

Contributions toward a new taxonomy of Central European freshwater species of the lichen genus *Thelidium* (*Verrucariales*, Ascomycota)

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Abstract: The morphological circumscription of selected Central European freshwater species in the lichen genus *Thelidium* was re-examined against the background of a molecular phylogeny based on ITS sequences. Molecular data reveal a close relationship between *Thelidium methorium*, *T. pertusatii* and *Verrucaria latebrosa* contradicting the traditional separation of *Verrucaria* and *Thelidium* by ascospore septation. *Thelidium methorium* and *T. pertusatii* are closely related species. The distinction of *Thelidium methorium* and *T. submethorium* was supported both by differences in ITS sequences and morphology. *Thelidium minutulum*, *T. pluvium* and *T. zwackhii* form a distinct clade with small perithecia and a thin or absent involucrellum. *Thelidium rehmi* and *T. minutulum* differ morphologically only in larger variability of ascospore length and the arrangement of photobiont cells, but are clearly separated by their ITS sequences as well their ecological requirements. An identification key for epilithic freshwater species of *Thelidium* in Central Europe is given, a lectotype for *T. pertusatii* is chosen, and detailed remarks for each species are provided.

Key words: amphibious lichens, identification key, ITS, phylogenetic analysis, typification

Introduction

Crustose *Verrucariales* are exceedingly poor in taxonomically useful characters. Furthermore, many of these characters are highly variable and largely dependent on environmental influences (Santesson 1939; Keller 1996; Thüs 2002). The taxonomy of *Verrucariales* on all systematic levels, from species to genera, has often been the subject of debate, and nomenclatural changes have been frequent in the past. Molecular studies so far have mainly addressed the identification of the phylogenetic position of the *Verrucariales* among the Ascomycota (Lutzoni *et al.* 2001; Liu & Hall 2004; Schmitt

et al. 2005) or the circumscription of generic boundaries within the order (Gueidan *et al.* 2007, 2008; Savić 2007; Savić & Tibell 2008; Savić *et al.* 2008). Molecular studies focusing on the significance of morphological characters in species delimitation have so far only been published for *Atla* (Savić & Tibell 2008), *Dermatocarpon* (Heiðmarsson 2003), and *Sporodictyon* (Savić 2007).

Thelidium in its current circumscription differs from similar crustose genera, such as *Verrucaria* and *Polyblastia*, only by its transversally septate ascospores. However, ascospore septation has been shown to be an unreliable character in crustose *Verrucariales* and septation of the ascospores has evolved several times among different groups (Gueidan *et al.* 2007, Savić *et al.* 2008). Major changes in generic delimitations and the description of new genera are certainly to be expected, but at present no emendation of *Thelidium* has been suggested. The present study focuses on species delimitation but it may also help to identify species groups which may form distinct genera.

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The ecology of species of the genus *Thelidium* as currently circumscribed covers a large range of environmental situations, ranging from dry exposed limestone to frequently submerged acidic siliceous rocks, and includes endolithic as well as epilithic crusts. In this paper we focus on the epilithic freshwater species from Central Europe as a first step towards the treatment of the genus *Thelidium* in the forthcoming lichen volume of the Freshwater Flora of Central Europe. Some of the Central European freshwater species are known only from their type localities or from herbarium specimens, and many species have rarely been collected since their first description. However, for some of these poorly known taxa, such as *T. pertusatii* (Garov.) Jatta, *T. rehmi* Zschacke, *T. submethorium* (Vain.) Zschacke and *T. aethioboloides* Zschacke non (Nyl.) Vain., fresh material became available for DNA extraction as a result of extensive fieldwork in Central Europe (including the Alps) during the past three years. Due to the poor knowledge of their distinctiveness, a statement on their threatened status for national Red Lists (e.g. Wirth *et al.* 1996) and their need for conservation have been open to debate. A better understanding of the genetic structure of freshwater *Thelidium* will help to discover whether these little known taxa are likely to represent monophyletic units or just morphological variants of widespread variable species.

Material and Methods

Taxon sampling

The sampling focused on representatives of two principal morphological groups within the epilithic *Thelidium* species which occur in Central European freshwater habitats, including the Alps: the *Thelidium methorium* group with large perithecia, a well-developed involucrellum and large ascospores, and the *T. minutulum* group with smaller perithecia and ascospores and absent or a weakly developed involucrellum. Sequences from GenBank of two species of *Verrucula* (*V. granulosa*, *V. inconnexaria*,) were used as outgroup. The genus *Verrucula* has been shown to be the most basal group within the *Verrucariales* (Gueidan *et al.* 2008). Wherever possible, multiple accessions were used for each putative *Thelidium* species representing different geographic origins, morphological variants and ecology. The list of

sequenced taxa together with their GenBank accession numbers is presented in Table 1. Each sequence represents an individual lichen thallus and multiple collections of the same species from identical geographical localities were sampled from different rocks or pebbles. For some rare species herbarium material or the protologues and line drawings of type specimens only were available. These taxa were included in the key and in the taxonomic treatment, but could not be included in the molecular studies.

DNA extraction, PCR amplification and sequencing

DNA was extracted from freshly collected material within 12 months of collection. Four to ten mm² of the thalli were scraped off their substrata, subsequently frozen in liquid nitrogen and ground to a fine powder. DNA extraction was performed with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. From rock specimens with more than one taxon of crustose *Verrucariales* on the surface, direct PCR from hymenial tissue of selected perithecia within the area which was subsequently used for DNA extraction was also performed in order to test the correct assignment of species names to the DNA extracts. Undiluted DNA extracts were subjected to PCR using primers MY1700, ITS1 and ITS4 (Helms *et al.* 2003, White *et al.* 1990) in a reaction volume of 25 µl. Primer content in the Mastermix was raised to 4 pM µl⁻¹ as lower contents did not give sufficient product yield for direct sequencing. The cycling profile for amplification was 95° (1 min) followed by 25 cycles at 95° (1 min), 51° (45 sec), 72° (2 min) and 15 cycles at 95° (1 min), 51° (45 sec) and 72° (2 min) with an additional increment of 5 sec and a final extension step at 72° for 10 min. For direct PCR small amounts of hymenial tissue were picked from within hydrated, fully swollen perithecia by means of a sterile needle, put in a PCR reaction tube, cooled in liquid nitrogen and ground with a glass pestle. The ground tissue was suspended in 20 µl of nuclease-free water and 5 µl of the diluted DNA was added to the PCR cocktail. The amplification products of this first PCR were subjected to a second nested PCR with primers 1800f and ITS4 using the following cycling profile: 95° (2 min) followed by 20 cycles at 95° (30 sec), 52° (30 sec with an additional increment of 2 sec), 72° (1 min). All PCR products were cleaned with the QIAquick Purification Kit (Qiagen, Hilden / Germany) following the manufacturer's protocol. PCR products were sequenced directly using the BigDye labelling method with primers 1800f and ITS4 (Friedl 1996; White *et al.* 1990). Amplification products were sequenced bidirectionally on an ABI-Capillary-Sequencer. Electrophoresis and detection of fragments were performed on an ABI3100 (PE Biosystems, Foster City, CA). The sequences were assembled with ClustalW as implemented in BioEdit (Hall 1999).

Sequence alignment and phylogenetic analyses

Sequences were aligned with ClustalX (Thompson *et al.* 1994) as implemented in BioEdit (Hall 1999).

TABLE 1. Voucher and GenBank accession numbers for sequences included in this study.

Species	Specimen	Genbank accession number
<i>Verrucula inconnexaria</i>	France, <i>C. Gueidan</i> 584	EU006537
<i>V. granulosa</i>	France, <i>C. Gueidan</i> 592	EU006539
<i>Thelidium methorium</i>	Switzerland, <i>Thüs</i> 1102b	EU249468
<i>T. methorium</i>	Slovakia, <i>Thüs</i> W1243	EU249470
<i>T. methorium</i>	Italy, <i>Nascimbene</i> JN1990	EU249469
<i>T. pertusatii</i>	Italy, <i>Nascimbene</i> JN1541	EU249471
<i>T. pertusatii</i>	Italy, <i>Nascimbene</i> JN1542	EU249472*
<i>T. aethioboloides</i>	Germany, <i>Thüs</i> W1602	EU249475
<i>T. submethorium</i>	Italy, <i>Nascimbene</i> JN1991	EU249476
<i>T. rehmi</i>	Germany, <i>Thüs</i> W1276	EU249482
<i>T. rehmi</i>	Germany, <i>Thüs</i> W1646	EU249483*
<i>T. rehmi</i>	Germany, <i>Thüs</i> W1647	EU249484*
<i>T. rehmi</i>	Germany, <i>Thüs</i> W1648	EU249485
<i>T. minutulum</i>	Austria, <i>Thüs</i> W1386	EU249477
<i>T. minutulum</i>	Austria, <i>Thüs</i> W1387	EU249478*
<i>T. pluvium</i>	Austria, <i>Thüs</i> W1382	EU249481
<i>T. pluvium</i>	Austria, <i>Thüs</i> W1383	EU249480*
<i>T. zwackhii</i>	Germany, <i>Thüs</i> W1660	EU249479
<i>Verrucaria latebrosa</i>	Switzerland, <i>Thüs</i> W1097	EU249474
<i>V. latebrosa</i>	Switzerland, <i>Thüs</i> W1135	EU249473
<i>V. latebrosa</i>	Slovakia, <i>Thüs</i> W1213	EU249467

*Identical or near identical ($\geq 0.5\%$ similarity) sequences of duplicate specimens, collected at the same sampling site, but from different rocks. These sequences were excluded from the alignment prior to further analyses.

Gaps were treated as missing data. The alignment was analysed with PAUP4.0b10a (Swofford 2002) for parsimony analyses and MrBayes3.1 (Huelsenbeck & Ronquist 2001) for Bayesian inference of phylogeny. Heuristic searches using parsimony as optimality criterion, TBR branch swapping, random taxon addition and 1000 replicates were performed with Multrees option on, steepest descent off and collapse zero length branches on. Fitting of tree topologies to the underlying dataset was evaluated by performing 1000 Bootstrap replicates on the original data and counting relative abundances of individual nodes in the resulting most parsimonious trees. A Bayesian approach (Huelsenbeck & Ronquist 2001) was used to approximate posterior probabilities (PP) of nodes resulting from the calculation of topologies with Maximum Likelihood as optimality criterion. Settings for the best fitting model describing the dataset were estimated by Modeltest3.7 (Posada & Crandall 1998) and fixed to: revmatpr = fixed (1.0000, 3.1867, 1.0000, 1.0000, 5.7990, 1.0000), pinvarpr=fixed (0.5182), nst = 6 and rates = equal. MrBayes was run three times with 1 000 000 generations in five simultaneous chains without assuming a molecular clock for each run. Sampling frequency was set to every 100th tree. The log-likelihood values of the sampled trees were examined and 2500 trees generated before stationarity was achieved were discarded.

Assessment of morphological characters

Sections of thallus and perithecia were prepared by hand from freshly collected material within a week after collection using a razor blade or with a cryotome set at 10 μm . Observations of anatomical details were made at a magnification of $\times 1000$ with a Zeiss Axioskop compound microscope equipped with differential-interference-contrast (DIC). The presence of mucilaginous sheaths surrounding the ascospores was evaluated before and after application of a solution of Lugol's iodine and potassium hydroxide.

Results

PCR yielded 19 new sequences from nine putative species for the ingroup (*Thelidium* spp. and *Verrucaria latebrosa*, Table 1). Five of them were excluded from the alignment prior to further analyses since they are identical or near identical ($\leq 0.5\%$ differences) to those retrieved from duplicate specimens collected at the same site. Only sequences retrieved from one specimen for each site were therefore used in the analysis. The

alignment of these remaining 14 sequences together with the ITS and 5.8S sequences of two outgroup taxa (*Verrucula granulosaria* and *V. inconnexaria*) consisted of 593 positions. It is available from the first author on request. Flanking areas of the nuLSU and the initial first 40 positions of the ITS1, which were obtained only for a subset of specimens, were excluded before further analysis. The final alignment of 483 positions contained 154 parsimony informative sites. All epilithic *Thelidium* species with small perithecia and a weak or absent involucrellum (*Thelidium minutulum*, *T. pluvium*, *T. rehmi*, *T. zwackhii*) fall within a single clade (Fig. 1). Branches of multiple accessions for taxa within this group are rather short; the number of ascospore septa varies among species from one to three. In the second clade of epilithic freshwater *Thelidium* branch lengths are generally much longer between species as well as within species. Ascospores in this group are either uniseptate or unicellular. ITS data confirm a close relationship of *Verrucaria latebrosa* to *Thelidium methorium* and *T. pertusatii*. *Thelidium submethorium* is distinct from the species of the *T. methorium*-group with large perithecia and ascospores and is positioned on a long branch together with *T. aethioboloides*. The reproductive structures of these two taxa are small and of a similar size to those of the *T. minutulum*-group, but differ in their constantly well-developed and often laterally spreading involucrellum. The genetic similarity of these two taxa is very high in the ITS and 5.8S regions (98%), indicating that their grouping on a joint branch reflects true genealogical affinity and is not an artifact resulting from long branch attraction.

Discussion

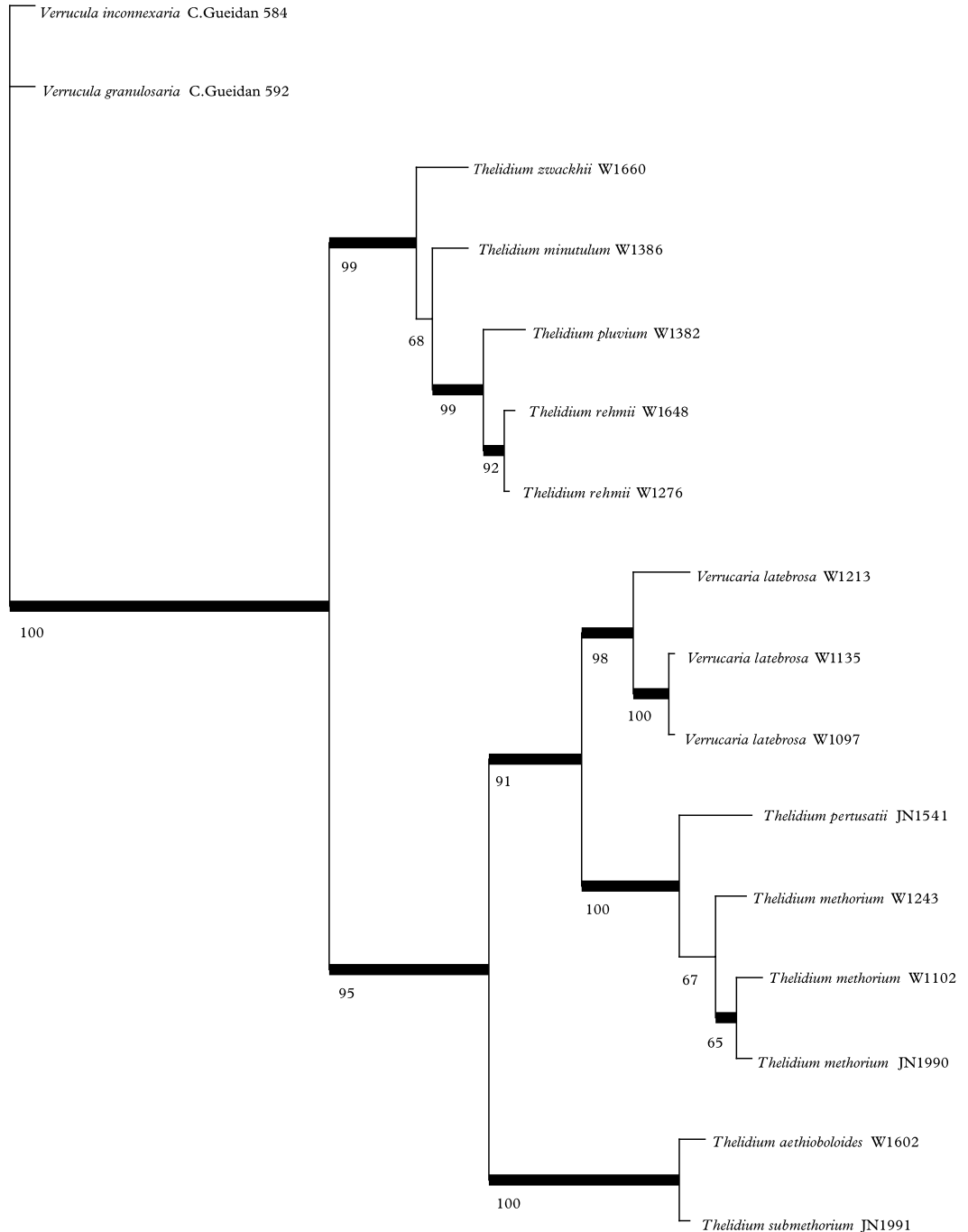
Diagnostic value of morphological characters

The dimensions of the reproductive structures appear to be generally good morphological characters for species delimitation in *Thelidium*. Even within species groups the

dimensions of ascospores and perithecia are rather conservative. Qualitative characters such as the presence of an involucrellum or ascospore septation are valuable at the species level, while variability even between closely related species can be high.

Taxonomy

Within the *Thelidium methorium* clade two morphologically distinct types can be recognized. Specimens with a thick involucrellum and small excipulum diameter have been described under the name *T. pertusatii* while typical *T. methorium* has a rather thin involucrellum and larger exciple. No evidence was found for the separation of *Thelidium methorium* and *T. aeneovinosum*, thus *T. aeneovinosum* is a younger synonym of *T. methorium*. However, the genetic heterogeneity of *T. methorium* s. l. suggests that with a wider sampling further infraspecific taxa may be recognized. *Thelidium methorium* s. l. (often under its synonym *T. aeneovinosum*) is known from high altitudes of most parts of the Northern Hemisphere and appears to be widespread in the subarctic region (Thomson 1997; Zschacke 1934). An extended sampling from non-European localities in order to explore the genetic structure of populations and distribution of morphological traits over the whole distribution range of *T. methorium* s.l. is needed. The genetic distance of *Thelidium methorium* and the closely related *T. pertusatii*, which both have 2-celled ascospores, to *Verrucaria latebrosa* with unicellular ascospores is smaller than to any other *Thelidium* species, indicating that the separation of the genera *Thelidium* and *Verrucaria* by ascospore septation is artificial; a result that also became evident in recent multigene studies with a wider taxon sampling (Gueidan *et al.* 2007; Gueidan *et al.* 2008; Savić *et al.* 2008). Morphologically, *Thelidium methorium*, *T. pertusatii*, and *Verrucaria latebrosa* differ from all other taxa included in these multigene studies by the character combination of completely epilithic thalli, large (usually >25 µm) unicellular to 1-septate ascospores and perithecia which are covered by a distinct



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FIG. 1. Phylogenetic tree based on ITS data of *Thelidium* spp. and *Verrucaria latebrosa* collected from freshwater habitats. Single most parsimonious tree with a length of 315 steps (CI 0.781, RI 0.862, RC 0.673, HI 0.219). Bayesian support values above 0.95 are indicated by thick branches; bootstrap values are shown below the branches. *Verrucula granulosa* and *V. incomexaria* were used as outgroup taxa.

involucrellum, but lack a thalline mantle. Studies on the delimitation of this species group with a wider taxon sampling and more conservative genetic markers are currently underway (H. Thüs *et al.*, unpublished).

The siliceous *Thelidium submethorium* with constantly 2-celled ascospores is closely related to *T. aethioboloides* Zschacke, a species from limestone with predominantly 4-celled ascospores. *Thelidium submethorium* and *T. aethioboloides* are a well supported monophylum within a larger clade including a second subclade with *T. methorium*, *T. pertusatii*, and *Verrucaria latebrosa*. *Thelidium submethorium* and *T. aethioboloides* differ from this second subclade by the smaller dimensions of their perithecia.

Small perithecia are also typical of a third species group of epilithic *Thelidium* species, but the involucrellum is only weak and thin or absent in this lineage, which includes the semi-aquatic species *T. pluvium*, the riparian *T. rehmi* as well as *T. minutulum* and *T. zwackhii*, which are both terrestrial species, but tolerant of short periods of inundation. Although having very similar morphology, *T. minutulum* and *T. rehmi* are not sister taxa. In the ITS phylogeny, *T. rehmi* is closer to *T. pluvium* than to *T. minutulum*. While morphological differences between *T. rehmi* and *T. minutulum* are small, niche separation of the two taxa appears to be distinct. *Thelidium minutulum* occurs on a wide range of substrata, but is usually restricted to well buffered habitats (carbonaceous rocks, basic silicates), tolerating both semi-aquatic (periodically inundated) and completely exposed (terrestrial) conditions in usually shaded but not necessarily very humid sites. *Thelidium rehmi* is restricted to substrata with little buffering capacity (pure sandstones) and is much more hygrophilic compared to *T. minutulum*. Typical habitats are small canyons or the borders of small creeks in shady ravines. Although it is restricted to sites with high air humidity and often found near small creeks, it apparently avoids sites with long periods of inundation. The preference for siliceous substrata and very humid sites is shared with its sister taxon *T. pluvium*, which is a highly specialized amphibious lichen.

Like many other amphibious *Verrucariales* with prominent perithecia, it develops a distinct and spreading involucrellum and the thallus is paraplectenchymatous, while in *T. rehmi* and *T. minutulum* the thallus varies from proso- to paraplectenchymatous, often in parts of the same thallus.

Many species of freshwater *Thelidium* are very rare (*T. aethioboloides*, *T. nigricans*, *T. pertusatii*, *T. rehmi*, *T. rivulicolum*, *T. submethorium*) or are known only from the type locality (*T. aquaticum*, *T. circumspersellum*, *T. klementii*, *T. suzaeanum*) and no fresh material of them was available. The type specimens of these taxa, however, appear to be in good condition. The delimitation of these species is discussed in detail in the taxonomic treatment below. All of them are in need of reconsideration as soon as molecular data from freshly collected specimens becomes available.

Ecological remarks and conservation needs

Most freshwater *Thelidium* species require stable substrata and prefer neutral or only slightly acidic water (Gilbert & Giavarini 1996; Gilbert 1997; Thüs 2002). Sediment deposition is tolerated only by *T. zwackhii* and, to a certain degree, also by *T. minutulum* (Thüs 2002). Both species have rather wide ecological amplitudes and are not specialized for an amphibious life-style. Specialized amphibious species of *Thelidium* generally appear to be poor competitors, and are typically restricted to sites with little growth of free-living algae or mosses. These requirements are fulfilled in cool, unpolluted and more or less shaded creeks. It is remarkable that freshwater *Thelidium* taxa in general seem to prefer much more shaded habitats than other amphibious *Verrucariales*. Whether this niche separation is due to a lower light compensation point or reflects the avoidance of locations suitable for stronger competitors requires detailed physiological experiments. Sites with a well-developed freshwater *Thelidium* flora (except those of the pioneer species *T. zwackhii* and *T.*

minutulium) are rare in Central Europe. Because of the much questioned taxonomy of *Thelethium*, their conservation status is largely unknown, although detailed investigations for *T. methorium* at least from the Black Forest indicate a strong decline of local populations (Thüs 2002), and *T. submethorium* is regarded as being extinct in this area (Wirth

et al. 1996). However, for most species the status of their populations reported in the older literature (Servit 1950, 1954; Zschacke 1934) needs checking. Certainly, the localities with recently discovered populations of rare species, such as *T. aethioboloides*, *T. pluvium*, *T. rehmii* or *T. submethorium*, are worthy of conservation.

Key to epilithic and semi-endolithic Central European freshwater species of *Thelethium*

- | | | |
|-------|---|------------------------|
| 1 | Ascospores 2-celled | 2 |
| | Ascospores 4-celled | 11 |
| 2(1) | On calcareous rocks | 3 |
| | On siliceous rocks | 5 |
| 3(2) | Thallus epilithic, up to 100 µm thick, perithecia 100–300 µm diam.; usually a terrestrial species, tolerating only temporary submersion | T. minutulum |
| | Thallus usually semi-endolithic, epilithic forms > 100 µm thick, amphibious species, usually submersed | 4 |
| 4(3) | Exciple up to 200 µm diam., involucrellum apical or absent | T. klementii |
| | Exciple 300–450 µm diam., involucrellum absent | T. rivulicolum |
| 5(2) | Involucrellum absent, or weak and indistinctly separated from the exciple | 6 |
| | Involucrellum distinctly separated from the exciple | 7 |
| 6(5) | Photobiont cells irregularly arranged, ascospores 20–30 × 8–15 µm, involucrellum absent or fused with the exciple and visible as a slight thickening in the upper part of the perithecium; on sandstone | T. rehmii |
| | Photobiont cells arranged in small aggregated groups, ascospores 13–32 × 4–15 µm, involucrellum absent or thin, indistinctly separated from the exciple and reaching the thallus base; on various (usually well buffered) substrata | T. minutulum |
| 7(5) | Ascospores 16–22 µm long | T. lahmianum |
| | Ascospores longer | 8 |
| 8(7) | Perithecia more than half covered by a thalline mantle, involucrellum usually weak (up to 30 µm thick) and often fading in lower parts | T. pluvium |
| | Perithecia naked, at least in upper two thirds, involucrellum thin to very thick (25–170 µm), evenly coloured throughout | 9 |
| 9(8) | Involucrellum very thick (115–170 µm in upper third), ratio of involucrellum thickness to exciple diameter >0.40 | T. pertusatii |
| | Involucrellum thinner (usually <90 µm in upper third), ratio of involucrellum thickness to exciple diameter <0.33 | 10 |
| 10(9) | Perithecia (430–) 500 (–1600) µm diam., ascospores (26–) 34 (–46) µm long | T. methorium |
| | Perithecia (250–) 361 (–510) µm diam., ascospores (26–) 28 (–39) µm long | T. submethorium |

- 11(1) Involucrellum present 12
 Involucrellum absent 16
- 12(11) Involucrellum thin, 10–30 (–35) μm , ascospores (22–) 30 (–36) μm long
 **T. fontigenum**
 Involucrellum thick (> 40 μm), ascospores 23–52 μm long 13
- 13(12) Thallus light coloured, whitish, grey to brownish; perithecia 300–800 μm diam.,
 (usually > 500 μm) 14
 Thallus dark coloured, brown to brown-black or green-grey to olive green-black;
 perithecia 200–485 μm 15
- 14(13) Ascospores (13–) 17 (–21) μm wide, base of perithecia forming pits in the sub-
 stratum, thallus thin and mostly endolithic to thick, with epilithic parts 10–80 μm ,
 black basal layer absent **T. papulare**
 Ascospores (16–) 19 (–23) μm wide, base of perithecia not forming pits in the
 substratum, thallus thick (> 100 μm), epilithic parts 50–100 μm , in parts with
 black basal layer **T. suzaeanum**
- 15(13) On siliceous rocks, thallus brown to brown-black, in parts with thin black basal layer,
 prothallus black **T. nigricans**
 On calcareous rocks, thallus greenish, greyish olive green-black to brown-
 black, basal layer absent or orange to brown coloured, prothallus white or
 absent **T. aethioboloides**
- 16(11) Ascospores (20–) 29 (–36) μm long, asci 90–120 μm long **T. zwackhii**
 Ascospores 16–27 μm long, asci 40–75 μm long 17
- 17(16) Exciple 200 μm diam., with black base, asci up to 75 μm long, thallus thin to
 semiendolithic; on calcareous substrata **T. circumspersellum**
 Exciple 80–160 μm diam., with colourless base, asci 40–60 μm long, thallus thin to
 thick (20–60 μm); epilithic on siliceous substrata **T. aquaticum**

Taxonomic treatment of the species

***Thelidium aethioboloides* Zschacke**

Hedw. 62: 144 (1920); type: [Switzerland, Grisons] Davos auf Kalkstein in einem Bache, nicht über Wasser, + *Thelidium zwackhii* A. Massal., 07.08.1916, *Zschacke* 3836 (B—syntype!).

Thallus black-brown, olive, dark greyish green, thin to thick (35–70 μm , up to 100 μm according to Zschacke 1920), \pm continuous with a few cracks. *Prothallus* whitish, little developed or absent. *Cortex* not differentiated, but uppermost parts of the thallus yellowish to brown, paraplectenchymatous, margins becoming \pm transparent when wet (subgelatinous). *Basal layer* locally present, with orange-brown colour. *Photobiont cells* 4–9 μm diam., occasionally also with elongated cells 8–11 \times 4–5 μm , dispersed in small aggregated groups.

Perithecia immersed in the \pm flat thallus or embedded in flat thalline warts, exposing only the naked ostiolum, basal parts often immersed in the substratum. *Exciple* (145–) 202 \pm 28.6 (–240) μm diam. ($n=7$, $N=7$). *Periphyses* up to 21 μm long. *Involucrellum* variable, apical to reaching the thallus base, (290–) 350 \pm 64 (–465) μm diam. ($n=7$, $N=7$), laterally extended in the thallus. *Asci* 73–93 \times 22–36 μm ($n=4$, $N=4$). *Ascospores* (1–) 2 (–3) septate, (25.2–) 31.6 \pm 3.5 (–40) \times (10–) 12.2 \pm 1.3 (–15.4) μm ($n=67$, $N=7$).

Habitat and distribution. Amphibious on limestone rocks, but usually not permanently submerged. Known only from a few localities in the Alps at upper montane to subalpine altitudes, always below the timber line and apparently avoiding sun exposed sites.

Nomenclatural notes. *Thelidium aethioboloides* Zschacke has priority over the heterotypic homonym *T. aethioboloides* (Nyl.) Vain. (= *T. minutulum* Körb.) based on *Verrucaria intercedens* var. *aethioboloides* Nyl. since this was published one year later than *T. aethioboloides* Zschacke. *Thelidium aethioboloides* Zschacke was regarded as a synonym of *T. minutulum* by Coppins (2002), but the latter taxon clearly differs by its weakly developed or absent involucrellum, by mostly having ascospores with only one septum, by a greyish to greenish instead of blackish-olive thallus colour and at least in part by having a prosoplectenchymatous instead of strictly paraplectenchymatous thallus architecture. The distinction of both taxa at species rank is further corroborated by the results of our analysis of ITS sequences.

Similar species. *Thelidium submethorium* can be similar to *T. aethioboloides*, but differs by having 2-celled ascospores. *Thelidium nigricans* is also similar, but has a brown-black thallus, a black basal layer, and occasionally a black prothallus. Furthermore, it grows on siliceous rocks. The thalline colour in *Thelidium* is much dependent on habitat conditions, thus the distinction of both taxa is in need of confirmation by molecular characters. In all specimens of *T. aethioboloides* small numbers of ascospores with one or three septa were observed, while in all specimens of *T. nigricans* only 2-septate ascospores were seen. Whether this difference is a reliable character for the distinction of the two taxa requires further studies of more material.

Additional specimens examined. **Germany:** *Bavaria:* Allgäu, Oberstdorf, Faltenbachtobel, in splash water zone, 950–980 m, *Thüs* W0862, W1602 (BM), 900–1000 m, inundated in a small creek, July 1918, *Lettau* (B); Garmisch-Partenkirchen, Lindertal, “7-Quellen”, 1100 m, periodically inundated, *M. Schultz* 08155c (hb. *M. Schultz*, Hamburg).—**Italy:** *Trentino-Alto Adige* (*Bolzano*): Sciliar Natural Park, Altopiano dello Sciliar, 2400 m, *Nascimbene* JN2027 (hb. *J. Nascimbene*). *Veneto* (*Belluno*): Val Tovanelle, La Stua, 950 m, *Nascimbene* JN1996 (hb. *J. Nascimbene*).

***Thelidium aquaticum* Servit**

Rozprawy Československé Akademie Věd 63 (7): 11, fig. 9 (1935); type: [Czech Republic:] Montes Rudohoří,

Hradiště prope Kadaň, 600 m, silic. In rivulo, 1950, *Servit* 842845 (PRM—holotype!).

Thallus dark olive greenish to brownish, continuous or with small cracks, 20–75 µm thick, paraplectenchymatous, margins not becoming transparent when wet. **Cortex** absent, but uppermost part of the thallus (–10 µm) in parts yellowish brown. **Basal layer** (?) developed in parts of the thallus (maybe remnants of other lichens which have been overgrown), light brownish, up to 20 µm high. **Photobiont cells** 6–9 µm diam., cells densely packed and homogeneously distributed, only in parts agglomerated.

Perithecia immersed to semi-immersed, exposed parts naked. **Exciple** 80–160 µm diam. **Periphyses** up to 12 µm long. **Involucrellum** absent or very thin and apical, covering exposed parts of the exciple up to 150 µm diam. **Asci** 40–60 × 9.6–16 µm. **Ascospores** (1–) 3 (–4)-septate, slightly constricted at the septa, (16–) 19.7 ± 4.2 (–27) × (4.8–) 6.1 ± 1.3 (–8) µm (*n*=7, *N*=3).

Habitat and distribution. The species is known only from the type locality in the Czech Republic where it grew in an amphibious situation in a river on siliceous substrata. The type specimen is associated with the aquatic *Verrucaria* cf. *funckii*.

Similar species. *Thelidium aethioboloides* has a similar thallus, but the exciple is protected by a distinct involucrellum and the ascospores are larger.

Taxonomic notes. The type material consists of three pieces of rock mounted on the same card. Most of *Servit*'s annotations refer to the large piece at the top of the card. A section of the thallus from all three pieces of rock revealed perithecia of similar morphology, but those from the smaller pieces had a slightly larger diameter compared to those on the largest piece of rock (145–160 vs. 80–120 µm). Ascospores were traced only on the large piece from the top of the card. *Alan Orange* (unpublished annotation and sections in the herbarium capsule of the type specimen) indicated the presence of a lichenicolous fungus with perithecia and

ascospores of the same size as *T. aquaticum* but with 6-septate instead of 1–4-septate ascospores and with a distinct constriction in the median septum on the type material. The perithecia in his sections appear to be more prominent and almost sessile on the thallus whereas the majority of the perithecia in all parts of the type specimen are completely immersed or only the upper third exposed. The documented section most probably represents either an unidentified lichenicolous fungus on the *Verrucaria* cf. *funckii* thalli on one of the smaller pieces of rock or an over-mature perithecium from a specimen of *T. aquaticum*. In the majority of the *Thelidium* thalli on all three pieces of rock, however, the perithecia are immersed in the thallus or exposed only in their uppermost part, the ascospores are 1–3-septate with only slight constrictions, the thallus shows a dense arrangement of paraplectenchymatous hyphal cells and contains densely packed algae without any signs of reduced vitality, indicating that *Thelidium aquaticum* is a lichenized and not a lichenicolous fungus.

***Thelidium circumspersellum* (Nyl.) Zschacke**

Verrucaria circumspersella Nyl., *Flora* 64: 536 (1881); type: [Romania] in rivulo prope Ponor-Ohaba, com. Hunyad in Transsylvania, Lojka: *Lich. Hung.* 114 (not seen). –*Thelidium circumspersellum* var. *lojkeanum* (Szatala) Szatala, *Folia Cryptog. I* (5): 371 (1927). –*Thelidium lojkeanum* Szatala, *Magy. Bot. Lap.* 24: 29 (1925); type: [Romania] ad saxa calcarea in rivulo ex antro minore oriundo prope Ponor-Ohaba, com. Hunyad in Transsylvania, 1874, *Lojka* 3384, (W—holotype!).

Thallus inconspicuous, with orange tinge in var. *lojkeanum* (due to the presence of *Trentepohlia* cells), semi-endolithic, very thin, of loosely interwoven, mostly vertically orientated hyphae, surface powdery, in parts minutely rimose. *Cortex* absent. *Basal layer* absent. *Photobiont* cells very sparse, irregularly dispersed or in small groups, 3–6 µm diam.; additional cells of *Trentepohlia* mostly on and near the surface; cyanobacteria scattered throughout the thallus but also completely endolithic below the thallus.

Perithecia sessile to semi-immersed, exposed parts naked. *Exciple* black-brown,

150–218 µm diam. *Periphyses* soon vanishing. *Involucrellum* absent. *Asci* (64–) 70.3 ± 3.8 (–75) × (17.4–) 19.6 ± 2.9 (–25) µm (*n*=6, *N*=2). *Ascospores* 3-septate, (18–) 23.1 ± 3.1 (–27) × (7.3–) 8.2 ± 0.9 (–9.6) µm (*n*=6, *N*=2) (data for var. *circumspersellum* adapted from Zschacke 1922).

Habitat and distribution. The species is known only from the type locality in Romania where it was collected on periodically inundated limestone.

Similar species. *Thelidium zwackhii* has larger ascospores and asci, a distinct thallus with a tight hyphal arrangement and the exciple is transparent in its lower parts. It is necessary to study fresh material and molecular characters in order to determine if *Thelidium circumspersellum* is more than a depauperate form of *T. zwackhii*. The co-occurrence of cyanobacteria, *Trentepohlia* and coccoid green algal cells among thin and little connected fungal hyphae may also indicate that *T. circumspersellum* is an algicolous fungus but not a lichenized species.

***Thelidium fontigenum* A. Massal.**

Miscell. Lich.: 31 (1856); type: [Germany, Bavaria] ad ligna irrigata in Franconia media (Dietenhofen), Anzi: *Lich. Rar. Venet.* 171, leg. Rehm, ex Hb. Mass. (W— isotype!, FR 0059100—syntype!).

Sagedia cataractarum Hepp, *Lich. Europ.* 442 (1857); type: [Switzerland?] an Sandsteinfelsen bei Wasserfällen, Hepp 442 (FR—syntype!).

Sagedia zwackhii β *toficola* Hepp, *Lich. Europ.* 443 (1857); type: [Switzerland?] auf Kalktufffelsen, häufig in Gesellschaft von *Scytonema heertanum* Näegeli und *Scytonema ambigua* Ktz., Hepp 443 (FR—syntype!).

?*Sagedia riparia* Hepp, *Lich. Europ.* 96 (1857); type: [Switzerland] Zürich, an entblößten Wurzeln alter Weidenstämme, Hepp 96 (not seen, see taxonomic notes).

?*Thelidium rodnense* Zschacke, *Mag. Bot. Lap.*: 299–298 (1912); type: [Romania] Rodnaborborek am Isvorul rosii auf Kalkgestein, Zschacke (not seen, see taxonomic notes).

Thallus white, whitish grey to greyish green, on wood occasionally with red, K+ purple patches, epilithic to semi-endolithic, 45–145 µm thick, proso- to paraplectenchymatous. *Cortex* not differentiated, but uppermost 10–15 µm of the thallus occasionally

yellowish brown. *Basal layer* absent. *Photobiont* a coccoid green alga, cells \pm clustered, 4–7 μm diam.

Perithecia immersed to semi-immersed in the thallus, epilithic or basal part to one third immersed in the substratum, naked. *Exciple* 200–320 μm diam., black-brown. *Periphyses* up to 30 μm . *Involucrellum* apical to almost reaching the thallus base, thin (10–35 μm), adjacent to the exciple or laterally extending in the thallus, 200–360 μm diam. ($n=7$, $N=7$). *Asci* 58–113 \times 13.4–50 μm ($n=8$, $N=4$). *Ascospores* eventually with three septa (1–2 septa in young ascospores), (22–) 30 \pm 4.9 (–36) \times (9.5–) 11.7 \pm 1.8 (–14) μm ($n=28$, $N=7$).

Habitat and distribution. This species occurs in damp to amphibious sites on base-rich substrata (limestone, lime-containing sandstone, on silt-impregnated wood, also on mica schist). It is widely distributed but rare, occurring from low mountain ranges to (sub-) alpine altitudes.

Similar species. *Thelidium zwackhii* does not have an involucrellum and its perithecia are usually largely exposed to almost sessile on the thallus surface.

Taxonomic notes. The isotypes of *Thelidium fontigenum* differ from those of *T. cataractarum* only in the development of the thallus (very thin in *T. cataractarum*, thicker in *T. fontigenum*), the reddish colour and the I+ blue reaction of the hymenium (I+ red in *T. cataractarum*). However, the reddish colour in the type specimens of *T. fontigenum* is not restricted to the lichen thalli but is also present within the wood and maybe caused by co-occurring saprobic fungi or bacteria. Thalloid features in *Thelidium* species from calcareous substrata are in general notoriously variable, often even within a single collection, and the iodine reaction of the hymenium alone appears insufficient for species delimitation in this genus. *Thelidium riparium* (Hepp) Zschacke, from submerged willow bark, differs from the type material of *T. fontigenum* only by the I+ red reaction instead of a blue reaction as in the type

specimens of *T. fontigenum* (according to the description given in Zschacke 1920). As in the case of *T. cataractarum* (from which it differs only by its substratum) this character alone is not sufficient for species delimitation. *Thelidium rodnense* was separated from *T. zwackhii* by the presence of an involucrellum. All anatomical details as given in the protologue are within the range of *T. fontigenum*. It is most probable that Zschacke did not consider *T. fontigenum* (nor its synonym *T. cataractarum*) when he described *T. rodnense*. He corrected this error in a subsequent paper (Zschacke 1920), where he placed *T. rodnense* in synonymy under *T. cataractarum* without further comment.

Additional specimens examined. **Germany:** Northrhine-Westphalia: in den Quellen der Alme, May 1865, Lahm, sub. *T. cataractarum* Hepp (B).—**Austria:** Tyrol: Matrei, bei der Waldrast, 5000 ft., Glimmer im Bach, Arnold, sub. *T. cataractarum* Hepp (B).—**Switzerland:** Bernese Oberland: Schattenhalb, Rosenlauri, 1400 m, amphibious in a small spring fed creek, *Thüs* W1749 (BM).

***Thelidium klementii* Servit**

Mitt. Bot. Staatssamml. München 11: 41–43 (1954); [Germany, Bavaria] Lechdruchbruch am Illasberg bei Roßhaupten, ad saxa temporaria inundata, calcarea-renaria, 1953, *Klement* (M—holotype!).

Thallus whitish grey, semi-endolithic, thick (120–230 μm), finely rimose to areolate. *Cortex* 10–25 μm high, colourless. *Basal layer* absent. *Photobiont* cells rounded, 4–9 μm , dispersed.

Perithecia semi-immersed in the thallus, naked. *Exciple* 200 μm diam. *Periphyses* up to 40 μm . *Involucrellum* absent or apical and much varying in thickness. *Asci* 70–80 \times 25–28 μm . *Ascospores* mostly uniseptate, occasionally unicellular, one apex rounded, the other acute, (17–) 22.7 \pm 4.5 (–30) \times (10–) 11.7 \pm 1.9 (–15) μm ($n=6$, $N=1$).

Habitat and distribution. Known only from the type locality from temporarily inundated calcareous rocks with high content of silica. The type locality is now destroyed following the construction of a large reservoir and the species is now possibly extinct.

Similar species. *Thelidium rivulicolum* can develop a similar thick thallus, but this species has much larger perithecia and asci and the ascospores are slightly larger.

Taxonomic note. Servit (1954) did not mention any involucrellum in his original description, but new sections from the type specimen revealed that at least in some perithecia a distinct apical involucrellum is present.

***Thelidium lahmianum* Lojka ex Zschacke**

Hedwigia 62: 131 (1920); type: [Poland, Tatra], an überfluteten Granitblöcken im Koprovalat, *Lojka* (W—holotype!).

Thallus black-brown, 50–85 µm thick, rimose, entirely paraplectenchymatous. *Cortex* not differentiated, but in parts of the thallus the uppermost layer is without algae. *Basal layer* black-brown, up to 50 µm thick. *Photobiont* cells sparse, rounded, 4–7 µm diam.

Perithecia semi-immersed to sessile. *Exciple* 200–300 µm diam. *Periphyses* up to 20 µm. *Involucrellum* adpressed to the exciple, thin and hardly discernable, extending to the thallus base, 290–350 µm diam. *Asci* 66–70 × 22–25 µm, 8-spored. *Ascospores* uniseptate, (16–) 18.4 ± 1.9 (21.7) × (7.3–) 9.0 ± 1.0 (10.1) µm (*n*=7).

Habitat and distribution. Known only from the type locality in the Koprova Valley (Slovakia) from inundated granite boulders.

Taxonomic note. This poorly known taxon is in need of revision. It may be conspecific with the terrestrial *Thelidium tirolense* from calcareous rocks. Based on the information given by Zschacke (1920), the two taxa differ only in their substratum and thallus colour.

***Thelidium methorium* (Nyl.) Hellb.**

Verrucaria methoria Nyl., *Öfvers. K. Vetensk-Akad. Förhandl.* 17: 296 (1860); type: [Norway, Sør-Trøndelag], Dronthjem, *W. P. Schimper* (H-NYL 2121—holotype, H-NYL 6933—istotype!).

Sagedia aeneovinosa Anzi, *Comment. Soc. Critogam. Ital.* 2 (1): 25 (1864); type: [Italy] ad rupes et saxa

granitica humida, vet saepe aqua adspersa in monitem alpius que vallis Tellinae alpe di Bodrio, Valle di Mello, Anzi: *Lich. Langob.* 342A (FR 0059094—syntype!).

Thelidium aeneovinosum var. *kutákii* Servit, *Beih. Bot. Centralbl.* 55 Abt B: 263 (1936); type: [Czechia] Krkonosé (Riesengebirge): in convalle Obří důl (Riesengrund) ad saxa schistosa, 1000 m, Kuták: *Lich. Bohemiae* 452, 1917, leg. *V. Kuták*, hb. Servit 858024 (PRM—holotype!; 858026, PRM—istotype!), Suza: *Lich. Bohemoslov. Exs.* 271, leg. *V. Kuták* (W—istotype!).

Thallus (black-) brown to grey, thin to thick, (10–) 67 ± 30 (–130) µm (*n*=29, *N*=19), continuous or finely rimose, paraplectenchymatous, ± subgelatinous and with transparent margins when wet. *Cortex* indistinct or composed of an algal-free layer, uppermost part of thalli from sunny locations often brown. *Basal layer* absent. *Photobiont* cells dispersed, 5–7 (–12) µm diam.

Perithecia prominent, naked, at least in upper half. *Exciple* (228–) 361 ± 70 (–465) µm (*n*=29, *N*=19) diam. *Periphyses* up to 60 µm. *Involucrellum* almost reaching to the thallus base, (430–) 668 ± 264 (–1600) µm (*n*=29, *N*=19) diam., (40–) 64 ± 16 (–100) µm thick (*n*=29, *N*=19), ratio of involucrellum thickness to exciple diameter <0.33 (0.1–0.32). *Asci* (69–) 91 ± 11 (–100) µm (*n*= 7, *N*=7). *Ascospores* uniseptate, (26–) 33.8 ± 4.3 (–46) × (10–) 14.4 ± 1.7 (–18.8) µm (*n*=195, *N*=19) (up to 21 µm wide according to Wirth 1995).

Habitat and distribution. This species was found on siliceous rocks that were frequently wetted but usually not submerged for more than a few months in a year (Keller & Scheidegger 1994). Terrestrial populations exist in regions with high precipitation. Mostly on well-lit to semi-shaded sites in upland areas. Widely distributed at sub-alpine to alpine altitudes, very rare in high mountain ranges (e.g. Black Forest) and here probably at high risk of extinction owing to climatic changes and reforestation of open meadows.

Similar species. *Thelidium submethorium* differs by its smaller perithecia and ascospores. *Thelidium pluvium* has perithecia which are covered by a thalline mantle. *Thelidium pyrenophorum* Mudd is a predominantly

terrestrial species, but amphibious populations are reported from Great Britain and may also be expected in upland regions of Central Europe. Zschacke (1920, 1934), however, did not regard this species as hydrophytic. It differs from *T. methorium* by its whitish to greyish colours and smaller ascospores (19–32 µm). *Thelidium schibleri* Zschacke is a non-amphibious species from calcareous rock with similar sized ascospores but smaller perithecia (240–480 µm, A. Orange pers. comm.). Further studies are required to determine whether it is conspecific with *T. methorium*.

Taxonomic and nomenclatural notes. There has been some controversy on the use of the names *T. methorium* and *T. aeneovinosum* (A. Massal.) Arn. The slight nuances in thallus colour as well as the occasional occurrence of uniseptate ascospores motivated Zschacke (1934) to regard them as separate taxa, but this was not followed by authors of recent flora projects, and it is also rejected by our analysis of ITS-data which placed specimens with combinations of both character states in different nested clades within *T. methorium* s. l. The isotype specimen seen from FR is characterized by a rather thin and spreading involucrellum compared to most other specimens of *T. methorium*, but forms intergrading to a more adpressed involucrellum do exist in the same specimen. Nimis (2003) regarded *T. methorium* as a synonym of *T. aeneovinosum* while Coppins (2002) considered *T. methorium* to be the correct name for this lichen. As *Thelidium methorium* is based on *Verrucaria methoria* from 1860 it has priority over *Thelidium aeneovinosum* which is based on the younger basionym *Sagedia aeneovinosa* from 1864. Servit separated *Thelidium kutakii* from *T. methorium* by the occasional presence of 2-septate rather than uniseptate ascospores. Some perithecia in the syntypes of *Thelidium kutakii* contain a minority of ascospores with up to three septa among a majority of 2-celled ascospores. As there are also perithecia from the same thallus with exclusively uniseptate ascospores, the occasional occurrence of single 3-septate

ascospores, without any other supporting characters is not a sufficient basis for species recognition.

Selected additional specimens examined. **Sweden:** Jämtland: Undersåker, Vällista, Malme: *Lich. Exsic.* 673 (W).—**Germany:** Baden-Württemberg: Black Forest, Zastler Bach, 1320 m NN, *Thüs* W0367 (FR).—**Switzerland:** Grisons: Davos, Dorfbach, 2000 m, splashwater zone, *Thüs* & Keller W1089 (BM); Flüela-river, 1965 m, *Thüs* & Keller W1131 (BM); Karlimatten, small ephemeral creek contributing to the Flüela-river, 2040 m, *Thüs* & Keller W1087 (BM); small ephemeral creek contributing to the Flüela-river, 2250 m, *Thüs* & Keller W1088, W1091, W1138, W1142, W1143 (BM); Schottensee, 2390 m, *Thüs* & Keller W1090 (BM).—**Italy:** South Tyrol: on porphyric rocks in a forest brook between Predazzo and Val Maor, 1882, [sub. *Thelidium diaboli* var. *aeneovinosum*], Arnold 952 (FR). *Sondrio:* Bormio, on mica-schist, Anzi [?, sub *Sagedia aeneovinosa* f. *depauperata*] (FR 0059093). *Trentino Alto Adige (Trento):* Paneveggio Nat. Park, Valbona, 1800 m, sub-alpine spruce forest, periodically submerged, *Nascimbene* JN1990 (hb. *f. Nascimbene*).—**Austria:** Salzburg: Preberkessel, rapids of a stream, 2010 m, *Thüs* W1804 (BM); ephemeral small creek in larch forest, *Thüs* W1822b (BM). *Tyrol:* Brenner, Griesberg valley, on wet mica-schist, 1524 m, Arnold 475b, (FR 0059096).—**Slovakia:** High Tatra: Bielovodská dolina, Litvorova Kotlina, 1500 m, *Thüs* W1243 (BM).

***Thelidium minutulum* Körb.**

Parerga Lichenol.: 351 (1863); syntypes: [Germany, Northrhine-Westphalia] auf Kalksteinen im Bagno bei Burgsteinfurth in Westphalen, leg. *Nitschke*, in hb. *A. Metzler* 15713 (FR 0059146—syntype!); Switzerland, auf schiefrigem Gestein bei Bern, leg. *Kemmler* (not seen).

Verrucaria intercedens var. *aethioboloides* Nyl. (type not seen).

Thelidium acrotellum Arnold; type: Herbarium Lichenum Nr. 10389, leg. Arnold (M—lectotype!).

Thelidium parvulum Arnold; type: [Germany, Northrhine-Westphalia] auf Steinen einer Straße zu Münster in Westphalen, 1868, *Lahm* 390, (M—isotype!).

Thelidium eimeri Zahlbr. = *Thelidium viride* Eitner (non Deakin); type: [Czech Republic, Chrudim] Chrast, auf überrieseltem Kalksandstein, *Eitner*, 07.1907, Acq.-Nr. 1923-4141 (W—isotype!); Acq.-Nr. 1929-12729 (W—isotype!).

Thallus thin to thick (30–100 µm), grey, brownish, greenish, very variable in appearance, from almost continuous to tartareous-rimose or granular. Hyphal arrangement proso- to paraplectenchymatous. *Photobiont* cells usually aggregated in small groups, 4–9 µm diam.

Perithecia usually prominent and naked, occasionally semi-immersed or basal parts covered by a thin layer of algae containing thallus. *Exciple* (100–) 194.2 ± 49.2 (–300) μm diam. ($n=20$, $N=15$), brownish in upper half. *Periphyses* up to 30 μm . *Involucrellum* absent, rarely thin (up to 30 μm) and covering the upper, exposed half of the exciple, 245–300 μm diam. *Asci* up to 90×23 μm . *Ascospores* uniseptate, (13.0–) 22.7 ± 4.0 (–31.9) \times (4.0–) 10.3 ± 2.1 (–15.3) μm ($n=111$, $N=15$).

Habitat and distribution. A terrestrial species, but occasionally in the splash water zone of creeks and rivers on limestone or basic siliceous rocks (especially metamorphic rocks). Widely distributed, but almost absent from regions with poorly buffered bedrock (here occasionally on man-made substrata, e.g. concrete). According to Cezanne *et al.* (2008) this species has clearly declined in the Odenwald region, Germany.

Similar species. *Thelidium minutulum* is a variable species, which in some forms can be very similar to *T. rehmi*, but the thallus is usually more granular compared to the rather even crusts formed by *T. rehmi*. In some morphs (including the type specimen) of *T. minutulum* the perithecia contain some small mature ascospores (16–18 μm) among larger ones, but morphs with consistently large ascospores exist. Such forms of *T. minutulum* (including the specimens which we sequenced in this study) differ from *T. rehmi* mainly by the arrangement of the photobionts in small aggregated groups, while in *T. rehmi* the algal cells are irregularly dispersed in the thallus. Whether this character is a reliable morphological marker for the separation of the two species requires further study. In some specimens of *T. minutulum* a weakly developed involucrellum is found. It can reach down to the exciple base and stretch slightly laterally in the thallus. In *T. rehmi* an involucrellum is usually absent, but the upper part of the exciple can be slightly thickened and may be indistinguishable from a weakly developed apical involucrellum. *Thelidium minutulum* as understood here is

restricted to well-buffered, basic substrata (limestone, metamorphic silicates), whereas *T. rehmi* is restricted to pure sandstones with low buffer capacity.

Taxonomic notes. *Thelidium eitneri* was separated only by its amphibious habitat from terrestrial forms of *T. minutulum*. *Thelidium minutulum* is interpreted here as a species with a wide ecological amplitude, including amphibious habitats and *T. eitneri* has thus to be regarded as a synonym. *Thelidium parvulum* was distinguished from *T. minutulum* by the presence of 3-septate ascospores among uniseptate ones. In his re-examination of the type specimen Zschacke (1934) found only uniseptate ascospores. Obviously there is a high variability in ascospore septation even among the perithecia of the type specimen, indicating that this character alone is not suitable for the separation of this morph from *T. minutulum*. *Thelidium viride* differs only in its greenish colour from *T. minutulum*. The colour of *T. minutulum* depends largely on the degree of insolation and many intermediate forms between grey and green can be observed. The intense green colour in the type specimens of *T. viride* is not caused by a specific pigment, but by the lack of any pigmentation in the cortex and a rather thin thallus.

Additional specimens examined. **Estonia:** Raplamaa, Kädva stream (58°54'N 25°05'E), on inundated stones, *Suija* 56c (TU).—**Germany:** *Northrhine-Westphalia:* Hösel, 95 m, spring contributing to the creek Dickelsbach, periodically immersed, *Thüs* W1840 (BM); Bensberg, 127 m, banks of the creek 'Kleiner Wahlbach', *Thüs* W0668 (FR); Bielsteinhöhle bei Lippspringe, *Beckhaus*, sub *T. parvulum* (B); Münster, on pebbles on a street, 1868, *Lahm* 390 (FR 0059145). *Hesse:* Taunus, Wisper, 155 m, on boulders bordering the river, *Thüs* W0060 (FR); Frankfurter Wald, 110 m, 1864, *A. Metzler* 2614 (FR 0059137). *Rhineland-Palatinate:* Pfauengrund, 190m, ephemeric creek, periodically inundated, *Thüs* W0265 (FR). *Saarland:* Mettlacher Saarschleife, Steinbach, on temporarily inundated sandstone, *Thüs* W1711 (cited in *Thüs* 2006 as *T. cf. parvulum*). *Baden-Württemberg:* Odenwald, Zwingenberg / Neckar, Wolfschlucht, on carbonaceous gravel on a path, *Thüs* W1654 (BM); north of Schlossau, west of Bönigfeld, small stone on the slope of a path, 1998. *Cezanne & Eichler* 4876a/b (hb. *Cezanne & Eichler*); Wachenberg, east of Weinheim, on porphyric pebbles on the ground, 390 m NN, 2004, *Cezanne & Eichler* 6778 (hb. *Cezanne & Eichler*). *Bavaria:* Allgäu,

Oberstdorf, Faltenbach, 980 m, on boulders in the splashwater zone, *Thüs* W1613 (BM); Garmisch-Partenkirchen, Lindertal, '7-Quellen', 1100m, periodically inundated, *M. Schultz* 08155c (hb. *M. Schultz*).—**Austria:** *Steiermark*: small creek contributing to the River Teigtisch, 430–480 m, *Thüs* W1386, W1387 (BM).

***Thelidium nigricans* Zschacke**

Magy. Bot. Lap.: 300 (1912); type: Romania, Südkarpathen, Bach unterhalb des Bullea Sees, July 1910, *Zschacke* 2843 (B—holotype!).

Thallus black-brown, thin (30–35 μm); according to *Zschacke* (1912) with single photobiont cells protruding up to 100 μm below the thallus surface (chasmoendolithic), finely rimose, paraplectenchymatous. *Cortex* absent, but uppermost layer of the thallus yellowish to brownish. *Black basal layer* very thin and developed only in parts of the thallus, partially reduced to isolated black patches. *Photobiont* cells 5–8 μm diam., mostly arranged in small groups.

Perithecia at first immersed in the thallus, but finally upper half exposed. *Exciple* 190–230 μm diam. *Periphyses* up to 30 μm . *Involucrellum* 200–485 μm diam., reaching the base of the thallus and in some perithecia connected with the black basal layer. *Asci* 70 \times 30 μm . *Ascospores* 3-septate, (21.9–) 32.3 \pm 3.4 (–40) \times (10.0–) 12.2 \pm 1.1 (–14.5) μm ($n=35$, $N=3$).

Habitat and distribution. This species is known only from two widely separated collections from Romania and Switzerland, but is possibly overlooked elsewhere. It grows on siliceous rocks in the splash water zone of small creeks.

Similar species. *Thelidium nigricans* is very similar to *T. aethioboloides* and molecular data are needed in order to clarify if they are genetically distinct species. In addition to their different substratum preferences they may be distinguished by the colour of the thallus, prothallus and basal layer.

Additional specimen examined. **Switzerland:** Berner Oberland, Schattenhalb, Rosenlauri, 1400 m, small spring fed creek, *Thüs* W1747 (BM).

***Thelidium papulare* (Fr.) Arnold**

Verrucaria papularis Fr., *Lichenogr. Europ. Reform.*: 434 (1831); type: ad rupes calcareas silvae nigrae in regno Württembergiae, leg. *Hochstetter*; not seen.

Thallus grey, brown, occasionally with whitish pruina, thin and \pm semi-endolithic to thick and epilithic (epilithic parts 10–80 μm). *Black basal layer* absent. *Photobiont* cells 6–9 μm , aggregated in small groups.

Perithecia half-immersed, often with the basal part in small pits in the rock. *Exciple* (370–) 410 \pm 39.4 (–485) μm diam. *Periphyses* up to 70 μm . *Involucrellum* thick (60–85 μm), (570–) 636.7 \pm 48.7 (–685) μm diam. ($n=5$, $N=6$), 300–800 μm in terrestrial specimens according to *Wirth* (1995); apical to reaching the base of the exciple, often extending laterally in the thallus. *Asci* 90–110 \times 36–43 μm . *Ascospores* (1–) 3-septate, (34.8–) 47.3 \pm 4.5 (–56.6) \times (13–) 17.1 \pm 1.8 (–21.2) μm ($n=73$, $N=6$); 30–65 \times 12–20 μm in terrestrial specimens according to *Wirth* (1995).

Habitat and distribution. This is a common terrestrial species but in upland regions it also colonizes temporarily submerged sites, especially in limestone areas with high amplitudes of water level changes. It is frequent on calcareous rocks but rarely also found on siliceous substrata, often in shaded locations. It is a widespread species, but amphibious populations are \pm restricted to cool (micro-) climates.

Similar species. *Thelidium suzaeanum* differs mainly in its slightly wider ascospores, which are in the range of (16–) 18–23 μm . In *T. papulare* such wide ascospores may occur in small numbers among a majority of narrower spores; most spores are less than 18 μm wide. The thallus in *T. suzaeanum* is usually thicker than in *T. papulare*, i.e. semi-endolithic, but mostly developed above the substratum surface, and the bases of the perithecia never form pits in the substratum. The two species are sometimes difficult to distinguish and further studies including molecular characters are needed in order to verify their status.

Selected specimens examined. **Germany:** *Hessen:* Lahntal, Villmar, Bodensteinerlei, on rarely inundated dolomite, *Thüs* W0944 (FR). *Bavaria:* Allgäu, Oberstdorf, Breitach, on periodically immersed boulders, *Thüs* W0824, W0830, (FR), W1604 (BM); Nebelhorn, ephemeric spring of the creek 'Wengenbach' *Thüs* W0859 (FR).—**Austria:** *Upper Austria:* Rofleiten, creek close to 'Pießlingursprung', on moist rocks, 750 m, *F. Berger* 21401 (hb. *F. Berger*); *ibid.*, on rocks wetted by splash water, *F. Berger* 21416 (hb. *F. Berger*).

***Thelidium pertusatii* (Garov.) Jatta**

Verrucaria pertusatii Garov., *Tent. disp. Lich. Langob.* 61: plate 4, fig. 4 (1865); type: ad rupes graniticas in Alpibus Valsesia, leg. *Garovaglio* (PAV—lectotype, hic designatus).

Thallus thin to rather thick (30–145 µm), grey, brown to brown-black, continuous to minutely rimose, paraplectenchymatous. *Photobionts* scattered, up to 6 µm diam.

Perithecia prominent, naked. *Exciple* 230–295 µm diam. *Periphyses* up to 45 µm. *Involucrellum* reaching the thallus base, 514–570 µm diam., very thick (115–170 µm), ratio of involucrellum thickness to exciple diameter >0.4. *Ascospores* uniseptate, (26.1–) 32.1 ± 4.2 (–40) × (11.6–) 13.5 ± 1.4 (–16) µm (*n*=16, *N*=3), fresh ascospores in part halonate.

Habitat and distribution. An amphibious species on frequently wetted rocks in alpine rivers and irrigated rocks in the Central and Western Alps, possibly endemic.

Similar taxa. *Thelidium pertusatii* differs from the closely related *T. methorium* by the small diameter of the exciple which is covered by a massive involucrellum.

Taxonomic note. The drawing in the protologue (also reproduced in Zschacke 1934) is schematic and does not represent the correct proportions of exciple and involucrellum in the type material.

Additional specimens examined. **Italy:** *Trentino Alto Adige (Trento):* Stelvio National Park, Val de la Mare, 46°25'16, 1°N, 010°41'04.3"E, 2200 m, alpine environment, well-lit site above the tree-line, *Nascimbene* JN1541, JN1542 (hb. *J. Nascimbene*).

***Thelidium pluvium* Orange**

Lichenologist 23: 101 (1991); type: Anglia, Westmoreland, prope Newby Bridge, Yew Barrow, Ellerside,

54°16'N, 2°59'W (34/355867), 120 m, on acidic stone in small shaded stream in woodland, 19 February 1988, *A. Orange* 6647 (NMW—holotype!).

Thallus greyish, brown, greenish, continuous or with small cracks, 35–85 µm high, paraplectenchymatous. *Photobiont* rounded, up to 8 µm diam. or elongated 5.5 × 9 µm, irregularly arranged.

Perithecia in prominent thalline warts. *Periphyses* up to 40 µm long. *Exciple* 100–320 µm diam. (up to 350 µm, *Orange* 1991). *Involucrellum* apical, to reaching the thallus base, 150–500 µm diam., thin (up to 30 µm) but distinct, often spreading ventrolaterally, leaving a transparent area between exciple and involucrellum, upper part dark coloured, lower part fading, covered by a thin thallus mantle, involucrellum pigment K–. *Asci* 75–100 × 25–30 µm (*Orange* 1991). *Ascospores* uniseptate, (19.4–) 27.6 ± 4.7 (–36.3) µm × (8.1–) 11.6 ± 2.1 (–17.4) µm (*n*=27, *N*=4).

Habitat and distribution. This species grows in the splash zone of small rivers and creeks on siliceous rocks and pebbles, in usually shaded situations. In Central Europe it is recorded from the Alps (*Berger & Priemethofer* 2000; *Keller* 2000), Black Forest (*Wirth* 1999) and the Odenwald (*Cezanne et al.* 2008), but should be looked for in other mountain regions. It is also known from the British Isles, Norway (*Orange* 1991) and Tasmania (*McCarthy* 1994).

Similar species. No other freshwater *Thelidium* with small uniseptate ascospores has perithecia with a thalline mantle. The terrestrial *T. fumidum* (B—isotype!) has a dark brown thallus, a well-developed involucrellum reaching the exciple base and a black basal layer. The involucrellum has a reddish brown pigment, which turns grey after application of K. The representatives of the *T. methorium* group have larger perithecia and a well-developed and thicker involucrellum.

Specimens examined. **Germany:** *Baden-Württemberg:* Reichenbach, small creek contributing to the 'Seebach', 320 m, on temporarily inundated sandstone together with *T. rehmi*, *Thüs* W1647 (BM).—**Austria:** Teigitsch, 430–480 m, on frequently inundated mica schist, *Thüs* W1382, W1383 (BM), *Keller* 2098 (teste *A. Orange*, hb. *Christine Keller*).

***Thelidium rehmsii* Zschacke**

Hedw. 62: 116 (1921); type: [Germany, Bavaria] Deutenheim in Mittelfranken im Keupersandsteinbrüche, Rabenhorst: *Lichenes Europaei* 594, leg. *Rehm*, sub *Thelidium nylanderii* (Hepp) Kremp. (B—syntype!, W—syntype!).

?*Thelidium margaceum* Zschacke *Hedwigia* 62: 121 (1921); type: England, Yorkshire, in a creek near Ayton, sub *Verrucaria margacea* var., in hb. *Lahm*, leg. *Leighton* (W—isotype!).

Thallus a whitish grey to green-grey, thin tartareous crust (15–110 µm) with fine cracks, proso- to paraplectenchymatous. *Photobionts* dispersed, not aggregated in small groups, ± globose, 3–10 µm diam.

Perithecia semi-immersed to almost sessile. *Exciple* 200–320 µm ($n=19$, $N=15$) diam., dark brown in upper parts, hyaline below. *Periphyses* up to 30 µm. *Involucrellum* absent or indistinguishable from the exciple, forming a slight apical thickening of the perithecium. *Asci* (67–) 80.4 ± 6.5 (–93) × (17–) 25.8 ± 5.4 (–40) µm ($n=16$, $N=14$). *Ascospores* uniseptate, (20–) 25.4 ± 2.3 (–29.9) × (7.8–) 10.1 ± 1.4 (–14.5) µm ($n=104$, $N=15$).

Habitat and distribution. This species is restricted to sandstone rocks in humid and more or less shaded places, often in the vicinity of little creeks but usually restricted to micro-sites without or with only short submersion periods. *Thelidium rehmsii* is known only from a few regions in southern Germany (Frankonia, Odenwald, Black Forest). A distribution map for the Odenwald is given in *Cezanne et al.* (2008).

Similar species. *Thelidium minutulum* is a variable species, which in some forms can be very similar to *T. rehmsii*. The thallus is usually more granular compared to the rather even crusts formed by *T. rehmsii*. In some morphs (including the type specimen) of *T. minutulum* the perithecia contain some small mature ascospores (16–18 µm) among larger ones, but morphs with consistently large ascospores also exist. Such forms of *T. minutulum* (including the specimens which we sequenced in this study) differ from *T. rehmsii* only by the arrangement of the photobionts in small aggregated groups, while in *T. rehmsii*

the algal cells are irregularly dispersed in the thallus. Whether this character is a reliable morphological marker for the separation of the two species requires further study. *Thelidium minutulum* as understood here is restricted to well buffered, basic substrata (limestone, metamorphic silicates), while *T. rehmsii* is restricted to pure sandstones with low buffer capacity.

Taxonomic note. *Thelidium margaceum* Zschacke differs from *T. rehmsii* s. str. mainly in its entirely paraplectenchymatous thallus which becomes gelatinous when wet and growing at temporarily inundated sites. This species is known from England and Scandinavia and a single collection from the Czech Republic (Servít 1954). Zschacke (1920, 1934) mentioned a distinct involucrellum closely attached to the exciple in *T. margaceum*. Re-examination of the isotype specimen from Vienna made it clear that there is no separate involucrellum but only a slight apical thickening of the exciple, very similar to the perithecia of the isotype specimens of *T. rehmsii*. The basal part of the exciple is colourless in both forms. Whether the two taxa should be separated at species rank requires further studies, including the collection of molecular data for *T. margaceum*.

Selected specimens examined. **Germany:** *Hessen:* Würzburg, Römerbad, shaded walls, on bricks, 500 m, *O. Behr* 3626 (B). *Baden-Württemberg:* SE Mittelberg, Eichelbachtal, on temporarily inundated sandstone in a creek, *Cezanne & Eichler* 5258 (hb. *Cezanne & Eichler*); NNE of Ünglert, Ünglertstal, on sandstone at the bank of the creek Mudbach, 320 m NN, *Cezanne & Eichler* 2215 (hb. *Cezanne & Eichler*); small valley NE Märzenbrunnen, NW Waldbrunn Trienzbachtal, on rarely inundated sandstone, *Cezanne & Eichler* 762 (hb. *Cezanne & Eichler*); Heuweg NE Sattelbach, on rocks in a creek, *Cezanne & Eichler* 3415 (hb. *Cezanne & Eichler*); W Wohlfahrtsmühle, NO Höpfingen, on temporarily inundated sandstone in a creek *Cezanne & Eichler* 5264 (hb. *Cezanne & Eichler*); SE Hirschhorn/Neckar, “Morsklinge”, on temporarily inundated sandstone *Cezanne & Eichler* 2725 (hb. *Cezanne & Eichler*); Reichenbach, small creek contributing to the ‘Seebach’, 320m, *Thüs* W1646–1648 (BM); Zwingenberg (Neckar), Wolfschlucht, 200 m, *Thüs*, W1652 (BM); Reisenbacher Grund, Antonlust, 360 m, *Thüs* W1276 (BM); Black-Forest, Vorbergzone, Schirnburg b. Maulburg (Wiesental), *Arnold*, in herb. *Zschacke* 3821 (B); Black-Forest?, on sandstone, *Lettau* 187 (B).

Bavaria: Odenwald, Kaltenbrunn, Kaltenbach, on sandstone boulders above the splashwater zone, *Thiis* W0921 (FR), on temporarily inundated sandstone *Cezanne & Eichler* 5187 (hb. Cezanne & Eichler).

***Thelidium rivulicolum* (Nyl.) Migula**

Verrucaria rivulicola Nyl., *Flora de Ratisbonne* 25: 13 (1875); type: supra lapides cretaceos (saepius submersus) rivuli prope Lenharrée in regione Catalaunensi (Marne), *M. Brisson* (W—syntype!).

Thallus epilithic or semi-endolithic, thick (100–200 µm), continuous to rimose, with powdery surface, whitish to greenish, with colourless cortex or cortex absent.

Perithecia semi-immersed to sessile, naked. *Exciple* 300–450 µm diam. *Periphyses* not seen (soon vanishing?). *Involucrellum* absent. *Asci* 90 × 35 µm. *Ascospores* uniseptate, 23–42 × 7–15 µm.

Habitat and distribution. Known only from the type locality in France and an old collection from Bavaria, both on temporarily inundated carbonaceous rocks.

Similar species: *Thelidium klementii* has much smaller perithecia, which often have an apical involucrellum and a semi-endolithic thallus. *Thelidium zwackhii* has 3-septate ascospores.

Taxonomic note. All the available herbarium specimens are in very poor condition and are either sterile or with only one or a few perithecia. A good illustration of the anatomy of the Bavarian material however, is given in Migula (1931) and detailed anatomical data for the type material are given in the annotation to the syntype in the Vienna herbarium.

Additional specimens examined. **Germany:** *Bavaria*: on pebbles in the headwater of a creek near Kofel in the vicinity of Oberammergau, leg. *Schnabl* = Arnold, *Lich. Exs.* 1633 (B, FR, W).

***Thelidium submethorium* (Vain.) Zahlbr.**

Verrucaria aeneovinosa subsp. *submethoria* Vain., *Medd. Soc. Fauna et Flora Fenn.* 19: 170 (1883); type: [Russia, Karelian ASSR] Kuusamo, Paanajärvi, Kiekkipuro [ad saxa gneissacea in rivulo Kiekkipuro], 1877, *E. A. Vainio* (TUR-V 3116—holotype; H—isotype!).

Thallus thin to thick (10–130 µm), continuous or finely rimose, paraplectenchymatous, ± subgelatinous and with transparent margins when wet. With olive, brown or brown-grey colours.

Perithecia prominent. *Exciple* (150–) 222.6 ± 41 (–275) µm ($n=11$, $N=5$) diam. *Periphyses* up to 30 µm. *Involucrellum* variable, even within a single thallus from apical and adpressed to the exciple to spreading and reaching the thallus base, (250–) 361.1 ± 87.5 (–510) µm ($n=11$, $N=5$) diam., thin to thick (25–85 µm); ratio of involucrellum thickness to exciple diameter < 0.33. *Ascospores* uniseptate, rarely unicellular, (26.1–) 28.1 ± 3.7 (–39) × (8.7–) 13.1 ± 1.8 (–18) µm ($n=54$, $N=5$), without perispore.

Habitat and distribution. This species occurs on siliceous substrata in clean creeks and rivers of high mountain ranges. Apart from the Finnish type locality it is known only from historical records from the Black Forest and the Italian region of Trentino-Alto Adige (Southern Alps). Scandinavian records of *T. aethioboloides* from siliceous substrata (e.g. Nordin 2002) may also refer to this species, but are in need of confirmation. Due to its scarce recent records and its need for unpolluted, cool creeks, *T. submethorium* is a species with high risk of extinction in Central Europe.

Similar species. *Thelidium pertusatii* is distinguished by its very thick involucrellum, reaching 30% or more of the diameter of the excipulum. *Thelidium methorium* is easily distinguished from *T. submethorium* in Central Europe by its distinctly larger perithecia and ascospores. On the same piece of rock from which *T. submethorium* JN1991 was sampled, we also found *T. methorium*, which differed clearly in its perithecium and ascospore size. The thalli of both species from this piece of rock were sequenced. *Thelidium methorium* JN1991 clusters together with sequences of *T. methorium* from other regions while *T. submethorium* JN1991 is a sister taxon to *T. aethioboloides* (Fig. 1). The type specimens of *T. submethorium* and *T. methorium* from northern Karelia and Norway respectively,

are much more similar to each other, with slightly overlapping extreme sizes of ascospores and perithecia. The possibility cannot be excluded at present that the type specimen of *T. submethorium* is a depauperate form of *T. methorium* and that the Central European specimens represent a separate taxon. Molecular data for Nordic specimens of *T. submethorium* may be necessary to verify the identity of our specimens and the nordic *T. submethorium*.

Specimens examined. **Germany:** Baden-Württemberg, Black Forest, Zastler Bach, 1953, Ried 140 (FR), Zell im Wiesenttal, Hohe Möhr, 700 m, at and in a small creek in forested area, 25 v 1922, Lettau (B).—**Italy:** Trentino Alto Adige (Trento): Paneveggio Nat. Park, Valbona, subalpine spruce forest, periodically submerged, 1800 m, 2005, Nascimbene JN1991 (hb. J. Nascimbene).

Thelidium suzaeanum (Servit) Servit

Involucrothele suzaeana Servit, *Rozprawy Československé Akademie Věd.* 63 (7): 23 (1953); type: [Slovakia] Tatry Bielské, ad saxa calcarea inundata in rivo Havran, 950 m, Servit 858027 (PRM—holotype!), Servit 858028 (PRM—isotype!).

Thallus dark olive to light brownish, thick (100–180 µm), semi-endolithic, epilithic part 50–100 µm, surface smooth, continuous to partially rimose-areolate, proso- to paraplectenchymatous. *Cortex* brownish. *Black basal layer* locally present, 20–120 µm. *Prothallus* dark or indistinct. *Photobiont* cells up to 8 µm diam., arranged in small groups. *Perithecia* semi-immersed in the thallus, not forming pits in the substratum. *Exciple* 300–510 µm diam. *Periphyses* up to 70 µm. *Involucrellum* thick (50–80 µm), apical to dimidiate but protruding a little laterally, up to 630 µm diam. *Asci* 70–85 × 22–25 µm. *Ascospores* eventually 3-septate, (31·7–) 33·4 ± (–55·1) × (16–) 18·6 (–23·2) µm ($n=15$, $N=2$).

Habitat and distribution. The species is known only from the type locality in the Tatry Bielské (Slovakia) from inundated limestone rocks.

Similar species. The majority of ascospores in the perithecia of *Thelidium papulare* are less than 18 µm wide, the thallus is thinner, always without a black basal layer, and the

bases of the perithecia form pits in the substratum.

Thelidium zwackhii (Hepp) A.Massal.

Sagedia zwackhii Hepp., *Lich. exs.*: 96 (1853); type: an Nagelfluhblöcken des Zürichsees, Hepp, *Lich. exs.* 96 (FR—syntype!).

Verrucaria xylospila Nyl. (type not seen, see taxonomic notes).

?*Thelidium subgelatinosum* Zschacke; type: [Great Britain] Cleveland, in a stream near Ayton, Mudd 281 (W—syntype!).

Thallus thin, 35–65 µm; greyish, whitish to brownish, proso- to paraplectenchymatous, occasionally with a pale, algal free basal layer. *Photobiont* cells arranged in small groups.

Perithecia at first more or less immersed in the thallus, finally usually almost sessile on the thallus. *Exciple* 180–260 µm diam. *Periphyses* up to 20 µm. *Involucrellum* absent or indistinct, occasionally the exciple is slightly swollen (up to 23 µm) in the uppermost third. *Asci* 90–120 × 29–40 µm (according to Servit 1954). *Ascospores* 3-septate, (20·3–) 29·1 ± 3·9 (–34·8) µm × (8·7–) 12·9 ± 4·1 (–15·4) µm, up to 36 µm long according to Wirth (1995).

Habitat and distribution. This species is most frequently found at terrestrial sites, but amphibious populations exist although it does not occur in permanently submerged sites. It is found on a wide diversity of substrata, often as a pioneer species on silt incrustated limestone, lime impregnated sandstone or hard wood, but generally does not occur on acidic substrata. Although little recorded it is presumably widely distributed but often overlooked.

Similar species. Among the 3-septate species, *Thelidium fontigenum* is most similar, differing only by the presence of a thin involucrellum. *Thelidium aethioboloides* has an olive-black to dark green-grey thallus and a well-developed involucrellum. *Thelidium circumspersellum* and *T. aquaticum* have smaller asci and ascospores.

Taxonomic notes. The separation of *Thelidium xylospilum* (Nyl.) Zschacke is based only

on the substratum type (lignum instead of rock or soil) and Zschacke (1920) commented that *Thelidium xylospilum* has to be regarded as a 'form' of *T. zwackhii*, thus contradicting his own combination. The substratum alone should never be used as the only basis for separating a taxon at specific level, if there are no other characters supporting this view. According to Zschacke (1933), *Thelidium subgelatinosum* differs mainly by the paraplectenchymatous instead of a prosoplectenchymatous thallus, but intergrading forms do exist, indicating that this character alone is not sufficient for recognizing this form at the species level. The syntype specimen in the Vienna collection (W), however, has mainly 2-celled ascospores. This lichen is most probably an over-mature specimen of *T. minutulum*. As other syntype specimens have not been checked so far, it is still possible that Mudd 281 is a heterogeneous exsiccate and different parts may belong to either *T. minutulum* and *T. zwackhii*.

Additional specimens examined. **Germany:** *Northrhine-Westphalia:* Teutoburger Wald, Lienen, spring in a beech forest, on calcareous pebbles above water level, *Thüs* W0954 (FR). *Rheinland-Pfalz:* Kaiserlautern, Botanical Garden of the Technical University, sandstone in the spray zone of an artificial waterfall, *Thüs* W1627, W1649, W1660 (BM); creek, close to the clay mine at the village Nohfelden-Eisen, on rocks at the river bank, *Thüs* W0028 (FR). *Saarland:* Orscholz, Saarschleife, Steinbach, on lime-impregnated sandstone close by the creek, *Thüs* W1702 (BM).—**Italy:** *Veneto (Belluno):* Pedavena, Val Porcilla 600 m, N 46°03'39", E 011°50'32", deciduous forest, in shaded conditions and periodically inundated rocks, *Nascimbene* JN1992 (hb. J. Nascimbene).

Excluded Taxa

Thelidium epomphalum (Nyl.) Zahlbr.

Verrucaria epomphala Nyl.; *Flora* 64: 536 (1881): [Romania] Super lapides calcareos subinundatos prope pagum Ponor Chába [=Ohába] in Transsylvania, *Lojka* Lich. Hung. Fasc. II Nr. 99, B600140923, B600140845, B600140847, B600140889 (B—syntypes!).—*Polyblastia epomphala* (Nyl.) Zschacke.

Thelidium epomphalum (Nyl.) Zahlbr is very similar to *T. papulare*. This taxon was described from temporarily irrigated rocks in Romania and no other Central European

records from creeks or rivers are known. It was first placed in *Verrucaria* and then transferred to *Thelidium* by Zschacke (1920), who observed exclusively 3-septate ascospores in a collection of specimens from the type locality. Nylander (1881) mentioned only a single ascospore with one additional longitudinal septum in the protologue, and Zschacke (1934) transferred the taxon to *Polyblastia* in his comprehensive treatment of *Verrucariaceae*. This generic affiliation is certainly not settled and future changes are to be expected (Gueidan *et al.* 2008; Savić *et al.* 2008). Apart from the occasional occurrence of longitudinal septae, *T. epomphalum* differs from *T. papulare* mainly by its thin and mostly endolithic thallus with short chains of oil-cells 15 µm diam. contrasting with the majority of hyphal cells that do not exceed a diameter of 3.6 µm. Zschacke (1920) also mentioned the flattened perithecia with depressed ostiolum as a diagnostic character, but this does not hold true, as there are populations of *T. papulare* that share this character, but lack oil cells and longitudinal septae (e.g. *Thüs* W0859).

Thelidium jizerae (Servít) H. Riedl

Involucrothele jizerae Servít; *Československé Lisejníky Čel-edi:* 187–188 (1954); type: [Czech Republic] Bohemia, saxa inundata schistosa flum. Jizerae pr. Urb. Semilyi 300 m, 1925, Servít 858029, 858030, 858031, 858032 (PRM—syntypes!).—*Verrucaria jizerae* (Servít) J. Nowak & Tobolewski.

Servít (1954) based his placement of this taxon in the genus *Involucrothele* on the observation of a single two-celled ascospore among a majority of unicellular ascospores. Nowak & Tobolewski (1975) regarded this placement as too schematic and proposed the new combination *Verrucaria jizerae* (Servít) J. Nowak & Tobol. This view is supported by the results of our re-examination of the syntypes from the collection at PRM, which revealed exclusively unicellular ascospores. The thalli are blackish brown with a rough and uneven surface which is continuous in parts but mostly cracked to almost areolate. Large areas appear to be eroded. Many perithecia are largely exposed and have the same roughened surface as the thallus. Most

ascospores have collapsed cytoplasm, but all of the ascospores ($n=20$) seen were unicellular. However, the size and form of the ascospores ($17\text{--}28\ \mu\text{m} \times 7\text{--}12\ \mu\text{m}$), the dimidiate involucrellum with the triangle between the involucrellum and the exciple base being dark coloured, as well as the vertical arrangement of the photobiont cells in short chains, make it most probable that the material represents an over-mature or damaged thallus of *Verrucaria funckii* (Spreng.) Zahlbr. and not a separate species.

Thelidium spadatum B. de Lesdain

Bull. Soc. Bot. Belg. 47: 43 (1910); type: [Belgium], Theux bei Spa auf Schiefer in einem Graben, leg. *Bouly*; Torfondry, auf Kieselsteinen in einem Bache, leg. *Lochenies* (material presumably lost during the second world war).

Thallus grey-brown, thin, fissured; perithecia 0.3 mm, semi-immersed in a thalline wart. Ascospores $24\text{--}30\ \mu\text{m} \times 12\ \mu\text{m}$ (leg. *Bouly*) & $27\text{--}31\ \mu\text{m} \times 15\text{--}16\ \mu\text{m}$ (leg. *Lochenies*). In the original description there is no information on the structure of the involucrellum, and *T. spadatum* remains a *nomem dubium*.

Thelidium subcontinuum (Nyl.) Servit

Verrucaria delita f. *subcontinua* Nyl., *Flora* 64: 189 (1881); type: Romania, Transsilvania, lacum Tenoga, saxa garantit. in und. 1872, leg. *Lojka* (W—holotype!).—*Verrucaria zenogensis* Zschacke.

According to Servit (1954) there is a small number of 2-celled ascospores among a majority of unicellular spores in the type specimen. Re-examination of the holotype revealed only unicellular ascospores and the older name *Verrucaria subcontinua* (Nyl.) Zschacke should therefore be applied instead of *Thelidium subcontinuum*. For the same type specimen, Zschacke (1933) replaced the name *V. subcontinua* (Nyl.) Zschacke with the superfluous new name *V. zenogensis* without further comments explaining the reasons of his decision. The ascospores of the type specimen are $23\text{--}38 \times 11\text{--}15\ \mu\text{m}$ (up to $40 \times 17\ \mu\text{m}$ according to Servit 1954), the involucrellum is dimidiate and laterally spreading, leaving a colourless triangle between the

involucrellum and the base of exciple. With these characters, this taxon is apparently conspecific with *Verrucaria applanata* Hepp. It differs only by transparent exciple bases, while in *V. applanata* the exciple base is reported to be dark coloured in some (!) perithecia (Zschacke 1933). Since in several syntype specimens of *V. applanata* (FR, W) only transparent exciples were found, a separation of the two taxa based on this character is not justified.

Thelidium sublacteum Eitner

Jahresber. schles. Gesellsch. vaterl. Kultur 88: 60 (1910); type: Bohemia, Chrudim, infra ruinam Rabštejn in rivulo, schist., 1908, leg. *Kalenský* (FR, W, PRM—syntypes!).

Re-examination of several syntype specimens from different herbaria showed that most of the perithecia have distinct and persistent periphyses. Furthermore, the photobiont was identified as *Trentepohlia*. This lichen can thus no longer be considered a representative of the *Verrucariales*, but has to be transferred to *Anisomeridium*. It is very similar to *Anisomeridium carinthiacum* (J. Steiner) R. C. Harris and the relation between both taxa will be discussed in more detail elsewhere.

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