

Family *Protospathidiidae* nov. fam.

Diagnosis: Cylindroidal to very narrowly spatulate or obclavate Spathidiina with temporary cytostome. Circumoral kinetofragments separated from each other by minute gaps and attached to the somatic ciliary rows. Anterior end of right side ciliary rows directed dorsally, left side row ends directed ventrally. Oral bulge obovate or cuneate, small, that is, usually as long as or shorter than widest trunk region. Brush located dorsolaterally and very diverse.

Type genus: *Protospathidium* DRAGESCO & DRAGESCO-KERNÉIS, 1979.

Remarks: We discovered several new *Protospathidium* and *Protospathidium*-like spathidiids in the literature and in our unpublished material from soils globally – altogether 14 species. These species form two groups which are considered as distinct genera and are united in the new family Protospathidiidae. Likely, many further species remain to be discovered.

The most important feature of the species united in this family are the oral kinetofragments which are not aligned to a “continuous” circumoral kinety, but separated from each other by gaps one to four dikinetids wide. Unfortunately, the feature is of varying distinctness and occurs also in some species and/or specimens of other genera, for instance, in *Spathidium extensum* and cultivated specimens of *S. turgitorum*. On the other hand, separation of the circumoral kinetofragments may be indistinct, for instance, in the Antarctic population of *P. terricola*. The allocation of such species and populations remains doubtful and is usually based on additional features, such as the shape of the body and oral bulge. Most protospathidiids are very narrowly spatulate or cylindroidal (length:width ratio > 6:1) and 70–150 µm long, while others resemble small *Spathidium* or middle-sized *Arcuospathidium* species, especially *A. namibiense*. The nuclear pattern, the extrusomes, and the dorsal brush are highly diverse, and thus the most important features for species recognition. Brush row 1 is reduced to a few dikinetids in several species, and the dikinetids of row 3 are comparatively widely spaced. In sum, none of the family features is distinct in all species, but taken together, they define the generic home of most populations rather unambiguously.

As in the Spathidiidae and Arcuospathidiidae, part of the Protospathidiidae has the dorsal brush located laterally, especially *Edaphospathula paradoxa*, *E. espeletiae*, and *E. minor*; *Protospathidium serpens* and *P. muscicola* show at least a tendency. If such distinction is confirmed by further investigations, at least *Edaphospathula* should be split, like *Spathidium* and *Latispathidium*, respectively, *Arcuospathidium* and *Cultellothrix*.

Interestingly, most *Edaphospathula* (family Protospathidiidae) and *Latispathidium* (family Spathidiidae) species have ovate extrusomes and short left lateral kinetofragments usually composed of ≤ four dikinetids. Likely, these features evolved convergently, possibly driven by environmental constraints, such as body narrowing due to soil life.

Key to genera (requires protargol impregnation)

- 1 Extrusomes $\leq 4 \mu\text{m}$ long, basically ovate and massive. Right side oral kinetofragments composed of ≤ 3 dikinetids. Circumoral kinety usually cuneate *Edaphospathula*
- Extrusomes $\geq 4 \mu\text{m}$ long, basically rod-shaped and comparatively fine. Right side oral kinetofragments composed of ≥ 3 dikinetids. Circumoral kinety usually elliptical or obovate *Protospathidium*

Key to species of *Protospathidium* and *Edaphospathula*

The species of the two protospathidiid genera are often difficult to distinguish because some features are not easily recognized in these tiny organisms. Thus, reliable identification usually requires both careful in vivo observation (extrusomes, brush details!) and protargol impregnation (number of ciliary rows, details of dorsal brush and oral kinetofragments!).

- 1 Length usually $\geq 200 \mu\text{m}$ in vivo 2
- Length usually $\leq 170 \mu\text{m}$ in vivo 3
- 2 Size about $210 \times 20 \mu\text{m}$. Extrusomes fine and rod-shaped. On average 9 ciliary rows. Oral bulge hemispherical and about half as long as widest trunk region *P. namibicola*
- Size about $230 \times 30 \mu\text{m}$. Extrusomes bluntly fusiform. On average 13 ciliary rows. Oral bulge ordinarily convex occupying about 38% of maximum body width *P. arenicola*
- 3 Oral bulge extrusomes oblong or rod-shaped and $2\text{--}5 \mu\text{m}$ long 10
- Oral bulge extrusomes lacking or present and massive (ovate, fusiform etc.) 4
- 4 Extrusomes lacking *E. inermis*
- Extrusomes present, minute but massive, that is, bluntly fusiform, ovate or ampulliform and $1\text{--}2 \times 0.8\text{--}1.5 \mu\text{m}$ in size 5
- 5 Macronucleus ellipsoidal to cylindroidal 6
- Macronucleus a long, tortuous strand or in many scattered nodules 8
- 6 Body bottle-shaped to elongate ovoidal, length:width ratio about 4:1 *E. minor*
- Body cylindroidal, vermiform, or elongate clavate, length:width ratio $\geq 7:1$, on average 9–11:1 7
- 7 Size about $100 \times 12 \mu\text{m}$ in vivo. Extrusomes ovate to ampulliform, $1.5 \mu\text{m}$ long. Dorsal brush occupies about 30% of body length; individual brush dikinetids widely spaced in all rows *E. brachycaryon*
- Size about $130 \times 12 \mu\text{m}$ in vivo. Extrusomes bluntly fusiform, $3 \mu\text{m}$ long. Dorsal brush occupies about 15% of body length; individual brush dikinetids narrowly (ordinarily) spaced in all rows *E. gracilis*
- 8 Many scattered macronucleus nodules *E. fusioplites*
- Macronucleus a long, more or less tortuous and twisted strand 9
- 9 About 7 ciliary rows. Right side oral kinetofragments each composed of 3 dikinetids *E. paradoxa*