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Polyozellus vs. *Pseudotomentella*: generic delimitation with a multi-gene dataset

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Abstract: *Polyozellus* and *Pseudotomentella* are two genera of closely related, ectomycorrhizal fungi in the order *Thelephorales*; the former stipitate and the latter corticioid. Both are widespread in the Northern Hemisphere and many species from both genera seem to be restricted to old growth forest. This study aimed to: a) identify genetic regions useful in inferring the phylogenetic relationship between *Polyozellus* and *Pseudotomentella*, b) infer this relationship with the regions identified and c) make any taxonomic changes warranted by the result. *RPB2*, *mtSSU* and nearly full-length portions of *nrlSU* and *nrSSU* were found to be comparatively easy to sequence and provide a strong phylogenetic signal. A STACEY species tree of these three regions revealed that *Polyozellus* makes *Pseudotomentella* paraphyletic. As a result, nearly all species currently placed in *Pseudotomentella* were recombined to *Polyozellus*. *Pseudotomentella larsenii* was found to be closer to *Tomentellopsis* than *Polyozellus*, but its placement needs further study and it was hence not recombined.

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INTRODUCTION

Thelephorales is a large order of basidiomycetes with a vast number of undescribed species; according to Kirk *et al.* (2008) 269 described species belong to the order, but following the UNITE ITS sequence database it contains 4 305 Species Hypotheses (SHs), at 1.5 % minimum distance between sister species (Kõljalg *et al.* 2013, Nilsson *et al.* 2018).

Nearly all *Thelephorales* species are ectomycorrhizal, notable exceptions being *Lenzites*, *Odontia* and possibly *Amaurodon* (Miettinen & Kõljalg 2007, He *et al.* 2019). *Thelephorales* often constitutes a large proportion of the ectomycorrhizal symbionts found on root tips in any ecosystem where such are present, and are hence important facilitators of forest and shrub growth in for example tundra, boreal and temperate ecosystems in large parts of the world (*e.g.* Kõljalg *et al.* 2000, Brundrett 2002, Sønstebo 2002, Taylor & Peterson 2005, Mühlmann & Peintner 2008, Ryberg *et al.* 2009, Bücking *et al.* 2012, Botnen *et al.* 2015). Some genera also form orchid mycorrhiza (*e.g.* Bidartondo *et al.* 2004, Jacquemyn *et al.* 2017).

Although most *Thelephorales* species are resupinate (*Amaurodon*, *Odontia*, *Pseudotomentella*, *Tomentella*, *Tomentellopsis*), some are stipitate hydroid (*Hydnellum*, *Phellodon*, *Sarcodon*), stipitate poroid (*Boletopsis*), stipitate

smooth (*Thelephora*) or cantharelloid (*Polyozellus*, *Thelephora*; Stalpers 1993, He *et al.* 2019). A few species are finger-like (*Thelephora*) and two are lamellate (*Lenzites*; Stalpers 1993, He *et al.* 2019). A morphological feature common to all species, except possibly *Amaurodon mustialaensis* (fine ornamentation sometimes visible in SEM), is the verrucose to echinulate spores (Ginns 1989, Stalpers 1993, He *et al.* 2019).

Polyozellus was described by Murrill (1910) to accommodate a blackish, cantharelloid fungus, which up until then had been known as *Cantharellus multiplex*. The genus remained monotypic until Voitek *et al.* (2018) showed that the morphological concept of this species, *Po. multiplex*, comprised five molecularly distinct species, four of which were hence previously undescribed.

All presently described *Polyozellus* species have spatulate to funnel-shaped basidiomata, with a ridged underside, which usually fuse at the base to form irregularly rosette-shaped clumps (Voitek *et al.* 2018; Fig. 1). They overlap in colour and range from brown to blue, purple or black, depending on species and stage of maturity. The pilei of young specimens are woolly or hirsute on top but become smooth with age, while the hymenium has a consistently matte appearance. Their consistency is soft and brittle. Microscopically all *Polyozellus* species are similar and share the features of hyaline, inamyloid spores covered in irregular lobes and nodules, thread-like hyphidia and clamped



Fig. 1. Basidiomata of species currently placed in *Polyozellus* and *Pseudotomentella*. **A.** *Ps. mucidula*. **B.** *Ps. media*. **C.** *Ps. griseopergamacea*. **D.** *Ps. humicola*. **E.** *Po. multiplex*. **F.** *Po. mariae*. Scale bars = 2 cm. Photos A–D. Urmas Kõljalg; E. Michael Burzynski; F. Andrus Voitk.

hyphae. The hyphae have a bluish black pigment in the walls, which produces a bluish green solution in KOH. As a genus *Polyozellus* is easily recognised in the field and by a combination of macroscopical characters and spore size it is possible to distinguish between species. Two species with smaller spores, *Po. multiplex* and *Po. atrolazulinus* can be separated from three species with larger spores: *Po. mariae*, *Po. marymargaretae* and *Po. purpureoniger* (Voitk *et al.* 2018).

Based on ITS sequences, the small-spored *Polyozellus* species form separate phylogenetic clades, while the large-spored species comprise one clade (Voitk *et al.* 2018). The species have different but overlapping geographical distributions and range from North America to Asia but are not present in Europe

(Voitk *et al.* 2018). The conservation statuses of the new species are not known but prior to their description *Po. multiplex* was considered a rare but locally abundant species in North America and a good indicator of old-growth forest (United States Forest Service 1994, Baroni 2017). It is also used in wool-dyeing and as a potential medicine against Alzheimer's disease (Hwang *et al.* 1997).

Pseudotomentella was described by Svrček (1958), with the pale brown, corticioid species *Ps. mucidula* as type. Svrček (1958, 1960) described and recombined a further eight species into the genus. Larsen (1967a, b, 1968, 1971a, b, 1974, 1983) subsequently described an additional 13 *Pseudotomentella* species. Hjortstam (1970, 1974) recombined three of the species

described by Svrček into *Tomentellopsis* and Kõljalg & Larsson (Kõljalg 1996) moved two into *Amaurodon*. Kõljalg (1996) also synonymised many of the species described by Larsen, and hence reduced the number of names to eight: *Ps. atrofusca*, *Ps. flavovirens*, *Ps. griseopergamacea*, *Ps. humicola*, *Ps. mucidula*, *Ps. nigra*, *Ps. tristis* and *Ps. vepallidospora*. Four species have later been described by various authors in separate publications: *Ps. armata* (Martini & Hentic 2002), *Ps. larsenii* (Kõljalg & Dunstan 2001), *Ps. ochracea* (Kõljalg & Larsson 1998) and *Ps. rhizopunctata* (Martini & Hentic 2003). Svantesson *et al.* (2019, 2021) described 12 new species from basidiomata previously identified as *Ps. tristis*, de-synonymised *Ps. umbrina* from the same and showed in a multi-gene phylogeny that these species form a clade sister to a clade containing *Ps. rhizopunctata* and *Ps. atrofusca*.

All *Pseudotomentella* species have corticioid, resupinate basidiomata, with a quite dense but soft and felt-like texture (Fig. 1). In similarity to *Polyozellus*, their hymenia have a matt appearance, but vary in colour from white to nearly black, past yellow, green, blue, purple, brown and grey. Microscopically, *Pseudotomentella* species display considerable variation: some species are simple-septate (e.g. *Ps. flavovirens*, *Ps. griseopergamacea* and *Ps. mucidula*), whereas others have clamped hyphae (e.g. *Ps. humicola*, the *Ps. tristis* group and *Ps. vepallidospora*); some species are monomitric (e.g. the core *Ps. tristis* group) and others are dimitic (e.g. *Ps. humicola*, *Ps. mucidula* and *Ps. rhizopunctata*); some species have dark-coloured spores (the *Ps. tristis* group) and others have hyaline spores (e.g. *Ps. griseopergamacea*, *Ps. mucidula*); a few species have chlamydospores (*Ps. rhizopunctata* and *Ps. vepallidospora*) but the rest do not. All species, however, have spores with bi- or trifurcate verrucae to echinuli. This feature in combination with the texture characteristics of their basidiomata makes *Pseudotomentella* readily recognisable under the microscope and to the trained eye they can be identified to species group already in the field. For identification to the level of species a combination of macro- and microscopical features is often needed (Kõljalg 1996, Kõljalg & Larsson 1998, Kõljalg & Dunstan 2001, Martini & Hentic 2002, 2003, Svantesson *et al.* 2019, 2021).

Pseudotomentella basidiomata are formed on the underside of dead wood, stones and turf, often very close to the ground (Kõljalg 1996, Svantesson *et al.* 2019). The spores of *Tomentella subllilacina* have been shown to be insect-dispersed (Lilleskov & Bruns 2005) and the similarity in spore-shape and growth habit of basidiomata indicate that this is likely the case for *Pseudotomentella* as well.

With the exception of *Ps. larsenii*, *Pseudotomentella* species are only naturally occurring in the Northern Hemisphere (Kõljalg 1996, Kõljalg & Larsson 1998, Kõljalg & Dunstan 2001, Martini & Hentic 2002, 2003, Svantesson *et al.* 2019, 2021). Less is known about their habitat than for *Polyozellus*, but many species are thought to only occur in old-growth forest. Svantesson *et al.* (2019) showed that all species in the *Ps. tristis* group, except the very widespread *Ps. umbrina*, are limited to ground with medium to high pH. Eight *Pseudotomentella* species are Red Listed in Sweden, three in Denmark and two in Estonia (Moeslund *et al.* 2019, Saar *et al.* 2019, SLU Artdatabanken 2020).

Vizzini *et al.* (2016) published an ITS phylogeny of *Thelephorales* indicating that, with the exception of *Ps. ochracea* and *Ps. larsenii*, *Polyozellus* and *Pseudotomentella* form a weakly supported clade together, while the clades of most other

Thelephorales genera were strongly supported. Preliminary analyses based on ITS and partial LSU sequences for the current study also displayed a close phylogenetic relationship between the genera *Polyozellus* and *Pseudotomentella*. These have, however, in addition shown that the partial LSU region alone has too weak a signal to resolve this part of the *Thelephorales* tree, while the majority of ITS is too variable to be reliably aligned. The purpose of this article is to explore what genetic markers can resolve the relationship between *Polyozellus* and *Pseudotomentella*, establish this relationship with a multi-gene species tree more densely sampled for the taxa of interest and make any nomenclatural changes warranted by the conclusions.

MATERIALS AND METHODS

Taxon sampling

The ingroup consisted of all described species of *Polyozellus* and *Pseudotomentella* or representatives from already known clades of described species where such exist, except *Ps. nigra*, *Ps. tenebrosa* and the heterotypic synonyms of *Ps. flavovirens*, *Ps. griseopergamacea* and *Ps. mucidula*. The latter are so similar to the species with which they are currently synonymised that it is unclear whether they are separate species. This approach was taken in order to minimise costs and keep sequencing efforts within the time-frame set by a PhD project (pursued by the first author). The identities of *Ps. nigra* and its heterotypic synonym *Ps. tenebrosa* are unclear and will be addressed in a separate publication. The sampling hence included one species from each of the *Polyozellus* clades identified by Voitek *et al.* (2018), the eight *Pseudotomentella* species accepted by Kõljalg (1996), except for *Ps. nigra*, the four *Pseudotomentella* species subsequently published by various authors, as well as four species from the *Ps. tristis* group, as delimited by Svantesson *et al.* (2019). In addition, *Tomentella italica* was added to the dataset, since Tedersoo *et al.* (2016) suspected it of belonging to *Pseudotomentella*. Sequencing was attempted for the types of *Ps. mucidula*, and *Ps. griseopergamacea*. In cases (other than for *Ps. nigra*) where the phylogenetic identity of a species was unclear and several genetically different contenders existed for a name, a representative of each was included in the dataset and were referred to as e.g. *Ps. cf. vepallidospora* 1 and 2.

In order to further account for as yet undescribed *Polyozellus* and *Pseudotomentella* species when inferring the relationship between the two genera, the Compound Cluster function of the UNITE database was used (Kõljalg *et al.* 2013, Nilsson *et al.* 2018). Compound Clusters consist of DNA sequences with 80 % or less sequence similarity. The database was queried for sequences belonging to non-singleton Species Hypotheses at the 3 % level. The retrieved sequences were included in the dataset if their taxonomic identity was stated as *Polyozellus* or *Pseudotomentella* in UNITE, INSD or both and they were found to belong to other Compound Clusters than those containing sequences of formally described species (or contenders of such). To reveal the phylogenetic placement of the two focal genera two species (type species and one other) were selected from all other genera within *Thelephorales*. Two specimens of an undescribed species of *Auricularia* were chosen as outgroup, due to their easy sequenceability and inclusion in an earlier dataset (Table 1; see next subsection).

Molecular data

Seven genetic regions were targeted for DNA sequencing: nrLSU, nrSSU, β -tubulin, mtSSU, *Tef1 α* , *RPB1* and *RPB2*. All are unlinked except nrLSU and nrSSU. A majority of the nrLSU and nrSSU sequences were full-length and generated through Nanopore and PacBio sequencing, as part of Wurzbacher *et al.* (2019; Table 1). These sequences were complemented with sequences derived through Sanger sequencing. Approximately 2 500 bases were amplified from the nrLSU gene with the primers LR0R and LR7, LR7R and LR14 (Hopple & Vilgalys 1999); *ca.* 1 500 bases from the nrSSU gene with NS1 and NS4, NS3 and NS8 (White *et al.* 1990); *ca.* 500 bases from the β -tubulin gene with B36f and B12r (Nagy *et al.* 2011), *ca.* 600 bases from the *Tef1 α* gene with EF983F and EF1567R (Rehner & Buckley 2005), *ca.* 700 bases from the mtSSU gene with MS1 and MS2 (White *et al.* 1990) and *ca.* 1 100 bases were amplified from the *RPB2* gene with fRPB2-5F and bRPB2-7.1 (Liu *et al.* 1999, Matheny 2005). *RPB1* sequences were obtained from whole genome sequencing by Tedersoo *et al.* (2016).

The primers used for sequencing were: for nrLSU - Ctb6 (Garbelotto *et al.* 1997) LR3R, LR5, LR7R, LR8R, LR9, LR14R (Hopple & Vilgalys 1999), for nrSSU - NS1, NS2, NS3, NS4 and NS8 (White *et al.* 1990), for β -tubulin - B36f and B12r, for *Tef1 α* - EF983F and 1567R, for mtSSU - MS1 and MS2 and for *RPB2* - bRPB2-7R, bRPB-6f and fRPB-5f (Matheny 2005).

The DNA sequences were assembled with Sequencher v. 5.4.6 (Gene Codes, Ann Arbor, MI, USA) and lodged in GenBank (Table 1). Alignments were made in AliView v. 1.18 (Larsson 2014), utilising the L-INS-i strategy, as implemented in MAFFT v. 7.017 (Kato & Standley 2013). Introns and low-quality ends were manually trimmed from the sequences prior to analysis.

Molecular analyses

Gblocks v. 0.91b (Castresana 2000, Talavera & Castresana 2007) was used to trim the alignments of problematic character regions (*e.g.* missing data, saturated sites and sections with unclear homology). The program has not been evaluated for Bayesian inference (BI), which was employed in this study for generating gene and species trees, but for neighbour joining, parsimony and maximum likelihood (ML) methods. Although they are conceptually very different methods the results of ML and BI tend to be the most similar, and Gblocks was therefore run in the relaxed version of the program, as outlined in Talavera & Castresana (2007), which according to the same study is suitable for ML analysis of alignments created with MAFFT. The resulting alignments were unchanged in length for nrSSU, mtSSU, *Tef1 α* and *RPB1*, 2 609 bases long for nrLSU (3 405 before) and 1 042 bases long for *RPB2* (1 056 before).

RDP4 (Martin *et al.* 2015) was used to test for recombination. During a first round of testing the methods RDP, GENECONV, Chimaera and MaxChi were used and the significance level set to 0.01. Sequences with significant signs of recombination were submitted to a second round of testing that made use of all recombination methods. Any sequences with a positive result for more than two methods with p -values $\leq 10^{-5}$ in the second round were regarded as probable recombinants. Recombined sections of such sequences were removed from the alignments prior to further analysis.

In the phylogenetic analyses the following minimal partitions were assumed: nrLSU, nrSSU, mtSSU and for the protein-encoding

genes: first, second and third positions. The automated best-fit tests implemented in PAUP v. 4.0a (Swofford 2002) were used to select optimal substitution models and optimal substitution model partitions. In agreement with the substitution models available in the BI programs used (BEAST v. 2 and STACEY) the tests evaluated models with three substitution schemes and equal or gamma-distributed among-site rate variation, based on BIC score. The partitioning result provided the best fit for keeping all minimal partitions separate, except first and second positions of *Tef1 α* and *RPB1*. The substitution model GTR+G was output as the optimal model for all partitions except *Tef1 α* and *RPB1* first+second positions and *RPB2* second positions. For these partitions the optimal models were JC+G, JC+G and K80+G, respectively.

To generate gene trees and assess their concordance prior to the species tree analysis BEAST v. 2.6.2 (Bouckaert *et al.* 2014, 2019) was used. The xml-files were prepared in the associated software BEAUti v. 2.6.2 (Bouckaert *et al.* 2014, 2019). The alignments were assigned the optimal partitions and substitution models output by PAUP v.4, but the substitution model was set to HKY+G for *RPB2* second partitions, since it is the most similar model to K80+G available in the program. Test runs revealed that all trees retained the same topology if GTR+G was changed to HKY+G, but convergence was considerably faster. This change in substitution models was hence implemented, in order to ensure consistency with the ensuing species tree analysis, which is often slow to converge even under optimal circumstances and where enhanced speed is thus preferable. The trees of the partitions were set as linked inside each genetic linkage group but a separate clock model was assumed for each. The clock models were set as relaxed, lognormal, since all partitions had a coefficient of variation well above 0.1 (*i.e.* implying a relatively high rate variation among branches) in test runs. The clock rate of each partition was estimated in the run, using a lognormal prior with a mean set to 1 in real space. The growth rate prior was set to lognormal, with a mean of 5 and a standard deviation of 2. These priors were set according to the STACEY package documentation (Jones 2014). The Markov Chain Monte Carlo chains were run until analyses converged well in advance of the 10 % burn-in threshold, had ESS values well above 200 for all parameters, and satisfactory chain mixing, as assessed in Tracer v. 1.6.0 (Rambaut *et al.* 2014). After discarding the burn-in trees, maximum clade credibility trees were identified by TreeAnnotator v. 2.6.2 (Bouckaert *et al.* 2014, 2019).

A species tree inferred under the multispecies coalescent model was estimated in STACEY v. 1.2.5 (Jones 2017). Substitution and clock models as well as clock and growth rate priors were set the same as for the gene tree analyses. All individuals were assumed as minimal clusters. The Collapse Height prior was set to 10^{-5} and a lognormal prior with a mean of -7 and a standard deviation of 2 was set to the PopPriorScale parameter, as per the STACEY package documentation (Jones 2014). The length and result of the analysis was determined and summarized as for the gene trees. The phylograms were visually prepared in FigTree v. 1.4.4 (Rambaut 2012) and Inkscape v. 0.92.3. (<https://inkscape.org>)

RESULTS

Seven DNA regions were assessed for their functionality in inferring the relationship between the closely related genera

Table 1. DNA sequences included in the STACEY species tree analysis and their vouchers. GenBank (two-letter combination)/ENA (four-letter combination) numbers in boldface and italics indicate sequences generated for this study and as part of Wurzbacher *et al.* (2019), respectively.

| Species | Voucher | nrLSU 1st part | nrLSU 2nd part | nrSSU | RPB2 | mtSSU |
|--|------------------|---------------------|---------------------|---------------------|-----------------|-----------------|
| <i>Polyozellus atrolazulinus</i> | TUF117559 | MT737307 | MT732081 | MT732090 | MT724777 | OK586800 |
| <i>Polyozellus marymargaretae</i> | TUF117347 | MT737308 | MT732082 | MT732089 | MT724778 | OK586801 |
| <i>Polyozellus multiplex</i> | TUF115322 | <i>SAMEA4659525</i> | <i>SAMEA4659525</i> | <i>SAMEA4659525</i> | MT724779 | OK586802 |
| <i>Pseudotomentella flavovirens</i> | KHLarsson16727 | OK559566 | OK559602 | OK559682 | OK632648 | OK586803 |
| <i>Pseudotomentella griseopergamacea</i> | SSvantesson401 | <i>SAMEA4659512</i> | <i>SAMEA4659512</i> | <i>SAMEA4659512</i> | OK632653 | OK586810 |
| <i>Pseudotomentella cf. humicola</i> 1 | SSvantesson345 | MK290724 | OK559582 | OK559681 | OK632649 | MK290650 |
| <i>Pseudotomentella cf. humicola</i> 2 | SSvantesson539 | OK559565 | — | OK559680 | OK632650 | OK586804 |
| <i>Pseudotomentella larsenii</i> | TUF100440 | MT737309 | MT732084 | MT732093 | OK632665 | OK586811 |
| <i>Pseudotomentella cf. mucidula</i> 1 | SSvantesson132 | <i>SAMEA4659509</i> | <i>SAMEA4659509</i> | <i>SAMEA4659509</i> | OK632654 | OK586805 |
| <i>Pseudotomentella cf. mucidula</i> 2 | SSvantesson458 | OK559564 | OK559603 | OK559679 | OK632655 | OK586806 |
| <i>Pseudotomentella cf. rhizopunctata</i> 1 | EMartini 10413 | OK559563 | OK559601 | OK559678 | OK632668 | OK586807 |
| <i>Pseudotomentella cf. rhizopunctata</i> 2 | SSvantesson129 | MK290717 | OK559581 | OK559677 | OK632669 | MK290652 |
| <i>Pseudotomentella sciastra</i> | SSvantesson213 | <i>SAMEA4659508</i> | <i>SAMEA4659508</i> | <i>SAMEA4659508</i> | OK632659 | OK586812 |
| <i>Pseudotomentella tristis</i> | SSvantesson193 | MK290679 | OK559580 | OK559676 | OK632658 | MK290662 |
| <i>Pseudotomentella umbrina</i> | SSvantesson351 | <i>SAMEA4659515</i> | <i>SAMEA4659515</i> | <i>SAMEA4659515</i> | OK632667 | MK290659 |
| <i>Pseudotomentella umbrinascens</i> | SSvantesson335 | <i>SAMEA4659496</i> | <i>SAMEA4659496</i> | <i>SAMEA4659496</i> | OK632666 | MK290670 |
| <i>Pseudotomentella cf. vepallidospora</i> 1 | SSvantesson456 | OK559562 | OK559605 | OK559675 | OK632651 | OK586808 |
| <i>Pseudotomentella cf. vepallidospora</i> 2 | SSvantesson493 | OK559561 | OK559604 | OK559674 | OK632652 | OK586809 |
| <i>Amaurodon sumatranus</i> | TUF115407 | <i>SAMEA4659524</i> | <i>SAMEA4659524</i> | <i>SAMEA4659524</i> | OK632661 | OK586789 |
| <i>Amaurodon viridis</i> | TUF115739 | OK559560 | OK559555 | OK559673 | — | OK586790 |
| <i>Boletopsis leucomelaena</i> | MKrikorev140912 | MK602710 | OK559579 | OK559672 | OK632676 | — |
| <i>Hydnellum ferrugineum</i> | ELarsson312-16 | <i>SAMEA4659497</i> | <i>SAMEA4659497</i> | <i>SAMEA4659497</i> | OK632673 | OK586794 |
| <i>Hydnellum suaveolens</i> | ELarsson8-14 | <i>SAMEA4659503</i> | <i>SAMEA4659503</i> | <i>SAMEA4659503</i> | OK632672 | OK586795 |
| <i>Lenzitopsis daii</i> | HSYuan2959 | JN169795 | MT732078 | MT732087 | MT724774 | OK586796 |
| <i>Lenzitopsis oxycedri</i> | TUF115268 | <i>SAMEA4659519</i> | <i>SAMEA4659519</i> | <i>SAMEA4659519</i> | MT724775 | OK586797 |
| <i>Odontia ferruginea</i> | TUF124098 | <i>SAMEA4659527</i> | <i>SAMEA4659527</i> | <i>SAMEA4659527</i> | MT724776 | OK586798 |
| <i>Odontia fibrosa</i> | SSvantesson38 | <i>SAMEA4659510</i> | <i>SAMEA4659510</i> | <i>SAMEA4659510</i> | OK632664 | OK586799 |
| <i>Phellodon melaleucus</i> | RGCarlsson160924 | <i>SAMEA4659494</i> | <i>SAMEA4659494</i> | <i>SAMEA4659494</i> | OK632675 | — |
| <i>Phellodon violascens</i> | RGCarlsson14033 | <i>SAMEA4659505</i> | <i>SAMEA4659505</i> | <i>SAMEA4659505</i> | OK632674 | OK586793 |
| <i>Sarcodon imbricatus</i> | ELarsson384-10 | <i>SAMEA4659502</i> | <i>SAMEA4659502</i> | <i>SAMEA4659502</i> | OK632670 | OK586813 |
| <i>Sarcodon squamosus</i> | ELarsson248-12 | MK602767 | OK559578 | OK559671 | OK632671 | — |
| <i>Thelephora palmata</i> | ATaylor20136 | <i>SAMEA4659526</i> | <i>SAMEA4659526</i> | <i>SAMEA4659526</i> | OK632647 | — |
| <i>Thelephora terrestris</i> | SSvantesson404 | <i>SAMEA4659516</i> | <i>SAMEA4659516</i> | <i>SAMEA4659516</i> | OK632660 | OK586814 |
| <i>Tomentella asperula</i> | SSvantesson392 | OK559559 | OK559556 | OK559670 | OK632662 | OK586815 |
| <i>Tomentella ferruginea</i> | SSvantesson367 | <i>SAMEA4659514</i> | <i>SAMEA4659514</i> | <i>SAMEA4659514</i> | OK632663 | OK586816 |
| <i>Tomentellopsis echinospora</i> | TUF110333 | <i>SAMEA4659521</i> | <i>SAMEA4659521</i> | <i>SAMEA4659521</i> | MT724780 | OK586817 |
| <i>Tomentellopsis zygodesmoides</i> | TUF124075 | MT737311 | — | MT732091 | MT724781 | OK586818 |
| <i>Auricularia</i> sp. | ELarsson17030 | OK559557 | OK559606 | OK559668 | OK632657 | OK586791 |
| | ELarsson17032 | OK559558 | OK559607 | OK559669 | OK632656 | OK586792 |

Polyozellus and *Pseudotomentella*. Out of these four could be readily sequenced and had a serviceable phylogenetic signal: nrLSU, nrSSU, RPB2 and mtSSU (Table 1). The BEAST v. 2 gene tree analyses for RPB2, mtSSU and the combined nrDNA regions were run for 30 M, 10 M and 30 M generations, respectively. The gene trees were concordant for all supported nodes, with the exception of the relative placement of *Odontia* to *Tomentella*/*Thelephora*, and could hence be combined into a species tree

analysis. The β -tubulin gene proved to be very hard to sequence and RPB1 was found to have a very weak phylogenetic signal. Sequencing of these genes was therefore discontinued, and they were not included in the species tree dataset. *Tef1 α* was found to be paralogous above genus level and therefore unusable in this study. This result will be presented in full in a separate publication. The sequencing attempts of the types of *Ps. griseopergamacea* and *Ps. mucidula* were unsuccessful,

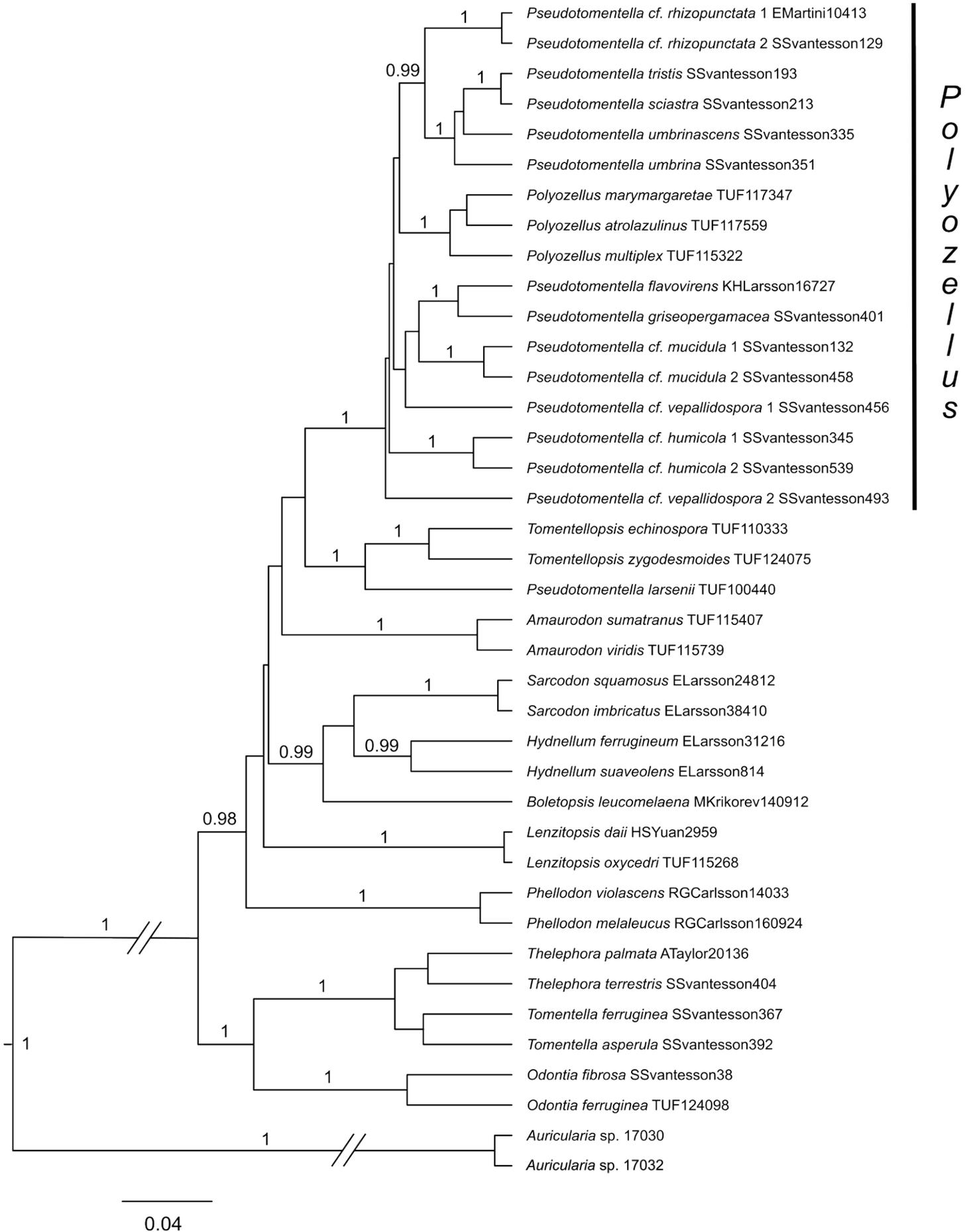


Fig. 2. STACEY species tree of *Thelephorales* with in-depth sampling of *Polyozellus* and *Pseudotomentella*, based on nrLSU, nrSSU, *RPB2* and mtSSU alignments. Only posterior probability values ≥ 0.95 are shown.

as was the sequencing of most genetic regions for *Ps. armata*, *Ps. ochracea*, *T. italica* and *Boletopsis grisea*. They were hence excluded from the analyses. No sequences were found to be recombinants.

The STACEY species tree analysis was run for 500 M generations. Its resulting phylogram strongly supports *Polyozellus* as a monophyletic genus but not *Pseudotomentella* (Fig. 2). The three species of *Polyozellus* included in the analysis as representatives of three main clades of *Polyozellus* (see *Materials and methods*) were retrieved as a fully supported clade and so was the *Ps. tristis* group. These clades along with all other species of *Pseudotomentella*, except *Ps. larsenii*, were found to reside in a fully supported clade with little additional internal structure, i.e. a group corresponding to *Polyozellus* together with the thus paraphyletic *Pseudotomentella*. *Pseudotomentella larsenii* was retrieved as closely related to *Tomentellopsis*. The sequences of *Ps. humicola*, *Ps. mucidula* and *Ps. rhizopunctata* notably keep together in well-supported clades, while the sequences of *Ps. vepallidospora* do not.

The *Polyozellus* and *Pseudotomentella* species included in the analyses were found to belong in eight UNITE Compound Clusters: UCL8_013290, UCL8_012144, UCL8_002955, UCL8_010302, UCL8_012755, UCL8_007778, UCL8_007844, UCL8_008897 and UCL8_018843. Many of these clusters included large numbers of Species Hypotheses with non-singleton DNA-sequences not currently attributed to formally described species. Non-singleton DNA sequences of undescribed species identified as *Polyozellus* or *Pseudotomentella* in UNITE or INSD, belonging to other Compound Clusters than the formally described species were not retrieved.

In order to retain monophyly and uphold nomenclatural priority all *Pseudotomentella* species included in the analysis, except *Ps. larsenii*, are recombined to *Polyozellus*, together with close relatives of them within the *Ps. tristis* group (as shown by Svantesson *et al.* 2019, 2021). The genus description of *Polyozellus* is revised accordingly.

Taxonomy

Polyozellus Murrill, *N. Amer. Fl.* **9**: 171. 1910, **emend.** Svantesson & Kõljalg

Type species: Polyozellus multiplex (Underw.) Murrill, *N. Amer. Fl.* **9**: 171. 1910.

Basionym: Cantharellus multiplex Underw., *Bull. Torrey Bot. Club* **26**: 254. 1899.

Description: Basidiomata annual, of two types:

1. Stipitate, multiple, complex, imbricately foliose; single pilei flabelliform to spatulate, sometimes funnel-shaped; terrestrial. *Pileus* surface downy to tomentose in active growth, becoming matt with concentric zonation or longitudinal ribbing; black, blue, purple, or brown; eventually glabrous and often shiny, darker in colour, often black. *Hymenium* composed of irregular, longitudinal, sinuous, anastomosing, decurrent folds, which can vary within basidiomata to be smooth, reticulate or almost poroid; various shades of blue, purple and grey, becoming darker with age. *Stipe* solid, fibrous, tapering downwards; often multiple, fused, converging to a common subterranean base; matt, scaly or shiny; blue, dark blue, dark purple, dark brown or black. *Context* soft, brittle, whitish, yellowish, pale grey, various

shades of purple and blue or black. *Odour* faintly pungent, chemical, fruity, mildly sweetish or unremarkable. *Taste* not recorded for most species, one species (*Po. atrolazulinus*) mild. *Spore deposit* white. Basidiomata resistant to decay and often last over a month in the field.

2. Corticioid, resupinate, membranaceous, effused; mature parts continuous, immature parts discontinuous; with a soft, somewhat fibrous and elastic (cottony) texture when fresh and a similar or soft yet compact, fibrous and ± elastic texture when dried; on the underside of wood, stones and debris lying on the ground, common in the roofs of rodent burrows. *Hymenium* smooth, but sometimes strongly undulating; colour ranging from nearly white, past yellow, green, blue, purple, brown and grey to nearly black. *Subiculum* well developed, loose, often fibrous, with whitish, yellow, orange, brown or black colours; often forms the outer edge of basidiomata, extending beyond the hymenium. *Odour* and *taste* not recorded. *Spore deposit* white to brown.

Hyphal system monomitoc or dimitic, hyphae simple-septate or clamped. Hyphal cords lacking or present. *Subicular hyphae*, when present, often thick-walled, forming a loose tissue, hyaline or with yellow, orange, brown or black colours. *Subhymenial hyphae* straight to somewhat sinuous, for some species interwoven and nodulose; thin to thick-walled; often forming a rather dense tissue; hyaline or pale green, yellow, orange or brown in KOH; in some species with a pigment in the walls which has a blue-green reaction in the presence of air and produces a similarly coloured solution; in some species amyloid. *Encrustation* present or absent, amorphous to granular; hyaline, or with green, orange, brown, purple or black colours in KOH, in some species sometimes dark blue green in the presence of air; when present occurring on the upper parts of subhymenial hyphae and on the lower parts of basidia. *Basidia* 4-sterigmate, occasionally 2-sterigmate; clavate, narrowly clavate or clavopedunculate, thin-walled, with 1–3 slight constrictions; sterigmata slightly curved; colours and reactions the same as for subhymenial hyphae, but in addition often with granular contents in KOH. *Hyphidia* present in some species; simple, filiform, not extending beyond basidia. *Basidiospores* in frontal face with a subcircular, subellipsoid or triangular basic shape; outline angular, nodulose, triangular, subcircular, subellipsoid, heart-shaped or cross-shaped; unlobed or with 3–7 lobes; lateral face with a subcircular, subellipsoid, ellipsoid or ovoid basic shape; outline evenly rounded, angular, lobed or nodulose; apiculus prominent to prolonged (possibly except for in *Po. mariae*); echinuli in most species long and prominent, bi- or trifurcate, sometimes singularly attached, in some species short and irregularly attached; colours and reactions the same as for subhymenial hyphae but often darker and reactions less frequent. *Chlamydospores* present or absent. Forming ectomycorrhiza.

Polyozellus abundilobus (Svantesson) Svantesson & Kõljalg, **comb. nov.** MycoBank MB 836134. UNITE SH: 1152984.08FU.

Basionym: Pseudotomentella abundiloba Svantesson, *MycKeys* **50**: 24. 2019.

Typus: Norway, Oslo (county), Oslo (municipality), Bygdøy, Hengsåsen, boreonemoral mixed forest on soil with high pH, 22 Sep. 2010, *S. Svantesson* (**holotype** O F110312).

Polyozellus alnophilus (Svantesson) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836135. UNITE SH: 1564303.08FU.
Basionym: *Pseudotomentella alnophila* Svantesson, *MycKeys* **50**: 26. 2019.

Typus: **Norway**, Buskerud, Ringerike, Juveren N, boreonemoral *Alnus incana* forest on soil with intermediate pH, 25 Sep. 2010, S. Svantesson & N. Svensson (**holotype** O F110313).

Polyozellus alobatus (Svantesson) Svantesson & Køljalg, **comb. nov.** MycoBank No.: MB 836136. UNITE SH: 1230089.08FU.
Basionym: *Pseudotomentella alobata* Svantesson, *MycKeys* **50**: 29. 2019.

Typus: **Sweden**, Dalsland, Mellerud, Skållerud, Norgekullen SW, coniferous forest on soil with high pH, 20 Sep. 2017, S. Svantesson 425 (**holotype** GB).

Polyozellus atrofuscus (M.J. Larsen) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836137. UNITE SH: 1230079.08FU.
Basionym: *Pseudotomentella atrofusca* M.J. Larsen, *Bull. Torrey Bot. Club* **98**: 39. 1971.

Typus: **USA**, Arizona, Fort Valley, Coconino Co., on *Pinus ponderosa*, 21 Sep. 1967, R.L. Gilbertson 7553 (**holotype** ARIZ; **isotype:** SSMF 685–4578).

Polyozellus badjelannanus (Svantesson) Svantesson & Køljalg, **comb. nov.** MycoBank MB 841511. UNITE SH: 1564288.08FU.
Basionym: *Pseudotomentella badjelannana* Svantesson, *Phytotaxa* **497**: 69. 2021.

Typus: **Sweden**, Lule Lappmark, Jokkmokk, Oarjep Slahpetjåhkkå, middle alpine *Dryas octopetala* heath on ground with high pH, on underside of stones and *Dryas* twigs, 18 Aug. 2016, S. Svantesson 303 (**holotype** GB).

Polyozellus flavovirens (Höhn. & Litsch.) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836138. UNITE SH: 1184825.08FU.
Basionym: *Tomentella flavovirens* Höhn. & Litsch., *Sitzungsber. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Cl., Abt. 1* **116**: 831. 1907.

Typus: **Germany**, Braunlage am Harz, auf nackter Erde [= on bare soil], Lindau, (**holotype** FH [v. Höhnel herb., sheet 1979]).

Polyozellus griseopergamaceus (M.J. Larsen) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836139. UNITE SH: 1184823.08FU.
Basionym: *Pseudotomentella griseopergamacea* M.J. Larsen, *Bull. Torrey Bot. Club* **98**: 38. 1971.

Typus: **USA**, New York, Highland Forest, Fabius P.O., Onondaga Co., on *Pinus resinosa*, 21 Oct. 1961, R. L. Gilbertson 3096 (**holotype** BPI; **isotype:** SSMF 695–4961).

Polyozellus humicola (M.J. Larsen) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836140. UNITE SH: 1236694.08FU, —.
Basionym: *Pseudotomentella humicola* M.J. Larsen, *Mycologia* **60**: 547. 1968.

Typus: **Canada**, Ontario, Algonquin Park, Opeongo Lake, on *Thuja occidentalis*, 18 Sep. 1939, R. F. Cain (**holotype** SSMF 8868; **isotypes:** TRTC 44362 and BPI).

Polyozellus medius (Svantesson & Køljalg.) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836141. UNITE SH: 1185287.08FU.
Basionym: *Pseudotomentella media* Svantesson & Køljalg, *MycKeys* **50**: 33. 2019.

Typus: **Estonia**, Valga, Otepää, Trommi, 12 Sep. 2012, U. Køljalg (**holotype** TUF 115609).

Polyozellus mucidulus (P. Karst.) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836142. UNITE SH: 1234281.08FU, 1234278.08FU.
Basionym: *Hypochnus mucidulus* P. Karst., *Bidrag Kännedom Finlands Natur Folk* **37**: 163. 1882.

Typus: **Finland**, Haarankorpi, in ligno mucido, 09 Oct. 1878, P. A. Karsten (**lectotype** H).

Polyozellus pinophilus (Svantesson) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836143. UNITE SH: 1185292.08FU.
Basionym: *Pseudotomentella pinophila* Svantesson, *MycKeys* **50**: 36. 2019.

Typus: **Sweden**, Småland, Jönköping, Svarttorp, Ramlaklint, boreonemoral, mixed, old-growth forest, on soil with intermediate pH, 12 Sep. 2016, S. Svantesson 358 (**holotype** GB).

Polyozellus plurilobus (Svantesson) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836144. UNITE SH: 1185290.08FU.
Basionym: *Pseudotomentella pluriloba* Svantesson, *MycKeys* **50**: 39. 2019.

Typus: **Finland**, Uusimaa, Loviisa, Rutosinpyhtää, Marinkylä, rotten trunk on the ground (*Picea*), 30 Sep. 2010, U. Söderholm 4263 (**holotype** H 6018127).

Polyozellus rhizopunctatus (E.C. Martini & Hentic) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836148. UNITE SH: 1230075.08FU, 1230078.08FU.
Basionym: *Pseudotomentella rhizopunctata* E.C. Martini & Hentic, *Bull. Trimestriel Soc. Mycol. France* **119**: 20. 2003.

Typus: **Switzerland**, canton Tessin, Someo, sur écorce d'une branche de *Pinus sylvestris*, 14 Nov. 1998, E. Zenone em-6886 (**holotype** PC; **isotype:** LUG).

Polyozellus rotundisporus (Svantesson) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836149. UNITE SH: 1185296.08FU.
Basionym: *Pseudotomentella rotundispora* Svantesson, *MycKeys* **50**: 41. 2019.

Typus: **Sweden**, Västergötland, Götene, Medelplana, Eriksberg, boreonemoral, mixed forest on soil with high pH, 17 Oct. 2016, S. Svantesson 413 (**holotype** GB).

Polyozellus sciastrus (Svantesson & Køljalg.) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836150. UNITE SH: 1230076.08FU.

Basionym: Pseudotomentella sciastra Svantesson & Køljalg, *MycoKeys* **50**: 44. 2019.

Typus: Sweden, Småland, Jönköping, Svarttorp, Ramlaklint, boreonemoral, mixed, old-growth forest, on soil with intermediate pH, 12 Sep. 2016, S. Svantesson 359 (**holotype** GB).

Polyozellus sorjusensis (Svantesson) Svantesson & Køljalg, **comb. nov.** MycoBank MB 841513. UNITE SH: 1185284.08FU.

Basionym: Pseudotomentella sorjusensis Svantesson, *Phytotaxa* **497**: 71. 2021.

Typus: Sweden, Lule Lappmark, Jokkmokk, Sårjås N, low alpine heath on ground with intermediate pH, on underside of stone, 17 Aug. 2016, S. Svantesson 298 (**holotype** GB).

Polyozellus tristis (P. Karst.) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836151. UNITE SH: 1230077.08FU.

Basionym: Hypochnus subfuscus subsp. *tristis* P. Karst., *Meddeland. Soc. Fauna Fl. Fenn.* **9**: 71. 1883.

Typus: Finland, Tavastia australis [= Etelä-Häme], Tammela, Mustiala, ad Betulam, 19 Aug. 1865, P. A. Karsten (**lectotype** H 6018703 [Herbarium P. A. Karsten 3036]); **epitype: Sweden**, Västerbotten, Vännäs, Orrböle, boreal, mixed forest on soil with high pH, 28 Aug. 2015, S. Svantesson 193 (GB).

Polyozellus tristoides (Svantesson & K.H. Larss.) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836152. UNITE SH: 1230081.08FU.

Basionym: Pseudotomentella tristoides Svantesson & K.H. Larss., *MycoKeys* **50**: 52. 2019.

Typus: Norway, Nord-Trøndelag, Snåsa, Bergsåsen, boreal, deciduous forest on soil with intermediate pH, 28 Aug. 2012, K.-H. Larsson (**holotype** O F110306).

Polyozellus umbrinus (Fr.) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836153. UNITE SH: 1185280.08FU.

Basionym: Thelephora umbrina Fr., *Elench. fung.* **1**: 199. 1828, *nom. sanct.*

Typus: Sweden, Småland, Femsjö, E. Fries (**neotype** UPS F003106 [Herb. Fries]); **epitype: Sweden**, Småland, Hylte, Femsjö, Femsjö Church Nature Reserve, boreonemoral, mixed forest on soil with intermediate pH, 7 Sep. 2016, S. Svantesson 351 (GB).

Polyozellus umbrinascens (Svantesson) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836154. UNITE SH: 1185297.08FU.

Basionym: Pseudotomentella umbrinascens Svantesson, *MycoKeys* **50**: 60. 2019.

Typus: Sweden, Bohuslän, Tanum (municipality), Tanum (parish), Greby Kleva, boreonemoral, deciduous forest on soil with high pH, RT90: E1236840, N6518916, 6 Sep. 2016, S. Svantesson 335 (**holotype** GB).

Polyozellus vepallidosporus (M.J. Larsen) Svantesson & Køljalg, **comb. nov.** MycoBank MB 836155. UNITE SH: 1191930.08FU, SH1244119.08FU.

Basionym: Pseudotomentella vepallidospora M.J. Larsen, *Canad. J. Bot.* **45**: 1299. 1967.

Typus: USA, Washington, Quinault, Olympic Peninsula, on rotten conifer log, 15 Oct. 1958, J. L. Lowe 10368 (**holotype** BPI; **isotype**: SYRF).

DISCUSSION

Among homobasidiomycetes there is a general evolutionary trend from structurally simple, corticioid basidiomata with flat hymenia to more complex, stipitate forms with gills, tubes, etc., occasionally followed by reversions to simpler forms (Hibbett & Binder 2002, Larsson *et al.* 2004). Most of these transitions occurred early during fungal evolution and the complex forms have since become well separated from the lineages that have retained a simple basidiome morphology. However, in a few cases species with complex basidiome forms occur nested within the same genus as species with simple forms, for example in *Trechispora*, as currently circumscribed (Ryvarden 2002, Meiras-Otoni *et al.* 2021) and in *Thelephorales* seemingly also in *Tomentella/Thelephora* (Vizzini *et al.* 2016). Here it is documented for *Polyozellus/Pseudotomentella*.

The authors choose to recombine the type species of *Pseudotomentella* along with most other species previously placed in the genus to *Polyozellus*, thus delimiting the latter as a monophyletic genus including species with both stipitate and resupinate basidiomata. Two other alternatives would have been possible: either retaining *Pseudotomentella*, in the knowledge that is a paraphyletic genus with regards to *Polyozellus* or keeping both monophyletic and describing many small genera. The first option was not viewed as a fitting solution to the present situation, given the evolutionary reality of basidiome transitions among homobasidiomycetes and the fact that paraphyletic genera are inadvisable in general. A more acceptable way would perhaps have been to create many small genera. However, as already implied genera are a subjective, human construct, to ease our understanding of the relationship between species. In this context it is the view of the authors that it is easier to handle a genus with two distinct but readily identifiable morphologies than to deal with seven genera, where many display small and often overlapping morphology (e.g. the two different contenders for the name *Po. vepallidospora*). Given additional molecular data from currently undescribed species, this view might change but given that no undescribed species were retrieved from any UNITE Compound Clusters other than those containing the formally described *Polyozellus* and *Pseudotomentella* species included in this study, it does not currently seem likely that major changes to the present phylogeny would arise with the inclusion of such.

Four DNA regions, *RPB2*, mtSSU and the combined nrLSU and nrSSU, were found to be usable in delimiting *Polyozellus* vs. *Pseudotomentella*. Additional regions would have been preferable to account for e.g. incomplete lineage sorting, but given the lack of conflict with respect to the ingroup the current dataset was deemed sufficient for the purpose of this study. The ease of sequencing and high information content of the regions used make them good candidates for further systematic studies within *Thelephorales*. In addition, this result further testifies to the usability of the full tandem repeat as a molecular marker for fungi, especially in cases where the ITS region is too variable to be reliably aligned but the partial LSU region normally used contains too little information to infer a satisfyingly resolved phylogeny (e.g. Krehenwinkel *et al.* 2019, Wurzbacher *et al.* 2019, Bradshaw *et al.* 2020).

The precise phylogenetic identities of *Po. humicola*, *Po. mucidula*, *Po. rhizopunctata* and *Po. vepallidospora* are currently unknown. Given the unsuccessful sequencing efforts of the types of *Po. griseopergamacea* and *Po. mucidula*, epitypification or sequencing with other methods are ways of resolution that should be explored. However, since the several available candidates for these four species were all shown to belong in *Polyozellus*, as here delimited, lack of knowledge about their definite identity does not pose a problem for their recombination.

The phylogenetic meaning of the heterotypic names currently synonymised with *Po. flavovirens*, *Po. griseopergamacea* and *Po. mucidula* is also unclear. Many of these species, e.g. *Pseudotomentella fumosa*, *Ps. kaniksuensis*, and *Ps. molybdea*, are very similar to the species currently given nomenclatural priority and are therefore unlikely not to belong in *Polyozellus*. Even so, their possible recombination needs clarification of their status as separate species.

Pseudotomentella larsenii is clearly more closely related to *Tomentellopsis* than to *Polyozellus* and is hence not recombined into the latter. This decision means that *Polyozellus* remains a genus restricted to the Northern Hemisphere. The possible inclusion of *Ps. larsenii* in *Tomentellopsis* needs to be addressed by further taxon sampling.

Pseudotomentella armata, *Ps. ochracea* and *T. italica* could not be included in the analyses, since sequencing of several gene regions failed. Their phylogenetic placement thus remains to be revealed by further studies.

Concerning conservation, *Pseudotomentella* is a larger genus than *Polyozellus* and the recombination of its species into the latter hence warrants more name changes than if the opposite were to be pursued. However, since *Polyozellus* is both older and has a wider usage – as a food source and indicator of old-growth forest, in dyeing *etc.* – this was not pursued.

It is clear that the identities of many members of what was formerly *Pseudotomentella*, and now constitute corticioid *Polyozellus* species, are deficiently known and will need further study in order to be clarified. Many of these species are rare and probably threatened by extinction due to loss of old growth forest. It is therefore the hope of the authors that their phylogenetically merited incorporation in *Polyozellus* will bring renewed interest to them and thereby facilitate the amount of attention and study that they deserve.

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