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Re-discovery of *Lieberkuehnia wageneri* Claparède *et* Lachmann, 1859 (Rhizaria, Foraminifera): Taxonomical and Morphological Studies Based on a Slovak Population

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Summary. *Lieberkuehnia wageneri* Claparède *et* Lachmann, 1859, a poorly known freshwater foraminiferan, was re-discovered after 38 years in a moss sample from an oak-hornbeam forest in Malé Karpaty Mts. (Western Slovakia). The species was taxonomically revised, re-described using light microscopical observations, and its occurrence and ecology were reviewed. Its locomotive form is typical with membranous and flexible ovoid test having a single terminal aperture from which a broad pseudopodial network emerges on a pseudopodial peduncle. Additional data and details are described, illustrated and discussed. *Lieberkuehnia wageneri* is known from various freshwater, soil and marine habitats in Western and Middle Europe, suggesting a high ecological tolerance and wide distribution. It is hoped that the observations reported in this paper will aid the identification of this species based on the locomotive morphology at the light microscopical level.

Key words: Alpha taxonomy, Foraminifera, nomenclature, moss, amoebae.

INTRODUCTION

The majority of representatives of Foraminifera are typically marine protists and few genera have been described from freshwater and soil environments (Lee *et al*. 2000, Meisterfeld *et al*. 2001). Species of the genus *Lieberkuehnia* Claparède *et* Lachmann, 1859 are marine, freshwater and soil foraminiferans (Rhizaria, Foraminifera) characterized by an ovoid membranous test with a smooth surface and a single aperture from which arise anastomosing pseudopodia on a short internal prolongation of the cytoplasm (Claparède and Lachmann 1859, Penard 1902, Cash *et al*. 1915). Only two species are known from freshwater or semiaquatic habitats: *Lieberkuehnia wageneri* Claparède *et* Lachmann, 1859, which has been reported several times in the past, and *Lieberkuehnia paludosa* Cienkowski, 1876, which is a rare species. Since the initial description of *Lieberkuehnia wageneri* by Claparède and Lachmann (1859), it was recorded and investigated several times around the turn of the 19th and early 20th centuries (Siddal 1880; Penard 1902, 1907, 1909; Cash *et al*. 1915). Detailed list of these early publications are given by Cash *et al*. (1915). Subsequent publications, mainly monographs or handbooks, have often merely adopted descriptions and illustrations from the earlier works (Doflein 1916,

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Schoenichen 1927, Bartoš 1954, Kudo 1966, Taylor and Sanders 2001). Among them, the short film with accompanying text by Netzel (1971), who documents the locomotion and reproduction of this species, stands out. After that, no any further morphological information or illustrations were published in spite of many years of research on freshwater and soil protists worldwide.

The present study reports the re-discovery of *Lieberkuehnia wageneri*, a rare and poorly-described species. The nomenclature and taxonomy of this species are revised, new data on the morphology of the locomotive form based on light microscopy are presented, and its occurrence and ecology are reviewed.

MATERIALS AND METHODS

During a study (2000–2002) of amoeboid protists in terrestrial habitats in the oak-hornbeam forests of Malé Karpaty Mts. (Western Slovakia), a total of 243 samples of soil and mosses were collected monthly from five localities. Amongst them, *Lieberkuehnia wageneri* was found in a single sample of moss grown on soil collected from the Lošonec-lom quarry (17°23′E, 48°29′N) on 5th of June 2001. This locality has a typical forest cover composition with *Quercus dalechampii*, *Q. cerris*, *Carpinus betulus*, *Crataegus monogyna*, *Acer campestre* and *Prunus spinosa*. The age of the forest has been estimated to be about 80–100 years. The soil in the sampling locality was a rendzina with a pH of 6.55, a carbon content of 2.7%, a nitrogen content of 0.29%, and a maximum humus content of 4.65%. The locality is situated just above the quarry and is affected by carbonate dust. This is reflected in the higher pH value compared with the other localities under investigation. For more details of the ecological characteristics of the locality, see Zlinská *et al*. (2005).

After transportation to the laboratory, the samples were left to dry. A modified method used for investigating soil ciliates (Foissner 1987, Aescht and Foissner 1995, Vďačný and Tirjaková 2006) was used to analyse the dry sample material. In brief, dried moss was placed in a Petri dish, saturated with distilled water and incubated for 5 days at laboratory temperature under undirected light. Amoebae were examined in a subsample of the suspension pipetted from the saturated sample, using a Nikon Labophot microscope equipped with phase contrast optics.

RESULTS AND DISCUSSION

Lieberkuehnia wageneri Claparède *et* Lachmann, 1859

1859 *Lieberkuehnia Wageneri* Claparède *et* Lachmann, *Mém. L' Inst. Genèvois* **6:** 465

1880 *Lieberkühnia Wageneri* – Siddal, *Q. J. Microsc. Sci.* **78:** 141 (morphological study, feeding behaviour)

1882 *Lieberkuehnia* – Maupas, *C. R. Acad. Sci. (Paris)* **45:** 191 (morphological study, feeding behaviour, reproduction)

1889 *Lieberkühnia Wageneri* – Verworn, Psychophysiologische Protistenstudien, p. 23 (feeding behaviour)

1902 *Lieberkühnia Wagneri* Claparède *et* Lachmann – Penard, Faune rhizopodique du bassin du Léman, p. 552 (morphological study)

1907 *Lieberkühnia wageneri* Clap. *et* Lachm. – Penard, *Arch. Protistenkd.* **8:** 225 (morphological study, reproduction and comparison with *L. paludosa*)

1909 *Lieberkühnia Wageneri* Clap. *et* Lachm. – Penard, *Arch. Protistenk.* **17:** 265 (comparison and proposed synonymization with *Gromia fluviatilis* Dujardin, 1841 and *G. terricola* Leidy, 1874)

1915 *Lieberkuehnia wageneri* Claparède & Lachmann – Cash, Wailes and Hopkinson, British freshwater Rhizopoda and Heliozoa. Vol. 3, p. 133 (description, list of literature)

1954 *Lieberkühnia wageneri* Claparède a Lachmann, 1859 – Bartoš, Rhizopods of the order Testacea, p. 172 (adopted description, habitats)

1971 *Lieberkühnia wageneri* Claparède & Lachmann – Netzel, Encyclopaedia Cinematographica, p. 3 (morphological study of German population, feeding behaviour, reproduction)

Nomenclature: Claparède and Lachmann (1859) named this species *Lieberkuehnia wageneri* as a tribute to two scientists Lieberkühn and Wagener who found and illustrated a specimen of this foraminiferan. As the Latin language does not include "ü", the vowel was transliterated as "ue" in the genus name. Several years after the type description, most published works referring to this species incorrectly spelled the genus name with the vowel "ü", *i.e*., *Lieberkühnia wageneri* (Siddal 1880, Penard 1907, Schoenichen 1927, Bartoš 1954, Netzel 1971). In other publications, even the species name was incorrectly spelt; the vowel "e" was omitted, changing the name to *Lieberkühnia wagneri* (Penard 1902, Doflein 1916, Kudo 1966). These spelling mistakes were noted for the first time by Cash *et al*. (1915) who provided a list of different variants of the name and pointed out the correct spelling of the genus name. In recent publications, the genus name is usually correct, although not always the species name (Meisterfeld *et al*. 2001, Taylor and Sanders 2001). However, according to the Principle of Priority and the Formation and

treatment of names in the International Code of Zoological Nomenclature (Chapters 6 and 7, respectively) these spelling mistakes are characterized as "incorrect subsequent spellings" (ICZN 1999), and the sole correct name of this species is that introduced by Claparède and Lachmann (1859).

Improved diagnosis (includes all data available)**:** Test flexible and membranous with ovoid, ellipsoidal or pyriform shape, length 32–900 μm. Single terminal aperture with internal septum separating pseudopodial peduncle from the internal cytoplasm. Large pseudopodial network, 350–1800 μm and more in extent, emerging from the peduncle and from the thin layer of cytoplasm enveloping the test. Cytoplasm sometimes yellowish or greenish, multinucleate, with 30–200 vesicular nuclei with up to three nucleoli. Numerous contractile vacuoles and food vacuoles. Reproduction by binary and multiple fission.

Habitat and distribution: Fresh water in Berlin (Germany), fresh water aquarium in Tübingen (Germany), in the Lake Geneva (Switzerland), on algae in freshwater and marine habitats in United Kingdom and Ireland, in submerged mosses near Geneva (Switzerland) and moss in Malé Karpaty Mts. (Slovakia).

Remarks: Differs from another freshwater species *Lieberkuehnia paludosa* Cienkowski, 1876 in the appearance of cytoplasm and the number and type of nuclei. In *L. paludosa* the cytoplasm is opaque and filled with many small granules and possesses a single nucleus or up to 30 nuclei with numerous endosomes.

Type locality: Unknown locality in Berlin, mentioned by Claparède and Lachmann (1859) only as a fresh water sample from Berlin, Germany, 13°24′E, 52°31′N.

Type material: Plate 23 in Claparède and Lachmann (1859). No slides or cultures of the species are available.

Description of Slovakian isolate: Three specimens were examined. The test was ovoid or ellipsoidal, smooth, membranous, flexible, thin and transparent, so cytoplasm was clearly visible through it. The length of the test was 66–70 μm, breadth 47–52 μm. The single aperture was clearly visible and its position was terminal. The colourless cytoplasm of the cell body was continually streaming or circulating. It was finely granular with about 30 spherical indistinct nuclei, about 4 μm in diameter, each one having a central nucleolus. There were up to three contractile vacuoles localized in various parts of the cell. From the cell body, a bent peduncular pseudpodial trunk, which was connected asymmetrically with the body, projected outwards. The trunk was separated from the internal space of the test by a distinct septum about 20 μm long. From the pseudopodial peduncle arose a thin layer of cytoplasm partially enveloping the external surface of the test near the aperture, where it also formed an expanded base for the pseudopodial network. The pseudopodia emerged mainly from the pseudopodial trunk but also from various parts of the cytoplasmic coat on the test (Fig. 1). Pseudopodia were 56–181 μm long, slightly broader at the base, of irregular shape, for most of their length very thin, and occasionally displaying areas of broader lamella-like cytoplasm. They showed the typical rapid bidirectional streaming of fine granules that were clearly visible with phase contrast optics. The lamelliform parts of pseudopodia were up to 30 μm long and were filled with granular cytoplasm, containing 1–3 small vacuoles or vesicles, probably food vacuoles. Numerous branching and anastomosing pseudopodia with many connections formed a broad pseudopodial network of circular shape around the test. The diameter of this network was about 350 μm (Fig. 1). Neither the resting stages nor the reproduction was observed.

Occurrence and ecology: *Lieberkuehnia wageneri* is known from freshwater, semiaquatic, soil and also marine habitats. In the present study, it was observed in moss grown on soil from near the Lošonec-lom quarry in June 2001 (Malé Karpaty Mts., Western Slovakia). The species was initially found in a fresh water sample from Berlin, Germany (Claparède and Lachmann 1859). Later, it was found in the Lake Geneva, Switzerland (Penard 1902) and in submerged mosses in the vicinity of the city of Geneva (Penard 1907, 1909). Although Bartoš (1954) did not find *L. wageneri* in the former Czechoslovakia, he mentioned it as a potential species on water plants, in sapropel, aerophytic mosses, soil and brackish waters. Netzel (1971) found this species on *Myriophyllum* in an outdoor aquarium in Tübingen, Germany. In marine settings, it was noted near Tenby (SW Wales, United Kingdom) by Siddal (1880), and Cash *et al*. (1915) reported it on algae from freshwater and marine habitats in the United Kingdom and Ireland. These records of *L. wageneri* in a wide variety of habitats suggest a high ecological tolerance and indicate its ubiquity. This is also supported by Netzel's (1971) experiment in which specimens isolated from freshwater survived in a mixture of sea water and demineralized water (ratio 63 : 37).

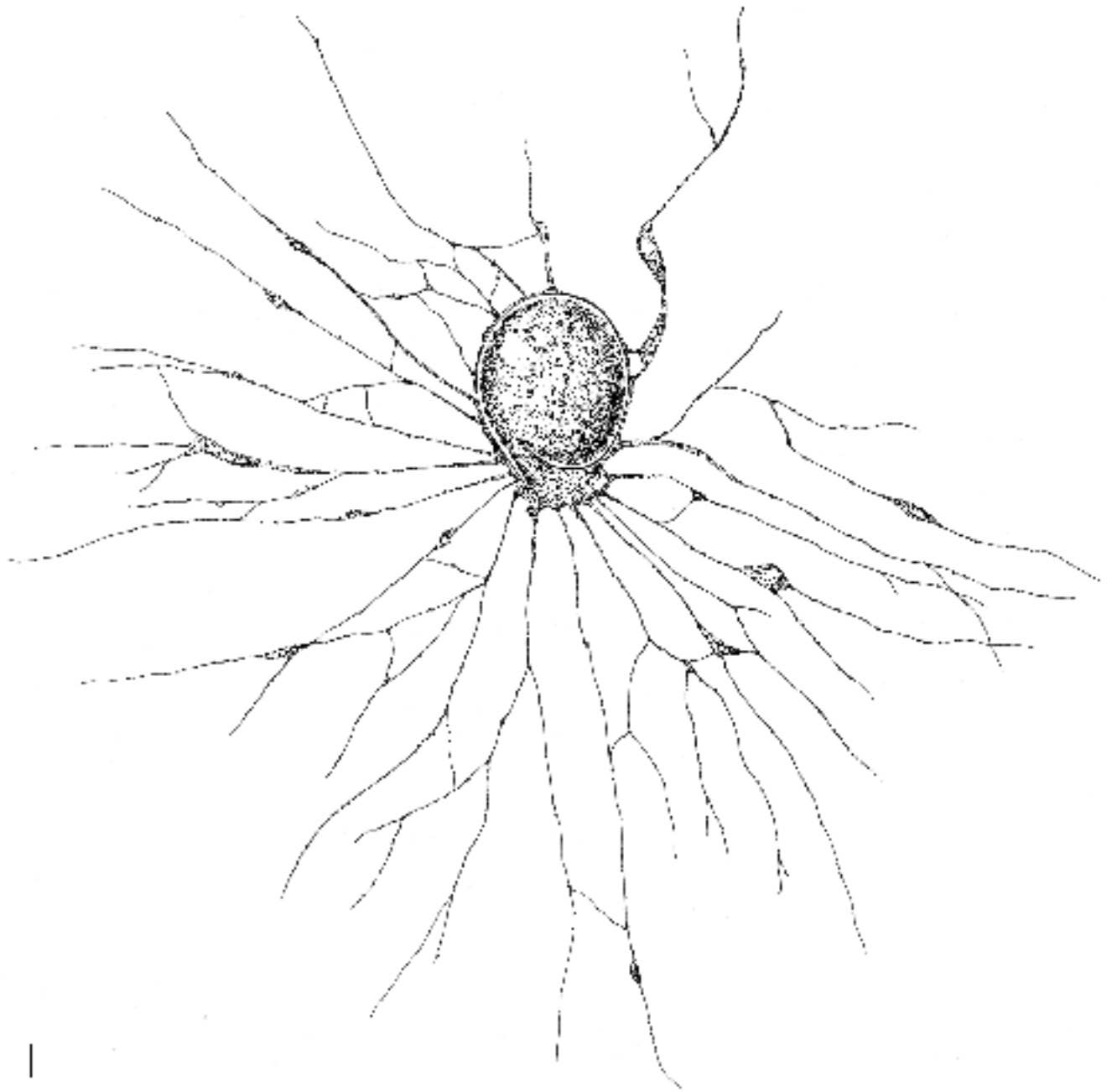


Fig. 1. *Lieberkuehnia wageneri* from life. Scale bar: 10 μm.

Feeding by the described specimens of *L. wageneri* was not observed directly. However, food vacuoles containing ingested bacteria, were noted on the lamelliform parts of pseudopodia (Fig. 1). On the other hand, information about feeding behaviour of *L. wageneri* is available in the literature. Generally, it is an omnivorous species feeding on flagellates, naked amoebae, ciliates and diatoms, but larger specimens can capture and consume even rotifers and nematodes (Siddal 1880, Penard 1902, Netzel 1971). The digestion occurs mainly within the pseudopodial network.

Comparison with original and later descriptions: The examined specimens of *L. wageneri* matched well the type description in the shape of the test and also

the typical pseudopodial trunk connected to the pseudopodial network. The size of the test (66–70 μm) is at the small end of the known range of dimensions for the species. In literature records, the size of *L. wageneri* varies considerably from 32 μm to 900 μm. In the type description, Claparède and Lachmann (1859) reported the length of the test as 160 μm. Penard (1902) found a 96 μm-long specimen and later he observed individuals 32–200 μm long (Penard 1907). Cash *et al*. (1915) gave a length range of 60–160 μm. The much larger size range (60–900 μm) mentioned by Bartoš (1954), is based on sources from earlier literature. This remarkably wide size range seems to be related to life cycle. Thus, Netzel (1971) observed that the length of the test shortly before multiple fission was 750–875 μm, compared with only 60–125 μm in the daughters. A large size range seems to be typical for this species and there are no doubts about the identification, even when populations differ considerably in size. Unfortunately, published data on the size of *L. wageneri* isolated from marine habitats (Siddal 1880, Cash *et al*. 1915) are incomplete or lacking and thus not allowing the comparison of test size between freshwater/soil versus marine populations.

The colourless cytoplasm in observed foraminiferan is in accordance with literature records. However, the cytoplasm may also be slightly yellowish or greenish in this species (Cash *et al*. 1915; Penard 1902, 1907; Bartoš 1954; Netzel 1971). Penard (1907) described oval refractile granules in the cytoplasm (Fig. 5) but they were not detected in the present specimens. The nuclei are only faintly visible in this species and for that reason they were not mentioned in the initial description (Claparède and Lachmann 1859). The number of nuclei (30) observed in the present specimens corresponds with published counts where it varies considerably from 30 up to 200. Similarly, the diameter of the nuclei (about 4 μm) was near by the minimal reported value of 4–13 μm (Siddal 1880; Penard 1902, 1907; Cash *et al*. 1915; Netzel 1971). The present specimens were only a little smaller than Penard's (1902) specimen (66–70 μm *vs*. 96 μm; Fig. 4), which possessed the highest number of nuclei known for this species (200). From these data, it seems that the nuclear number is not related to the size of a specimen.

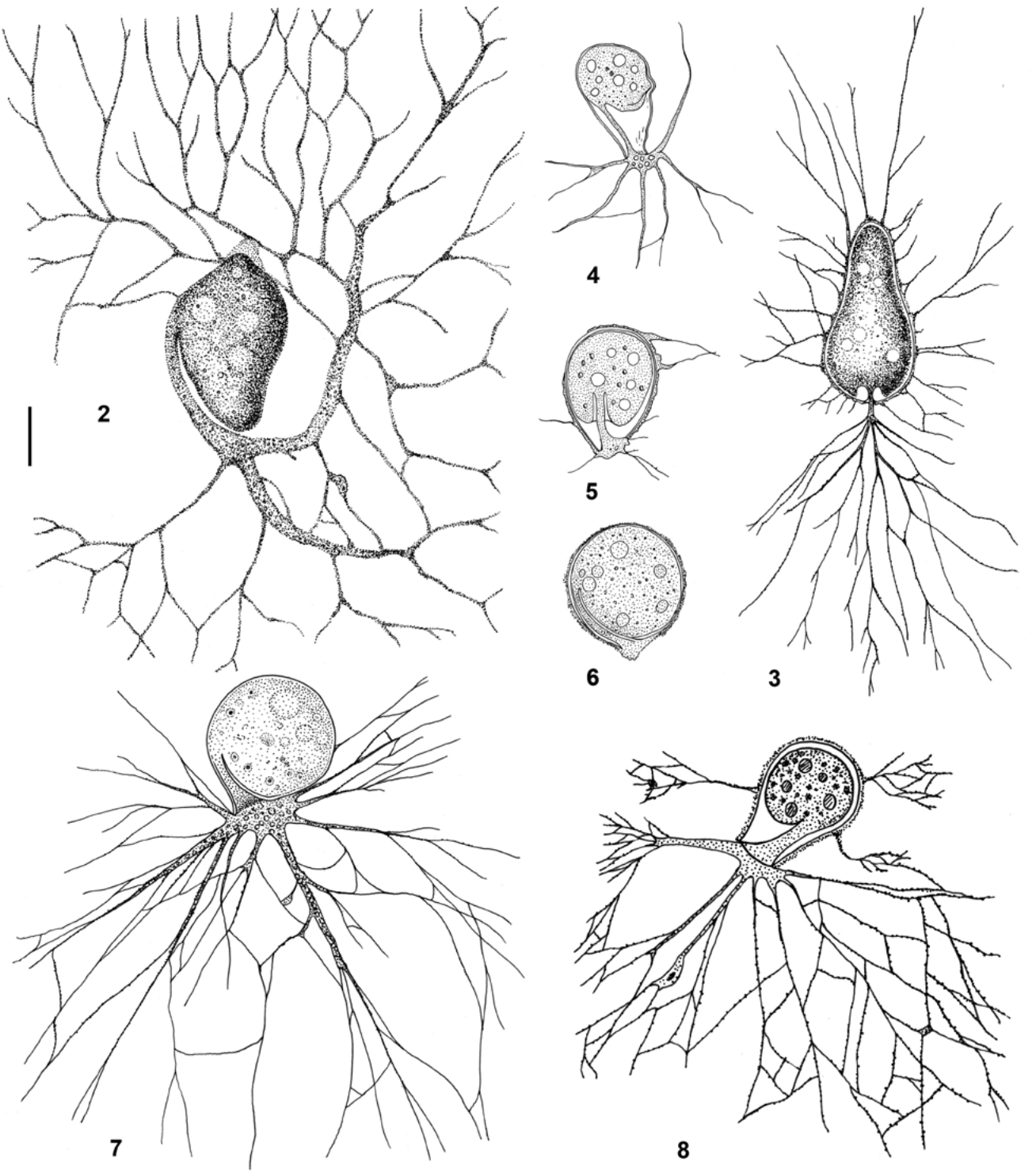
The broad anastomosing pseudopodial network around the test is characteristic of foraminiferans. The diameter of this network in *L. wageneri* from Malé Karpaty Mts. was about 350 μm. Unfortunately, the information on its size in the literature is poor and the data are incomplete. In the type description, the authors stated that the pseudopods are expanded to a distance three times bigger than illustrated on their Plate 23 (Claparède and Lachmann 1859): the size of the complete network estimated from their figure could be about 1500 μm (Fig. 2). Only Penard (1907) provided exact information about the network size in this species: the longest observed pseudopodium was 1800 μm in an 110 μm-long specimen and the pseudopodia could spread to a distance 15 or 16 times longer than the body of the cell. In other publications (Siddal 1880, Doflein 1916, Bartoš 1954), no information is provided on the size of the network (Figs 3, 7, 8). On the figures, the specimens are depicted either incomplete or resting with few and relatively short pseudopodia (Figs 4, 6; Penard 1902, 1909; Cash *et al*. 1915; Netzel 1971).

Although reproduction was not observed in the Malé Karpaty Mts. specimens, such data were published by some observers, who reported a successive and simultaneous binary or multiple fission into two or three individuals (Penard 1907, Cash *et al*. 1915), or even into five individuals (Netzel 1971). Typical is the formation of a new pseudopodial peduncle and pseudopodia at opposite side of the test before division. There is no evidence of sexual reproduction.

CONCLUSION

In general, foraminiferans are only rarely seen in freshwater or soil habitats, and this applies also to *L. wageneri*. The size and unusual appearance of *L. wageneri*, with its asymmetrical pseudopodial peduncle, makes this species easily recognizable and less likely to be overlooked. However, the number of records during many years of research worldwide is surprisingly low, and this species is considered as rare (Netzel 1971). Its rarity is also evident in the Malé Karpaty Mts., where *L. wageneri* was recorded only once in a total of 243 soil and moss samples examined during extensive research on amoeboid protists. However, recent analyses of DNA from environmental samples indicate a previously unknown diversity of freshwater foraminiferans (Holzmann *et al*. 2003) which should be studied in the future alongside re-descriptions and descriptions of new species.

The rare occurrence of *L. wageneri* has hampered further study, particularly of its ultrastructure and molecular characteristics. For this, it is essential to establish



Figs 2–8. *Lieberkuehnia wageneri*, after various authors. **2** – pyriform specimen from the type description (after Claparède and Lachmann 1859; modified); **3** – specimen from the dorsal view (after Siddal 1880; modified); **4** – Penard's specimen (after Penard 1902; modified); **5** – specimen with refractile granules in the cytoplasm from the dorsal view (after Penard 1907; modified); **6** – spherical specimen without depicted pseudopodia (after Penard 1909; modified); **7** – spherical specimen of *L. wageneri* after Doflein (1916), modified; **8** – ovoid specimen of *L. wageneri* after Bartoš (1954), modified. Scale bar: 50 μm (Figs 2, 4).

a new culture of the species. Diluted soil extract with the addition of nitrate and phosphate and *Chlorogonium elongatum* as a food organism was used by Netzel (1971) and provides a starting point.

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REFERENCES

Aescht E., Foissner W. (1995) Microfauna. In: Methods in soil biology, (Eds. F. Schinner, R. Öhlinger, E. Kandeler, R. Margesin). Springer, Berlin–Heidelberg–New York, 316–337

Bartoš E. (1954) Rhizopods of the order Testacea. SAV, Bratislava (in Slovak)

Cash J., Wailes G. H., Hopkinson J. (1915) British freshwater Rhizopoda and Heliozoa, Vol. 3. Ray Society, London

Claparède E., Lachmann J. (1859) Études sur les infusoires et les rhizopodes. Deuxième partie. Anatomie et classification des rhizopodes. *Mém. L'Inst. Genèvois* **6:** 413–467

Doflein F. (1916) Lehrbuch der Protozoenkunde. 4 Aufl. Fischer Verlag, Jena

Foissner W. (1987) Soil Protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators and guide to the literature. *Prog. Protistol.* **2:** 69–212

Holzmann M., Habura A., Giles H., Bowser S. S., Pawlowski J. (2003) Freshwater foraminiferans revealed by analysis of environmental DNA samples. *J. Eukaryot. Microbiol.* **50:** 135–139

International Commission on Zoological Nomenclature (1999) International Code of Zoological Nomenclature. 4th ed. Tipografia La Garangola, Padova

Kudo R. R. (1966) Protozoology. 5th ed. Thomas Publisher, Springfield-Illinois

Lee J. J., Pawlowski J., Debenay J. P., Whittaker J., Banner F., Gooday A. J., Tendal O., Haynes J., Faber W. W. (2000) Phylum Granuloreticulosa, Lee 1990. In: An illustrated guide to the Protozoa. 2nd ed., (Eds. J. J. Lee, G. F. Leedale, P. Bradbury). Allen Press, Lawrence, 872–951

Maupas E. (1882) Sur le *Lieberkuehnia*, Rhizopode d'eau douce multinucléé. *C. R. Acad. Sci. (Paris)* **45:** 191–194. Reference from Netzel (1971)

Meisterfeld R., Holzmann M., Pawlowski J. (2001) Morphological and molecular characterization of a new terrestrial allogromiid species: *Edaphoallogromia australica* gen. et spec. nov. (Foraminifera) from Northern Queensland (Australia). *Protist* **152:** 185–192

Netzel H. (1971) *Lieberkühnia wageneri* (Testacea) Bewegung und Fortpflanzung. In: Encyclopaedia Cinematographica, (Ed. G. Wolf). Institut für den wissenschaftlichen Film, Göttingen, 1–11

Penard E. (1902) Faune rhizopodique du bassin du Léman. Kündig, Genève

Penard E. (1907) Recherches biologiques sur deux *Lieberkühnia*. *Arch. Protistenk.* **8:** 225–258

Penard E. (1909) Sur quelques rhizopodes des mousses. *Arch. Protistenk.* **17:** 258–296

Schoenichen W. (1927) Einfachste Lebensformen des Tier- und Pflanzenreiches, Bd. 2 Urtiere, Rädetiere. Bermühler Verlag, Berlin

Siddal J. D. (1880) On *Shepheardella*, an undescribed type of marine Rhizopoda; with a few observations on *Lieberkühnia*. *Q. J. Microsc. Sci.* **78:** 130–145

Taylor W. D., Sanders R. W. (2001) Protozoa. In: Ecology and classification of North American freshwater invertebrates. 2nd ed., (Eds. J. H. Thorp, A. P. Covich). Academic Press, New York, 43–95

Verworn M. (1889) Psycho-physiologische Protistenstudien. Experimentelle Untersuchungen. Fischer Verlag, Jena. Reference from Doflein (1916)

Vďačný P., Tirjaková E. (2006) First records of soil ciliates (Protozoa, Ciliophora) from classes Prostomea, Nassophorea, Spirotrichea, and Colpodea in Slovakia. *Biologia, Bratislava* **61:** 509–516

Zlinská J., Šomšák L., Holecová M. (2005) Ecological characteristics of the studied forest communities of an oak-hornbeam tier in SW Slovakia. *Ekológia (Bratislava)* **24**, **Suppl. 2:** 3–19

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