The Lichenologist **48**(1): 3–12 (2016)
doi:10.1017/S0024282915000432

Collolechia revisited and a re-assessment of ascus characteristics in *Placynthiaceae* (*Peltigerales*, Ascomycota)

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Abstract: We investigated the phylogenetic relationships in the cyanolichen family *Placynthiaceae* to test the current generic delimitations, where the monotypic *Collolechia* is currently accepted as distinct, based on differences in ascospores, ascus apex characteristics and the leprose thallus. Bayesian and maximum likelihood phylogenetic analyses of two sequence marker datasets confirmed that *Collolechia caesia* is nested within *Placynthium*, and should be called *Placynthium caesium* (Fr.) Jatta. We reassessed the spore and ascus characteristics and showed that *Placynthium caesium* falls well within the variation in *Placynthium* and is thus yet another example of a species that differs from close relatives by its crustose-leprose thallus structure.

Key words: ascus structure, classification, lichens, morphology, phylogenetics, taxonomy

Accepted for publication 19 *August* 2015

Introduction

Lichenization is one of the most important life strategies among fungi in the Ascomycota, but only a comparatively small number of ascomycete lichen fungi (*c.* 1700 species; Rikkinen 2002) utilize cyanobacteria as photobionts. These lichens may still have a substantial impact on the ecosystems they inhabit by contributing fixed atmospheric nitrogen (Cornelissen *et al.* 2007; Nash 2008; Campbell *et al.* 2010), and many cyanobacterial lichens are sensitive to habitat disturbance such as changes in forest age, structure and composition (e.g. Price & Hochachka 2001; Scheidegger *et al.* 2002; Hedenås & Ericson 2008; Fedrowitz *et al.* 2012) and pollution (e.g. Goward & Arsenault 2000; Jovan 2008). The largest group of lichenized Ascomycota featuring cyanobacteria as the main or sole photobiont is *Peltigerales* in the Lecanoromycetes (Wiklund & Wedin 2003; Lumbsch *et al.* 2004; Wedin *et al.* 2005; Schoch *et al.* 2009; Miądlikowska *et al.* 2014; Rikkinen 2015). *Peltigerales* are a comparatively recent group of fungi, the ancestor of which diverged from its Lecidealean sister-group in the early Jurassic, and the group diversified towards the end of the Jurassic-early Cretaceous (Prieto & Wedin 2013). *Peltigerales* currently includes ten families (Wedin *et al.* 2007, 2011; Spribille & Muggia 2013), one of which is *Placynthiaceae*.

Placynthiaceae comprises comparatively small, flat, rosette-forming, crustose to squamulose-lobate species where the thalli often produce a prothallus (Czeika & Czeika 2007; Jørgensen 2007). After the recent reclassification of *Vestergrenopsis* into the newly described family *Koerberiaceae* (Spribille & Muggia 2013), *Placynthiaceae* currently includes two genera: *Placynthium* with *c.* 30 species worldwide, and the monotypic *Collolechia*. *Placynthium* and *Collolechia* differ in thallus structure (squamulose vs. leprose-crustose thallus), ascus apex structure (apical caps/sheets vs. apical ring/tube) and spore shape and septation

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(ellipsoid, 1–3 septate in *Placynthium* vs. elongate, fusiform-acicular, pluriseptate in *Collolechia*; Jørgensen 2005, 2007). These observations were made on *Placynthium nigrum* (the type for *Placynthium*), and *Collolechia caesia*, respectively (Jørgensen 2005). Several authors, however, have observed tube structures in the asci of *Placynthium*, including in *P. nigrum* (Keuck 1977; Rambold & Triebel 1992; Gilbert & James 2009; Øvstedal *et al.* 2009; Wirth *et al.* 2013). The presence of a tube in *Placynthiaceae* was pointed out as supporting the sister-group relationship with *Collemataceae* by Wiklund & Wedin (2003). Several authors have also noted large variation within the group, including cap- or sheet-like structures, and tube- or ring-structures (Czeika & Czeika 2007; Spribille & Muggia 2013).

In Scandinavia, *Collolechia caesia* was for many years confused with *Placynthium garovaglioi* (Fig. 1). The distinction between the species was clarified by Jørgensen (2005) who showed that in Scandinavia *C. caesia* was known only from a couple of localities on the Baltic island of Gotland, where it had not been collected since 1942. These are northern outposts for a species with an otherwise mainly southern warm-temperate distribution. True *Placynthium garovaglioi* is not known from Sweden. In 2014, we visited some of the known Swedish localities to assess the status of *C. caesia*, and collected fresh material for DNA-based studies. In this study, we investigated the phylogenetic relationships of *Collolechia* to test the current generic delimitations in *Placynthiaceae*. We reinvestigated herbarium material of

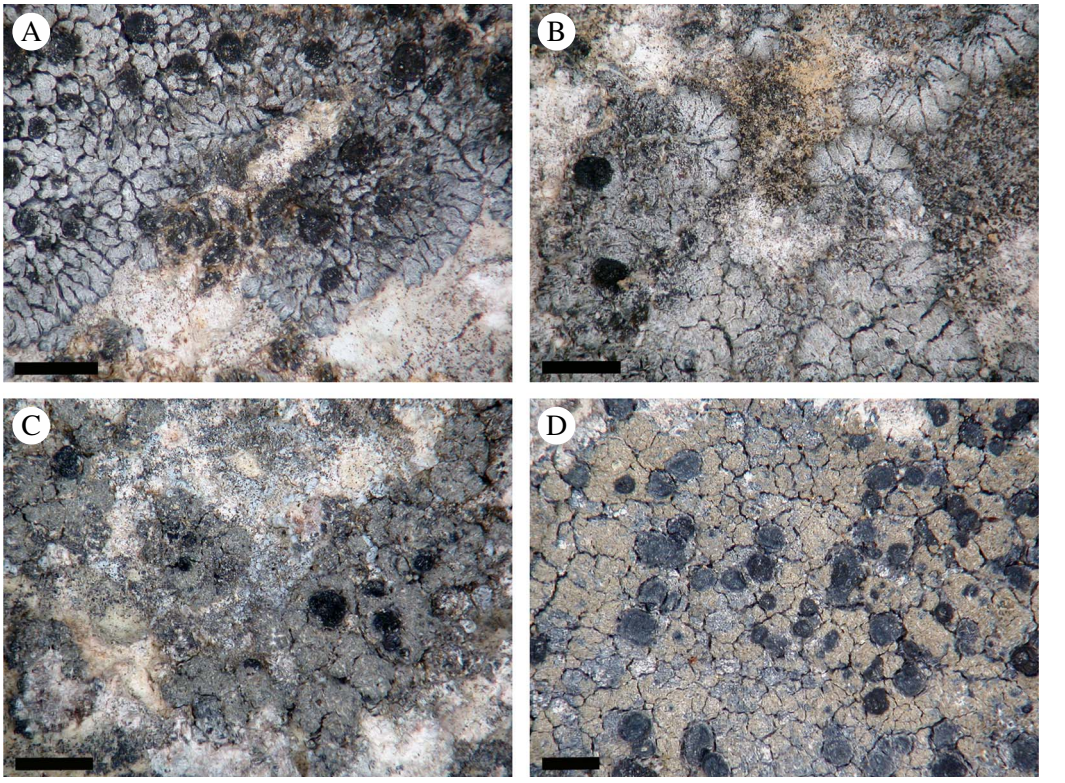

FIG. 1. Morphology of *Placynthium garovaglioi* and "*Collolechia*" *caesia*. A & B, thallus of *Placynthium garovaglioi* with distinct marginal lobes; C & D, "*Collolechia*" *caesia* with a leprose thallus lacking marginal lobes. Specimen origin. A, *Palice* 16564 (SAV); B, *Palice* 16954 (S); C, *Košuthová* GOT2 (S); D, *Cleve* s. n. (S). Scales = 1 mm. In colour online.

https://doi.org/10.1017/S0024282915000432 Published online by Cambridge University Press

Collolechia and *P. garovaglioi*, and studied the spore and ascus apex characteristics in these species and other selected *Placynthiaceae* in order to assess variation and potential natural groupings.

Material and Methods

Taxon sampling and morphological analysis

Material utilized for the phylogenetic study was mainly freshly collected specimens but was also supplemented with herbarium material from S, SAV and UPS and the personal herbaria of Z. Palice (hb. Palice) and J. Malíček (hb. Malíček). Origin of the material is summarized in Table 1. Asci were studied under oil-immersion, in hand-cut sections of apothecia which were pretreated with and squashed in KOH and subsequently stained with Lugol's solution.

DNA extraction, amplification and sequencing

Total DNA was extracted from fresh material and herbarium specimens, and isolated using the DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's instructions. We amplified ≈0·6 kb of the small subunit of the mitochondrial rDNA (mtSSU) and ≈1·2 kb of nuclear mini-chromosome maintenance complex component 7 (*Mcm7*). Primer combinations used in this study were: mrSSU1 and mrSSU3R (Zoller *et al.* 1999) for the mtSSU; and MCM7-709for and MCM7-1348rev for the *Mcm7* (Schmitt *et al.* 2009). Symmetric PCR amplifications were performed using Illustra^{TM} Hot Start PCR beads, according to the manufacturer's instructions. PCR reactions for mtSSU were performed using one of two cycling conditions, depending on what worked with particular samples. The first was 95 °C for 5 min followed by 35–40 cycles (95 °C for 1 min, 54 °C for 50 s, and 72 °C for 1 min), with a final extension of 72 °C for 8 min. The second was as follows: 95 °C for 5 min followed by 4 cycles (95 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min), 4 cycles (95 °C for 1 min, 56 °C for 1 min, and 72 °C for 1 min) and 34 cycles (95 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min) with a final extension of 72 °C for 8 min. For the amplification of the *Mcm7*, the following cycling conditions were used: 95 °C for 5 min followed by 4 cycles (95 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min), followed by 36 cycles (95 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min), with a final extension of 72 °C for 8 min. After examination by gel electrophoresis, amplification products were purified using ExoSAP-IT (USB Corp., USA). Sequencing of both

TABLE 1. *Specimen information and European Nucleotide Archive or GenBank accession numbers for the specimens included in the phylogenetic inference depicted in Figure 2. Sequences represented in bold font were generated in this study.*

			Accession number	
Species name	Isolate no.	Geographical origin, voucher	mtSSU	*Mcm7*
Collolechia caesia	AL147	Turkey, *Yazici* 1037 (S)	**LN876659**	**LN876678**
C. caesia	AL149	Sweden, *Košuthová* GOT1 (S)	**LN876660**	**LN876679**
C. caesia	AL150	Sweden, *Košuthová* GOT2 (S)	**LN876661**	**LN876680**
Placynthium asperellum	AL73	Sweden, *Westberg* (S F263269)	**LN876662**	**LN876681**
P. flabellosum	AL75	Norway, *Nordin* 5666 (UPS)	**LN876663**	**LN876682**
P. garovaglioi	AL146	Turkey, *Yazici* 1123 (S)	**LN876664**	**LN876683**
P. garovaglioi	AL148	Turkey, *Yazici* 1125 (S)	**LN876665**	-
P. garovaglioi	AL93	Slovakia, *Palice* 16564 (S)	**LN876666**	**LN876684**
P. garovaglioi	AL94	Slovakia, *Palice* 16954 (S)	**LN876667**	**LN876685**
P. hungaricum	AL95	Slovakia, *Palice* 12746 (hb. Palice)	**LN876668**	**LN876686**
P. hungaricum	AL96	Romania, *Malíček* 5625 (hb. Malíček)	**LN876669**	**LN876687**
P. nigrum	AL100	Czech Republic, *Svoboda* (S)	**LN876670**	**LN876688**
P. nigrum	AL79	Sweden, *Nordin* 5860 (UPS)	**LN876671**	**LN876689**
P. pannariellum	AL81	Sweden, *Nordin* 5787 (UPS)	**LN876672**	**LN876690**
P. pulvinatum	AL80	Sweden, *Westberg* (S F263268)	**LN876673**	**LN876691**
P. rosulans	AL76	Sweden, *Hermansson* 16011 (UPS)	**LN876674**	**LN876692**
P. rosulans	AL77	Norway, *Nordin* 5671 (UPS)	**LN876675**	**LN876693**
P. sp. A	AL91	Spain, *Malíček* 5616 (hb. Malíček)	**LN876676**	**LN876694**
P. sp. B	AL97	Albania, *Malíček* 4273 (hb. Malíček)	**LN876677**	**LN876695**
Collema nigrescens			EU982563	JX992989
Lobaria pulmonaria			AY340504	JX000169
Pannaria rubiginosa			AY340513	JX993042
Scytinium lichenoides			DQ923120	JX993021
Staurolemma omphalarioides			EU982560	JX993043

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strands was performed with the Big Dye Terminator technology kit v3.1 (ABI PRISM, USA) using the PCR primers, and the additional internal PCR primers mrSSU2 and mrSSU2R (Zoller *et al.* 1999).

Sequence alignments and analyses

Sequence fragments were assembled and edited using Sequencer 4.9 (Gene Codes Corp., Ann Arbor, MI) and Geneious version R8 (http://www.geneious.com, Kearse *et al.* 2012), and were subjected to BLAST searches (Zhang *et al.* 2000) for a first identity verification. Sequences were aligned manually in Aliview 1.09 (Larsson 2014). Introns and ambiguously aligned regions (*sensu* Lutzoni *et al.* 2000) were delimited manually and excluded from the analyses. We analyzed the mtSSU and *Mcm7* datasets separately using maximum likelihood (ML) as the optimization criterion, with GARLI v.2.0 (Zwickl 2006). Models of molecular evolution were estimated for each locus using the Akaike information criterion correction for finite sample sizes (AICc; Akaike 1973) implemented in jModeltest v.0.1.1 (Guindon & Gascuel 2003; Posada 2008). The models selected were TVM+I+G (Posada 2003) for mtSSU, TrNef+I (Tamura & Nei 1993) for *Mcm7* first codon position, F81+I (Felsenstein 1981) for *Mcm7* second codon position, and K80+G (Kimura 1980) for *Mcm7* third codon position. We performed ML searches setting the program to stop after 10 000 generations if no improvement of the Ln likelihood ≤0·01 was detected, with a maximum of 500 000. Topological incongruence between the two datasets was examined using the consensus trees from 1000 replicates of ML bootstrapping under the same models, on each locus separately (Mason-Gamer & Kellogg 1996). Because no incongruence was detected using a 70% reciprocal threshold, the two alignments were concatenated and one specimen (*Placynthium garovaglioi* AL148) for which we have only the mtSSU sequence included. The concatenated alignment was deposited in TreeBASE (accession number S18034).

Phylogenetic relationships and confidence were inferred on the combined dataset using ML and Bayesian inference (B). For the ML analysis, the same settings were used as in the individual gene analyses using GARLI v.2.0, with the same models specified for each partition, for both ML search and ML bootstrap analyses. The Bayesian inference of the phylogeny was carried out by a Metropolis coupled Markov chain Monte Carlo (MCMCMC), as implemented in MrBayes 3.2.3 (Ronquist *et al.* 2012). The substitution models estimated using the AICc implemented in jModeltest v.0.1.1 were GTR+I+G (Tavaré 1986) for mtSSU, SYM+I (Zharkikh 1994) for *Mcm7* first codon position (MCM7_c1), F81+I for *Mcm7* second codon position (MCM7_c2), and K80+G for *Mcm7* third codon position (MCM7_c3). The prior distributions settings were: all topologies equally probable and branch lengths followed an unconstrained gamma distribution (1, 0·1, 1, 1); the state frequencies followed a (1, 1, 1, 1) Dirichlet distribution for mtSSU and MCM7_c2 and were equally probable for MCM7_c1 and MCM7_c3; the rate matrix for mtSSU and MCM7_c1 followed a (1, 1, 1, 1, 1, 1) Dirichlet distribution, for the transition-transversion rates for MCM7_c3 a beta (1, 1) distribution and was equally probable for MCM7_c2; when applicable, proportion of invariable sites followed a uniform distribution (0, 1). Two parallel runs with four independent chains each were conducted for 20 million generations, with trees sampled at intervals of 500 generations. A burn-in sample of the first 10 000 trees was discarded for each run and the remaining trees were used to estimate branch lengths and posterior probabilities (PP). Convergence was monitored with the diagnostic tool provided by MrBayes 3.2.3., including the average standard deviation of splits between runs. All analyses were run in the CIPRES Science Gateway (Miller *et al.* 2010).

Hypothesis testing

We specified two hypotheses to be tested. One equals the classification where *Placynthium* and *Collolechia* are two separate accepted genera (H_0: *Placynthium* monophyletic excluding *Collolechia*). The alternative hypothesis (H_1) corresponds to the case where *Collolechia* is nested within a paraphyletic *Placynthium*. In order to contrast the hypotheses, we calculated Bayes factors by comparing the ratio of the marginal likelihoods of each hypothesis. One common approach is to estimate the marginal likelihoods from constrained and unconstrained Bayesian analyses (e.g., Nelsen & Gargas 2009; Otálora *et al.* 2014; Westberg *et al.* 2015). Here, however, we followed the novel approach proposed in Bergsten *et al.* (2013), in which the marginal likelihoods are calculated from two alternative topologies after the specification of equally informed priors (constraints). Interpretation of Bayes factor values followed Kass & Raftery (1995). We calculated the marginal likelihoods using the stepping-stone sampling algorithm implemented in MrBayes 3.2.3., which has proved to be a more accurate estimator of the model likelihoods than the harmonic mean estimator calculated in the MCMC output (Ronquist *et al.* 2011; Bergsten *et al.* 2013). We ran the stepping-stone sampling taking 50 steps for a total of 10 200 000 generations, sampling every 100th generation, and discarding the first 200 000 generations as burn-in. The contribution to the marginal likelihood in each step was estimated from a sample size of 2000.

Results

New sequences from two loci were produced for the 19 specimens, except for *Placynthium garovaglioi* AL148 for which only mtSSU was obtained. These were aligned with 10 sequences from five taxa representing several families of the order *Peltigerales* (i.e., *Collemataceae*, *Lobariaceae* and *Pannariaceae*), retrieved from GenBank. *Lobaria pulmonaria* was selected as outgroup to root the tree.

https://doi.org/10.1017/S0024282915000432 Published online by Cambridge University Press

Voucher information for newly produced sequences and accession numbers are listed in Table 1. The matrix of aligned sequences included 1588 sites (635 for *Mcm7* and 953 for mtSSU), which was reduced to 1311 sites (of which 297 were parsimony-informative) after the exclusion of the flanking primer regions, introns, and ambiguously aligned regions.

The most likely tree from ML (Fig. 2) with ln likelihood = −6126·8160 recovered a topology with 22 resolved internodes, of which 17 were significantly supported (i.e., ML-BS ≥ 70%). In the Bayesian analysis, the value of the standard deviation of splits between runs was 0·000632, below the threshold of 0·01 established for convergence (Ronquist *et al.* 2011). This was further confirmed as the PSRF of all parameters and bipartitions was close to 1·0. The 50% majority-rule consensus trees of the 60 000 trees showed 21 resolved internodes, of which 14 were significantly supported (PP ≥ 0·95). As the topologies had no significant conflicts, only the ML tree is shown in Fig. 2, with the support indicated for both analyses. In both phylogenetic analyses, *Collolechia* was recovered as monophyletic with strong support (BS = 95%, PP = 1·00), and nested within *Placynthium* (BS = 99%, PP = 1·00).

The two independent runs for each stepping-stone MCMC sampling conducted to calculate the marginal likelihood of each alternative topology reached convergence, as shown by the values of split frequencies <0·01. The estimation of the marginal log

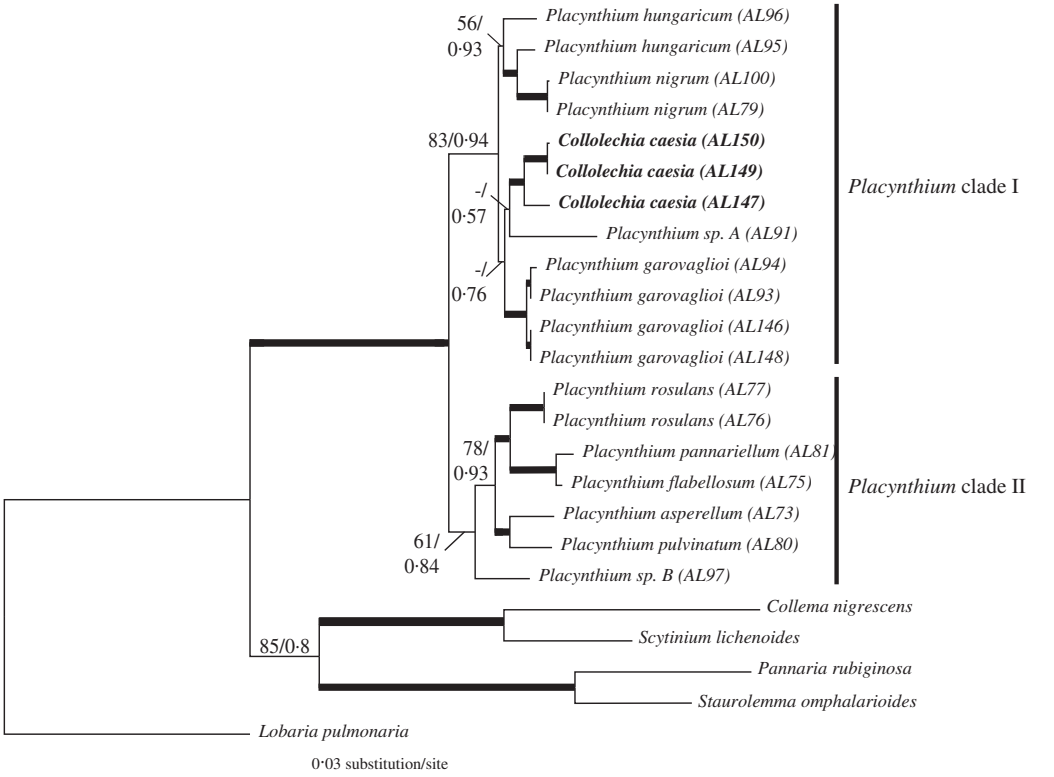

FIG. 2. Most likely tree (ln likelihood = −6126·8160) based on a combined matrix of mtSSU and *Mcm7* showing *Collolechia* nested within *Placynthium*. Internodes with bootstrap values ≥70% and posterior probabilities ≥0·95 are represented by thick lines. Numbers above other internodes indicate ML bootstrap support (only when values ≥50%) followed by posterior probabilities (only when values ≥0·5%) for the Bayesian analysis.

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likelihood was −6123·60 for the hypothesis in which *Placynthium* was constrained to be monophyletic and −6114·30 for the alternative hypothesis in which *Collolechia* was nested within *Placynthium*. The Bayes factor value was 18·6. We compared this value to the reference table provided by Kass & Raftery (1995, p.777) that states $2 \times \log_e (BF_{10})$ values >10 as strong evidence against H_0.

Discussion

Here, we show that *Collolechia* is clearly nested within *Placynthium* (Fig. 2), which is composed of two monophyletic subgroups, one of which contains both *Collolechia* and the type species of *Placynthium*, *P. nigrum*, as well as *P. garovaglioi*, *P. hungaricum*, and the potentially undescribed *Placynthium* sp. A. As a consequence, *Collolechia caesia* should be classified in *Placynthium*. The nomenclature of "*Collolechia*" *caesia* is complicated, but fortunately Jørgensen (2005) has clarified the situation and the reader is referred to this work for details regarding author citation, typification, and synonymy. When treated in *Placynthium*, the correct name for this species is *Placynthium caesium* (Fr.) Jatta.

Placynthium caesium (Fr.) Jatta

Syll. Lich. Ital.: 38 (1900).—*Lecidea contigua* var. *caesia* Fr., *Lich. Eur.*: 302 (1831).—*Collolechia caesia* (Fr.) A. Massal. *Geneac. Lich.*: 7 (1854); type: (France) Gallia merid., *Dufour* (UPS!— lectotypus, designated by Jørgensen 2005).

This re-synonymization contradicts the suggestion by Jørgensen (2005), who justified treating the two genera as distinct based on their different ascus, spore and thallus characteristics. The ascus characters are difficult to study in many *Placynthium* species; the asci are small and the structures indistinct, and the variation between ascus developmental stages within one hymenium is frequently quite confusing. We can still confirm that several *Placynthium* species do have a tube structure in their asci, a trait considered characteristic of "*Collolechia*" (Jørgensen 2005). *Placynthium caesium*, *P. garovaglioi*, *P. hungaricum*, *Placynthium* sp. A, and *P. nigrum* (the type of *Placynthium*) have a distinct amyloid tube structure in the ascus apex (Fig. 3). Spribille & Muggia (2013) provided a very useful overview of the ascus structures in *Peltigerales*, and included the amyloid tube structure in the *Micarea*-type. A tube structure is reported in a number of *Placynthium* species by Keuck (1977) and Czeika & Czeika (2007), and in the *Collemataceae* (Rambold & Triebel 1992), and hence could be seen as a synapomorphy for the *Placynthiaceae* and *Collemataceae* (Wiklund & Wedin 2003). The tube in *Placynthium* is frequently flaring and the apical opening is often visible only from above (Fig. 3A). Our observations confirm that other *Placynthium* species sampled here (i.e. *P. asperellum*, *P. flabellosum*, and *P. rosulans*) have an amyloid cap-like structure (Fig. 3D), corresponding to the *Vahliella*-type of Spribille & Muggia (2013), as previously reported by Keuck (1977) and Spribille & Muggia (2013). The asci in the potentially undescribed *Placynthium* sp. B were very difficult to interpret and we await more material to study this further. We have not found any apothecia in the samples of *P. pannariellum* and *P. pulvinatum*, both of which are very rarely fertile. Although we have only investigated a fraction of the species in *Placynthium*, each ascus character state is correlated with one of the two monophyletic groups identified within the genus. This, however, needs further study to confirm.

All investigated samples of *Placynthium caesium* and *P. garovaglioi* have long spores (*c.* 26–38 × 3–5 µm in *P. caesium* and *c.* 24–35 × 3·5–6·5 µm in *P. garovaglioi*) with 3(–5) septa. The difference in spore length between the two species appears less conspicuous than proposed by Jørgensen (2005). Long, pluriseptate spores are also produced in several other *Placynthium* species, and in our material, *P. flabellosum*, *P. rosulans*, and *Placynthium* sp. B have more than one septum. *Placynthium pulvinatum* which is shown here (Fig. 2) to be only distantly related to *P. caesium* is, according to the original description (Øvstedal *et al.* 2009), another species with long, pluriseptate

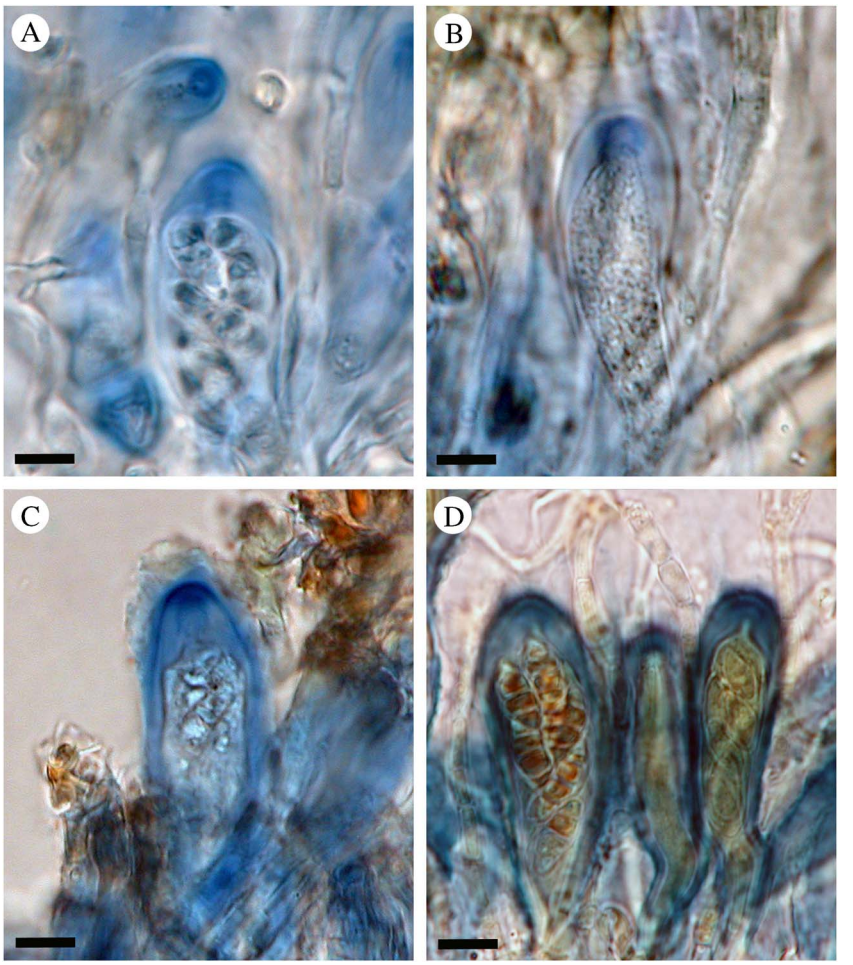

FIG. 3. Ascus characteristics in *Placynthium*. A–C, tube structures of the "*Micarea*-type"; D, cap-like structure of the "*Vahliella*-type". A, *Placynthium nigrum*, type species of *Placynthium* (*Nordin* 5860, UPS); B, *Placynthium* ("*Collolechia*") *caesium* (*Košuthová* GOT2, S); C, *Placynthium garovaglioi* (*Palice* 16954, S); D, *Placynthium flabellosum* (*Nordin* 5666, UPS). Scales = 10 µm. In colour online.

spores, again suggesting that this character state is widespread in the genus. *Placynthium nigrum* has shorter (*c.* 10–12 × 5 µm), mainly 1(–3)-septate spores.

In conclusion, when the phylogeny suggests that the two groups are not distinct and the claimed differences in ascus and spore do not hold up to scrutiny, it appears that "*Collolechia*" *caesia* should be better treated as a *Placynthium* species with a crustose-leprose thallus structure. It is not unusual to find examples of closely related lichens, including cyanolichens, which differ in thallus structure. *Caloplaca chrysodeta* and *Micarea leprosula* (Tønsberg 1992) are both examples of leprose representatives in green-algal crustose genera, and the cyanolichen "*Moelleropsis*" *nebulosa* was recently shown to be a leprose *Fuscopannaria* (Ekman *et al.* 2014). Also, in the spore and ascus characteristics, *Placynthium caesium* is not unique compared to other *Placynthium* species and the results of the molecular phylogeny are consistent with the morphology.

https://doi.org/10.1017/S0024282915000432 Published online by Cambridge University Press

Material investigated (Placynthium caesium): **France:** Gallia merid., *Dufour* (UPS L104293, lectotype).—**Germany:** *Bayern*: ad saxa jurassica prope in valle Wiesentthal Bavariae, *Arnold* s. n., (S F155260, F155943); Obersdorf (Tiefenbach) in Algäu, *Rehm* s. n. (S F155268); Streitberg Oberfranken, 1865, (S F15524); Eichstätt, 1956, (S F155248); Muggendorf, *Arnold* s. n. (S F155266); 1954, (S F155259).—**Italy:** *Massalongo* s. n. (S F155283).—**Slovakia:** Žilinský kraj, Kraľovany, 1882, *Lojka* s. n. (S F155283); Muránska planina Mts, Pohronská Polhora – Bánovo, 2014, *Guttová & Fačkovcová* s. n. (SAV).—**Sweden:** *Gotland*: *Ardre* par., Tviburg (v. Torsburgen), 1943, *Degelius* s. n. (S F155213); 1963, *Degelius* s. n. (UPS L159251); *Hangvar* par., Ire, *Floderus* (UPS L130317); Irevik, 2014, *Košuthová* GOT2 (S); *Kräklingbo* par., Torsburgen, 1857, *Stenhammar & Floderus* s. n. (S F155224); 1857, *Lönnroth* s. n. (S F155216, F155217, F155221); 1864, *Cleve* s. n. (S F155209, UPS L137454); 1871, *Molér* s. n. (S F155218, S F155215, UPS L137459); 1874, *Elmqvist* s. n. (S F155225, UPS L137458); 1880, *Blomberg* s. n. (S F155223; UPS L137452); 1889, *Hellborn* s. n. (S F155222); 1918, *Malme*, Malme, Lich. Suec. Exs no 743 (S F155214, UPS L109332); 2014, *Košuthová* GOT1 (S); Lojsta par., Lojsta, *Lönnroth* s. n. (S F155210); Lojstabergen, *Lönnroth* s. n. (S F155934; UPS L137455); Stenkyrka par., Lickershamn, 1869, *Laurer* s. n. (UPS L137460); *Vesterheide* par., Hallbros alvar, 1917, *Malme* (S F155220); 1918, *Malme* (S F155211); Kneippbyn, 1918, *Magnusson* 2330 (UPS L160437); Kneippbyn, 2014, *Košuthová* GOT3 (S).—**Switzerland:** Schwyz, Pilatus, *Hegetschweiler & Hegetschweiler* s. n. (S F155227).—**Turkey:** Bucak, 2013, *Yazici* 1037 (S).

Material investigated for comparison (Placynthium garovaglioi): **Slovakia:** W. Carpathians, Dolina Siedmych, 2013, *Palice* 16564 (SAV); W. Carpathians, Tisovec, 2013, *Palice* 16954 (S).—**Spain:** *Navarra*: Estella, 1983, *Santesson* 30775 (UPS L160445).—**Poland:** *Malopolskie*: Tatry, Giewont 1954, *Tobolewski* 76 (UPS L160450).—**Turkey:** *Burdur*: 2012, *Yazici* 1123 (S), 1125 (S).

This study was supported by funding from the Swedish Research Council grant 621-2012-3990, to M. Wedin. M. Westberg was funded by the Swedish Taxonomy Initiative, and A. Košuthová was funded by Štefan Schwarz Fund SAS, and EEA and Norway grants no. NF-CZ07-ICP-3-104-2015. We are grateful to the staff at the Molecular Systematics Laboratory at the Swedish Museum of Natural History, in particular Bodil Cronholm, for laboratory assistance. We thank Dr Johannes Bergsten for his helpful discussions and guidance regarding the hypothesis testing analyses. The staff at S, SAV, and UPS, and Z. Palice and J. Malíček, are gratefully thanked for the loan of material, and A. Aptroot kindly gave us samples from Turkey.

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