet, which occludes transapically elongated areolae, shows well-arranged rows of poroids, but they are aligned perpendicular to the virgae, for example, *Punctastriata* (Williams & Round 1987; Idei & Nagumo 1995). This supports the opinion that *Frankophila* should be removed from the Fragilariaceae and placed in a raphid order (Lowe *et al.* 2006).

Figs. 35,36. *Eunotia rabenhorstiana*. Scanning electron microscopy (SEM). 35. Resting cells (right) connected with a vegetative cell (left) by mucilaginous pad. Note two resting cells linked with interdigitating spines (arrow). 36. Partly broken resting cells showing bifurcations of linking spines.



Figs. 37,38. *Pinnularia debesii*. Scanning electron microscopy (SEM). 37. Zigzag colony formed by linking spine. 38. Enlargement of the interdigitating spines placed on one side of the valve shoulder.



The valvocopula with serrated extensions is present not only in *Hygropetra* but also in *Frankophila*. A series of slit-like openings in the pars exterior of the valvocopula has also been reported in *F. similoides*.

Krammer & Lange-Bertalot (in Krammer 2000) have briefly commented on the similarity between *Hygropetra* and *Frankophila*, but they distinguished the genera on the basis of differences in raphe branch length. Finding a short raphe in *H. gelasina* leads us to re-consider the synonymy of the two genera.

We have observed neither linking spines nor spinules on the valve shoulders of *Hygropetra*. Valves without spines were observed in some specimens of *F. similoides* and *F. loetschertii* (Lange-Bertalot & Le Cohu 1985; Lange-Bertalot 1997), and valves with spinules, which do not function to form chains, were observed in *F. maillardii* (Le Cohu) Lange-Bertalot (Lange-Bertalot & Le Cohu 1985), *F. similoides* (Lange-Bertalot 1997), and *F. horstii* Rumrich & Rumrich (Rumrich *et al.* 2000). However, previously published images of *Frankophila* valves were either single valves, or valves on one side of a frustule with well-developed spines present on the other side. In other words, frustules without any spines have not yet been reported. Frustules comprising two types of valves; namely a linking valve and a separation valve, are found in several genera, for example, *Aulacoseira* and *Skeletonema*. However, there is no further information on different character states of the spines in *Frankophila*.

Interdigitating spines are an ancestral characteristic found in both centric and araphid diatoms but is

unusual in raphid diatoms. The bifurcated linking spines of *Frankophila* may indicate its close relationship to the Fragilariaceae as mentioned by Krammer & Lange-Bertalot (in Krammer 2000). However, similar spines are present in the resting cells of *Eunotia rabenhorstiana* (Grunow) Hustedt (Figs 35,36), although the vegetative valves have only spinules. Moreover, vegetative cells of *Pinnularia debesii* Hustedt have interdigitating spines on one side of the valve shoulder, thereby forming a zigzag colony (Figs 37,38), previously reported from light microscopy observations by Hustedt (1926) as 'Verbindungsstücke'. Thus, linking spines are not a missing characteristic in raphid diatoms.

Possession of linking spines may be a characteristic that defines certain genera. Many species of *Staurosira* and *Pseudostaurosira* usually have apically expanded or bifurcated linking spines (Idei & Nagumo 1995); however, some *Fragilaria* species, for example, *F. vaucheriae* (Kützinger) J. B. Petersen and *F. capitellata* (Grunow) J. B. Petersen, are usually found solitary without linking spines, but with less well-developed spinules (Lange-Bertalot 1980; Kobayasi *et al.* 2006). *Staurosirella* includes several spineless species (Morales & Edlund 2003; Morales 2005; Morales & Manoylov 2006). Conversely, while common *Ulnaria* species have no linking spines, *U. pseudogaillonii* (H. Kobayasi & M. Idei) M. Idei has interdigitating spines and forms chains, although the other characteristics of this diatom correspond well with the usual characteristics of *Ulnaria* (Kobayasi & Idei 1979). This indicates that *Frankophila* is also not strictly defined by the possession of linking spines.

The raphe of *H. gelasina* is simpler than that of *H. balfouriana*, but still remains visible under light microscopy. On the other hand, raphe slits in *Frankophila* are more reduced or underdeveloped, difficult to recognize in the light micrographs of previous studies. Thus, the raphe slit structure seems to be much simpler in *Frankophila*. Raphe character states vary among raphid diatoms but are generally considered to be stable within a species and are useful criteria for classification of many taxa. However, some exceptional genera are also reported. *Nupela* is a genus composed of various species in which raphe branches vary from full or almost full length, to a complete lack of branches (Lange-Bertalot & Moser 1994; Metzeltin & Lange-Bertalot 1998; Lange-Bertalot & Genkal 1999; Monnier *et al.* 2003; Potapova *et al.* 2003; Kobayasi *et al.* 2006). *Diadsmis* also shows reduced raphe and raphe-less valves in field samples as well as in cultures (Granetti 1977, 1978; Lange-Bertalot 1980; Cox 2006). The reduction in the raphe branches progresses toward the apices in the above genera. This direction of reduction is also observed when comparing *H. balfouriana* and *H. gelasina*. Although we did not observe an extremely reduced raphe among our *H. gelasina* specimens, it is possible that the present range in slit length will be increased by finding shorter raphes in the genus if hitherto unknown species are identified, or the genus is combined with the other related genera.

In raphid diatoms, which acquired mobility during evolution, raphe reduction and the facultative raphe loss and spine development are intriguing phenomena. Cox (2006) hypothesized that colony production in *Diadsmis* may be an adaptation that allows the terrestrial species to grow submerged in a liquid medium rather than on damp surfaces, or to grow suspended under static conditions rather than on surfaces under flowing water. We add that colony formation in a liquid medium also allows the population to develop three-dimensionally. This is an advantage for the uptake of nutrition. However, the spatial development of the colony is difficult in subaerial conditions because the colony is affected by gravity and drying. Our specimens of *Hygropetra* were not collected from under running water but from the moss in misty sprays of waterfalls, whereas the specimens of *Frankophila* were obtained from not only subaerial environments but also shallow pool, lakes, and running rivers (see Lowe *et al.* 2006, tab 1). The records of the habits of *Hygropetra* and *Frankophila* may support the above-mentioned hypothesis, but further information from fields and a demonstration experiment are also required.

The taxonomic positions of *Hygropetra* and *Frankophila* within the raphid diatoms are still uncertain; however, the hexagonal arrangement of the areolae with the hymens present internally, which is observed in *Hygropetra*, may imply a close relationship with *Plan-*

othidium, which shares the same characteristics (Round & Bukhtiyarova 1996; Kobayasi *et al.* 2006). In recent phylogenetic trees (Bruder & Medlin 2008), the members of the monophyletic group that includes *Plan-othidium* contain a single plastid, although the shape of the plastid varies among the genera. Thus, *Hygropetra* can also be expected to have a single plastid, if its inclusion into this clade is verified.

In the present study, we could not find a conclusive reason to separate *Hygropetra* from *Frankophila*. However, we should be careful to synonymize them because all SEM photographs in the early studies of *Frankophila* do not show the vela in the areolae. Only one specimen showed a velum-like structure in an internal view of the valve (Lange-Bertalot & Le Cohu 1985, Fig. 32). Even though they are true vela, it is difficult to determine whether they are hymenes or volae. Detailed observations of the areola occlusion of *Frankophila* accompanied by plastid and molecular studies are needed to confirm the classification of this and related genera.

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