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Reassessing evolutionary relationships in the filamentous cyanolichen genus *Spilonema* (*Peltigerales*, Lecanoromycetes)

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Abstract: *Spilonema* was originally described to accommodate an unusual group of cyanolichens with thread-like, cushion-forming thalli, and has long been placed in *Coccocarpiaceae* based on ascomatal development. However, *Spilonema* is the only genus of *Peltigerales* to include species lichenized with the cyanobacterial genus *Stigonema*, and the evolutionary relationships of *Spilonema* to other genera in the family have yet to be tested using molecular data. We present evidence from combined nuclear 28S, 18S and mitochondrial 12S rDNA to confirm the placement of the core species of *Spilonema* (*S. paradoxum* and *S. revertens*) in *Coccocarpiaceae*. Our data further show that despite possessing a different genus of photobiont (*Scytonema*), the north Pacific endemic genus *Spilonemella* must be included within *Spilonema*, suggesting that closely related species of the genus have changed photobionts in the course of evolution. However, we recovered *Spilonema dendroides*, one of the only lichens known to associate with the cyanobacterial genus *Hyphomorpha*, as only distantly related to the *Coccocarpiaceae*. The evolutionary relationships of this species are as yet unclear but it may occupy a basal position in the *Peltigerales*. We create for this species the new genus *Erinacellus* T. Sprib., Muggia & Tønsberg.

Key words: body plan, cyanobacteria, *Erinacellus*, lichenization, photobiont, phylogenetics

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Introduction

Spilonema paradoxum was described by Bornet (1856) as a new genus and species to accommodate an enigmatic, thread-like, caespitose lichen occurring on granite in the mountains around Cannes in the south of France. In a lengthy discussion of the thalline and ascomatal morphology of the species, Bornet drew comparisons to the cyanobacterial genus *Stigonema* which was later revealed to be its photobiont, as well as the similarity to the lichen genus *Ephebe*, described thirty years prior by Fries (1825). The new species did not fit into either of these groups, nor *Collema* or *Leptogium*, as Bornet noted. *Spilonema* was later expanded to include a second species, *S. revertens*, by Nylander (1865) and then two species from the Asia-Pacific region almost a century later (Henssen 1963). In the latter work, the genus was placed in the Peltigeralean family *Coccocarpiaceae*, a placement later expounded in more depth based on anatomical studies of the ascomata (Henssen & Jahns 1973; Keuck 1977; Henssen *et al.* 1981). The family *Coccocarpiaceae* was, however, not nomenclaturally validated until later (Eriksson & Hawksworth 1986: 314).

The family *Coccocarpiaceae*, as defined by Henssen *et al.* (1981), occupies a special position among Lecanoromycetidae as a hot spot of body plan innovation and photobiont

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We dedicate this paper to Brian J. Coppins, friend and mentor, on the occasion of his 65th birthday.

affinity. Relatively few genera are assigned to *Coccocarpiaceae*, but those that are could hardly appear more different in gross morphology: a genus with a 'classical' dorsiventral, longitudinal, broadly attached body plan (*Coccocarpia*), a dorsiventral, umbilicate body plan (*Peltularia*), and two genera with thread-like thalli that form cushions (*Spilonemella* and *Spilonema*). What is more, these four genera as currently circumscribed include species that associate with photobionts deriving from four different cyanobacterial families: *Nostoc* (*Nostocaceae*, *Nostocales*; in *Peltularia crassa*), *Scytonema* (*Scytonemataceae*, *Nostocales*; in *Coccocarpia*, *Spilonemella*), *Stigonema* (*Stigonemataceae*, *Stigonematales*; in *Spilonema*) and *Hyphomorpha* (*Loriellaceae*, *Stigonematales*; in *Spilonema*), the last being the only known case of its occurrence in lichens (Henssen 1981). The genera assigned here also span the widest possible macroclimatic gradient, from polar permafrost soils to tropical rainforests. (A fifth lichen genus previously assigned to *Coccocarpiaceae*, *Steinera*, associated in part with *Nostoc* and *Scytonema*, has since been shown not to belong to *Coccocarpiaceae*; Spribille & Muggia 2013).

Recent molecular studies have not always lent support to past classifications of fungal genera with similar body plans but different photobionts. An example of this is *Polychidium*, treated in the same classical work by Henssen (1963) as uniting two groups of species that associated with *Nostoc* and *Scytonema*, respectively. Muggia *et al.* (2011) found in a multilocus phylogeny that the two groups are in fact only distantly related and achieved their dendroid thallus architecture through body plan convergence. This, and the finding that ascomatal ontogeny is not always a reliable predictor of relatedness, cast doubt on the circumscription of *Coccocarpiaceae* and the position of one of its key genera, *Spilonema*. *Spilonema* is furthermore the only genus of *Peltigerales*, and one of only a few *Lecanoromycetes*, to include species lichenized with *Stigonema* (illustrated in detail by Henssen 1963). *Spilonema* has not heretofore been sampled in a molecular phylogeny and fresh material can be challenging to acquire, especially in central Europe, where most collections are historical.

The present paper is the third in a series in which we test classical evolutionary hypotheses within the *Peltigerales* based on new molecular data, with special emphasis on small, often overlooked and poorly sampled species. In the present case, we assess three of the four described members of *Spilonema*, including the rare *Hyphomorpha*-associated species *S. dendroides*, as well as their relationship to the Pacific Rim endemic genus *Spilonemella* (Muggia *et al.* 2011). The phylogenetic relationships of these species to each other, and in the context of the *Peltigerales*, can be expected to inform views on photobiont and body plan diversity in *Coccocarpiaceae* and lay the groundwork for hypothesis testing in character evolution.

Materials and Methods

Taxon sampling

We sampled a total of 51 taxa representing all ten recognized families of the *Peltigerales* according to recent studies (Wedin *et al.* 2007, 2009, 2011; Muggia *et al.* 2011; Spribille & Muggia 2013). We acquired sequences for a total of 14 new isolates including one of *S. paradoxum*, six of *S. revertens* s. str., six of *S. dendroides* and one of what appears to be an undescribed taxon from British Columbia, Canada. The material studied is in the herbaria cited, following abbreviations used in Index Herbariorum.

DNA extraction, amplification and sequencing

DNA was extracted according to Cubero *et al.* (1999). The phylogenetic affiliation of the lichen mycobionts was studied with sequences of the nuclear 28S, partial nuclear 18S and mitochondrial 12S ribosomal subunits (hereafter 28S, 18S and mitochondrial 12S). The 28S fragment was obtained in two pieces using primers ITS1F (Gardes & Bruns 1993) and LR5 for the first half, and LR7 (Vilgalys & Hester 1990) and LR3R for the second (http://www.biology.duke.edu/fungi/mycolab/primers.htm). 18S was amplified using nuSSU0072 and nuSSU0852 (Gargas & Taylor 1992), and mitochondrial 12S was obtained with mtSSU1 and mtSSU3R (Zoller *et al.* 1999). PCR conditions were as in previous studies (Muggia *et al.* 2010, 2011). Complementary strands were sequenced by Macrogen Inc. (Korea) and were subjected to a blastn query against NCBI non-redundant nucleotide database (nr/nt) to confirm sequence similarity to *Peltigerales* and rule out association with other fungal groups such as Lichinomycetes and *Arctomiaceae* (Ostropomycetidae), species of which in the latter case

Table 1. *Newly analyzed specimens included in the phylogenetic analysis. Dashes stand for absence of sequence data*

Isolate	Taxon	Locality	GenBank Accession Numbers 28S	18S	mitochondrial 12S
L752	*Erinacellus dendroides*	USA, Alaska, Klondike Gold Rush National Historical Park, Chilkoot Trail, 3 August 2008, *T. Spribille* 27164 (KLGO)	—	KC893692	KC893683
L753	*E. dendroides*	USA, Alaska, Klondike Gold Rush National Historical Park, Chilkoot Trail, 28 July 2008, *T. Spribille* 26660 (KLGO)	KC893674	—	KC893682
L754	*E. dendroides*	USA, Alaska, Klondike Gold Rush National Historical Park, Chilkoot Trail, 30 August 2008, *T. Spribille* 28808 (KLGO)	KC893673	KC893691	KC893681
L755	*E. dendroides*	USA, Alaska, Klondike Gold Rush National Historical Park, Saintly Hill, 7 October 2007, *T. Spribille* 24968 (KLGO)	KC893672	—	—
L1728	*E. dendroides*	USA, Alaska, Glacier Bay National Park and Preserve, meadows at main entrance, on *Pinus contorta*, 5 September 2011, *T. Spribille* 36301 (GZU)	KC893671	KC893690	KC893680
L1729	*E. dendroides*	USA, Alaska, Glacier Bay main entrance, corticolous on *Pinus contorta*, 5 September 2011, *T. Spribille* 36300 (GZU)	KC893670	KC893689	KC893679
L863	*Spilonema paradoxum*	Greece, Epirus, Valia Kalda, near Vovousa, above Aoos River, on serpentine, 5 July 2005, *T. Spribille* 15920 (GZU)	—	—	KC893684
L943	*S. revertens*	Canada, British Columbia, central interior, slopes below Natural Bridge, *c.* 8 km north of Clearwater: 51°42′N, 120°01′W, 15 June 2009, *T. Goward* 09-628 (UBC)	KC893669	—	—
L919	*S. revertens*	USA, Alaska, Denali National Park, trail to Mt. Healy, 63°44·439′N, 148°57·193′W, 19 August 2008, *T. Spribille* 27944 *& C. Hampton-Miller* (GZU)	KC893668	—	—
L1727	*S. revertens*	USA, Montana, Sanders Co., Flathead River 1·6 km upstream of Perma bridge along Hwy. 200, on diabase sill, 47°21′43·60″N, 114°33′55·62″W, 9 March 2012, *T. Wheeler* 3798a (GZU)	KC893667	KC893688	KC893678
L1731	*S. revertens*	USA, Alaska, Kathul Mtn., Yukon River, 24 June 2007, *J. Scelza* 07-112 (GZU)	KC893666	KC893687	KC893677
L1733	*S. revertens*	USA, Alaska, Kathul Mtn., Yukon River, 25 June 2007, *J. Scelza* 07-135 (GZU)	KC893665	KC893686	KC893676
L1735	*S. revertens*	USA, Alaska, Kathul Mtn., Yukon River, June 2007, *J. Scelza* 07-154 (GZU)	KC893664	—	KC893675
L944	*S.* sp. 1	Canada, British Columbia, shore of Bute Inlet, Barge Facility 2, 14 September 2009, *C.R. Björk* 19757 (GZU)	KC893663	KC893685	—

TABLE 2. *Previously sequenced specimens included in the phylogenetic analysis, with their species name and NCBI accessions. For sequences originally produced in Graz, isolate numbers are also given. Dashes stand for absence of sequence data*

Species	Sample No.	GenBank Accession Number		
		28S	18S	mitochondrial 12S
*Cladia retipora**		AY340540	AF184751	AY340487
Coccocarpia erythroxyli	L806	JF938133	JF938160	JF938189
C. palmicola		GQ258987	—	GQ259016
Collema flaccidum		EU982618	EU360873	EU982578
C. tenax		EU982619	—	EU082580
Degelia plumbea		DQ912348	DQ912325	DQ912300
Erioderma verruculosum		DQ973041	DQ973017	DQ972990
Fuscoderma amphibolum		GQ258993	—	GQ259023
Fuscopannaria leucosticta (Harris 33159)	1	DQ900640	—	DQ900630
Fuscopannaria sp.	L854	JX464120	JX464152	JX464136
Koerberia biformis	L860	JX464117	JX464149	JX464133
*Lecanora polytropa**		DQ986792	DQ986701	DQ986807
*Lecidea fuscoatra**		DQ912332	DQ912310	DQ912275
*L. silacea**		AY756340	DQ986723	DQ986878
Leciophysma furfurascens		GQ258998	—	GQ259028
Leptochidium albociliatum	L795	JF938135	JF938163	JF938192
L. albociliatum	L796	JF938136	JF938164	JF938193
Leptogidium dendriscum	L807	JF938139	JF938170	JF938198
L. dendriscum	L741	JF938140	JF938171	JF938199
L. dendriscum	L742	JF938137	JF938168	JF938196
Leptogium imbricatum		GQ259001	—	GQ259030
L. lichenoides		DQ917412	DQ917413	DQ923120
Lobaria pulmonaria		AF183934	AF183935	AF069541
Massalongia carnosa	1	EU360858	EU360881	—
Nephroma arcticum		DQ973040	DQ973016	DQ972989
N. bellum		EU360859	EU360882	AY300895
Pannaria hookeri	L896	JX464118	JX464150	JX464134
Parmeliella triptophylla		EU360860	EU360883	AY652623
Peltigera didactyla		AF286807	—	AY124164
P. rufescens		AY257928	AY424239	—
Physma byrsaeum		GQ259010	—	GQ259039
Placynthium nigrum	L764	JF938148	JF938178	JF938209
P. pannariellum	L758	JF938153	JF938185	JF938215
Polychidium muscicola	L798	JF938157	—	JF938221
*Porpidia albocaerulescens**		DQ986757	DQ986716	DQ986871
Protopannaria pezizoides	1	DQ912350	DQ912326	DQ912301
Psoroma hypnorum		AY424210	AY424261	AY340523
Pseudocyphellaria aurata		AY340562	—	AY340520
Santessoniella saximontana	L761	JX464119	JX464151	JX464135
Solorina saccata		DQ973044	DQ973021	DQ972994
Spilonema americanum	L751	JF938159	—	JF938224
Staurolemma omphalarioides		GQ259014	—	GQ259044
Steinera radiata	L874	JX464121	JX464153	JX464137
S. symptychia	L872	JX464122	JX464154	JX464138
Steineropsis alaskana	L769	JX464123	JX464155	JX464139
Sticta beauvoisii		DQ986769	DQ986713	DQ986867
Vahliella leucophaea	L766	JX464125	JX464157	JX464141
V. leucophaea		EU360852	EU360874	AY652621
Vestergrenopsis isidiata	L756	JX464127	JX464159	JX464143
V. isidiata	L759	JX464128	JX464160	JX464144
*Xanthoria elegans**		DQ912352	DQ912329	DQ912304

* outgroups

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have been misclassified with *Peltigerales* in the past (e.g., Otálora & Wedin 2013). Base calls were proof-read and sequence alignments prepared in BioEdit (Hall 1999).

Alignment and phylogenetic analysis

For a number of specimens we were unable to generate the sequences of one of the three loci, and in other instances single sequences were unavailable from GenBank. We examined the heterogeneity in phylogenetic signal between the different genetic markers (Buckley *et al.* 2002). Using both Bayesian and Maximum Likelihood (ML) approaches, we first analyzed each locus separately and subsequently combined them in a multilocus alignment, as performed in previous studies (Kauff & Lutzoni 2002; Miądlikowska *et al.* 2006). The combined data set was used to infer the phylogenetic relationships of the taxa selected using both Bayesian and ML approaches. The optimal nucleotide substitution model was estimated with the program MrModeltest v3.7 for each locus individually. The Bayesian Markov Chain Monte Carlo (B/MCMC) algorithm of MrBayes 3.1.2 (Huelsenbeck & Ronquist 2003; Ronquist *et al.* 2005) was performed with the General Time Reversible substitution model (Rodriguez *et al.* 1990), with estimation of invariant sites and assuming a gamma distribution with four categories (GTR+I+Γ). The Bayesian algorithm ran with six chains simultaneously, each starting from a random tree, for 10 million generations, and trees were sampled every 100th generation for a total sample of 100 000 trees. A burn-in sample of 500 000 generations (50 001 trees) was discarded for each run and a majority rule consensus tree calculated for the remaining 50 000 trees. The burn-in period was determined after testing for stationarity of likelihood values (Ronquist *et al.* 2005) by plotting log-likelihood scores against generation time using Tracer 1.4 (Rambaut & Drummond 2007). The program RAxML 7.0.4 (Stamatakis *et al.* 2005) was used for ML analyses and estimation of bootstrap support. The ML analyses in RAxML were performed with a GTRMIX substitution model and 1000 bootstrap replicates. Gene partitions were applied in both Bayesian and ML analyses in the three-locus data sets. The phylogenetic tree graphics were produced with TreeView (Page 1996).

Morphological analysis

We studied thallus morphology using a Leica Wild M3Z dissecting microscope. Ascus type and other microanatomical features were studied using a Zeiss Axioskop light microscope at ×1000 magnification. Photographs were taken with a ZeissAxioCam MRc5 digital camera and accompanying software (Axiovision, Axio VS40, Zeiss); images of growth habit were digitally optimized using CombineZM open source image processing software (CombineZM, www.hadleyweb.pwp.blueyonder.co.uk/CZM/).

Scanning electron microscopy

Air-dried thalli were fixed on aluminium stubs with carbon-impregnated, double-sided tape and studied with a scanning electron microscope (XL30 ESEM, FEI). We either investigated samples directly without any further preparation using the low vacuum mode and detection of backscattered electrons (Stabentheiner *et al.* 2010), or sputter-coated them with gold (AGAR Sputter Coater) and studied them in high vacuum mode using secondary electron detection.

Results

We obtained a total of 30 new sequences for the three target loci for 14 taxa. When combined with previously sequenced taxa, this yielded an alignment of 4·8 kb of sequence and 65 taxa. Burn-in was reached after <1 000 000 generations in the Bayesian analysis, and the average standard deviation of splits with a frequency of at least 0·1 was 0·012565. The same topology was recovered in both the Bayesian and ML analyses of the combined loci.

Newly acquired sequences of *Spilonema* were recovered in different places in the order *Peltigerales* (Fig. 1). The type species, *S. paradoxum,* forms a strongly supported monophyletic group with the type species of *Spilonemella*, *S. americanum*, and is nested between samples of *Spilonema revertens* and a possibly undescribed *Spilonema* species from British Columbia, Canada. Together, sequences from these isolates form a strongly supported monophyletic group in *Coccocarpiaceae*. Likewise, all samples of *S. dendroides* form a strongly supported monophyletic group but, in contrast to the other *Spilonema* species, they are recovered within an unresolved *Peltigerineae* as poorly supported sister to *Koerberiaceae*.

The inclusion of *Spilonema* species in the present phylogenetic analysis weakens the backbone support for the major nodes of *Peltigerales* (*Collematineae* and *Peltigerineae*) compared to previously published analyses (Spribille & Muggia 2013), and this led us to try to isolate the cause of this. Experimentally deleting *S. dendroides* from the alignment and re-running the concatenated data set including *Spilonema* s. str. restored the support for high level groupings to nearly the same levels as observed in previous studies. We accordingly analyzed the data set for intralocus signal conflict by performing both ML and

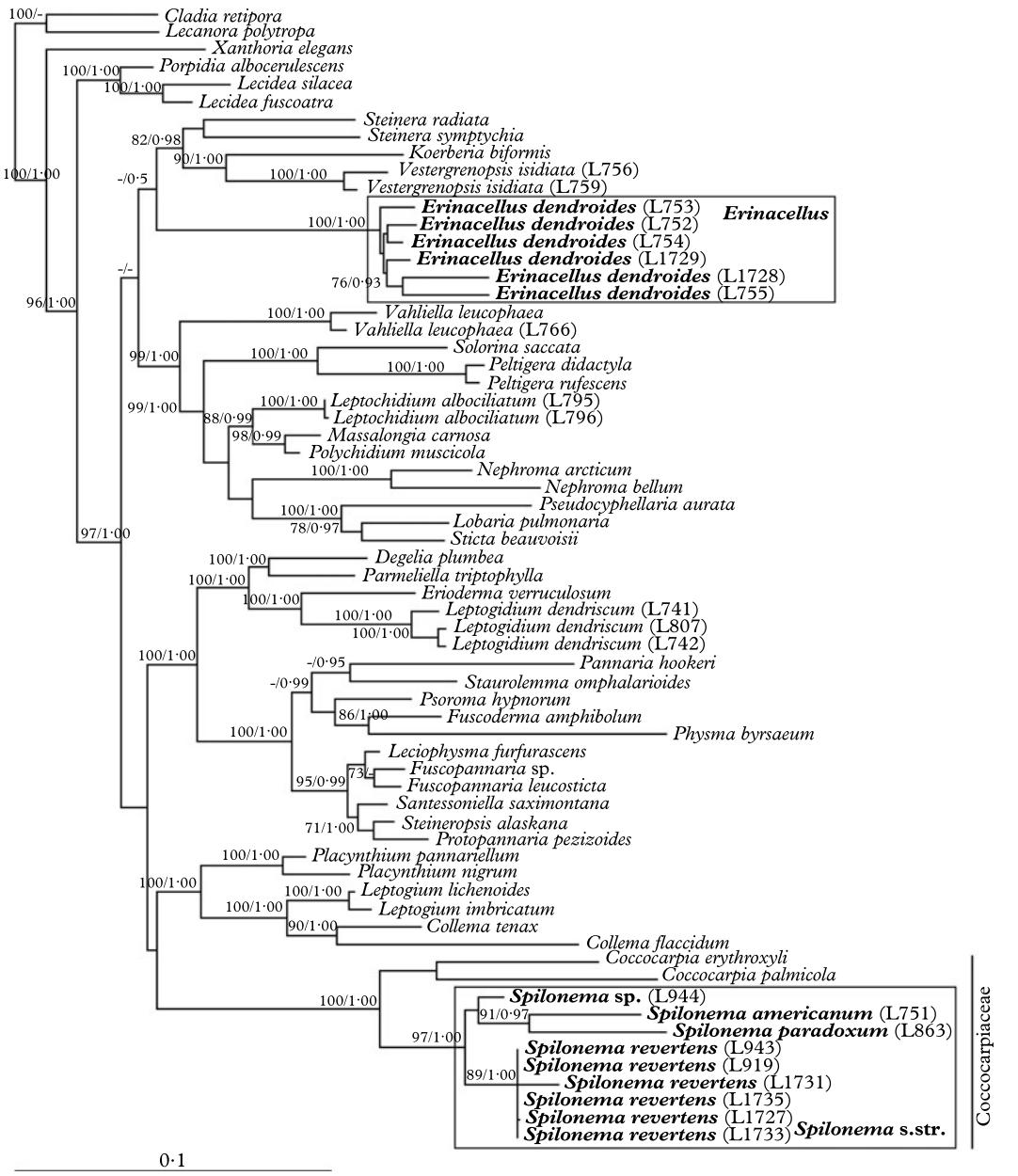

FIG. 1. Multigene phylogenetic reconstruction of *Peltigerales* showing position of *Spilonema* s. str. and *Spilonema dendroides* (= *Erinacellus*). ML analysis with branch length based on the combined 28S, 18S and mitochondrial 12S data sets (Table 1 and Table 2). Bootstrap support values >70% and Bayesian PP > 95% are reported above the branches.

Bayesian phylogenetic reconstructions of single locus data sets. Both the 28S and mitochondrial 12S data sets produced topologies broadly similar to the results of the three loci when concatenated (Fig. 2). The placement of *Koerberiaceae* changed in the 28S data compared to the concatenated analysis, and recovery of numerous families in 18S data,

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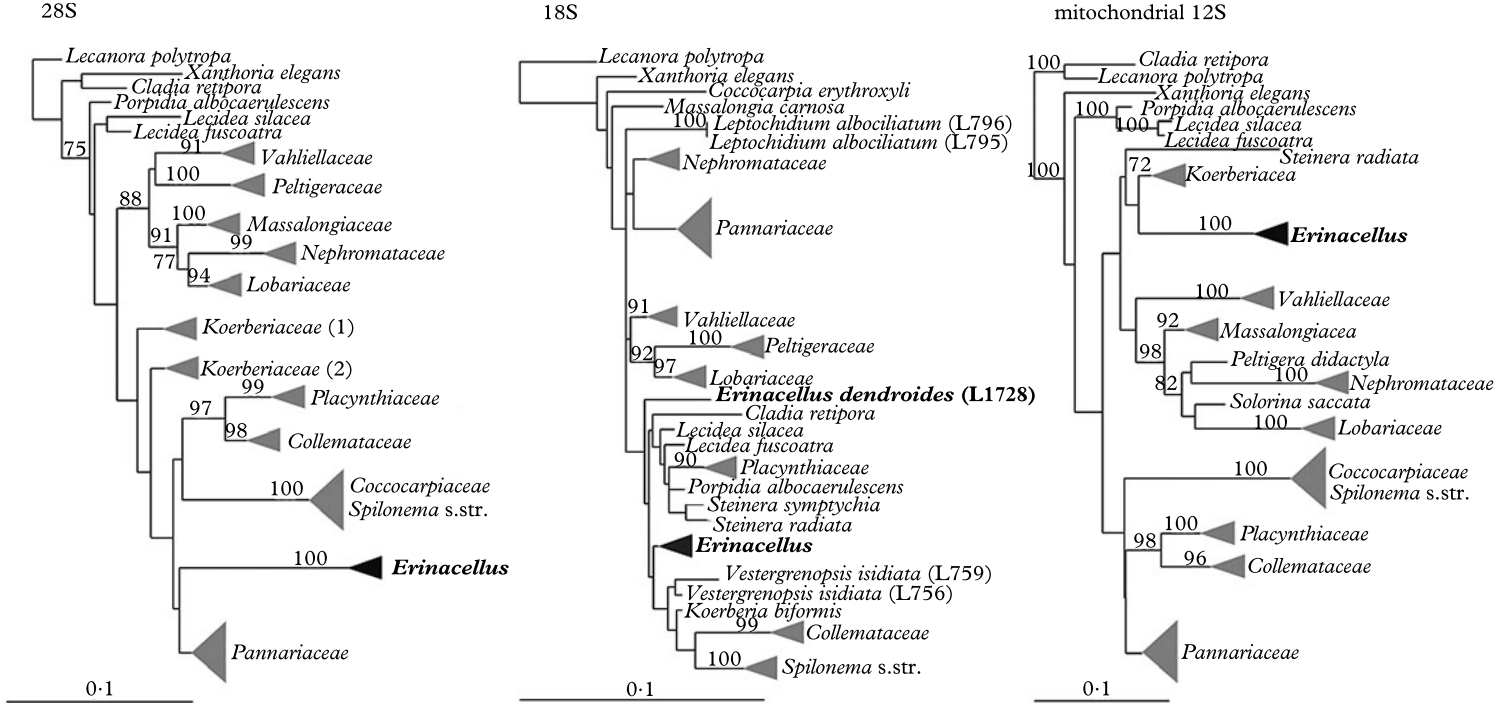

FIG. 2. Single locus phylogenetic trees of *Peltigerales*, with terminal clades collapsed to the family level to highlight position of *Erinacellus* in different loci. ML analyses individually based on 28S, 18S and mitochondrial 12S loci. Bootstrap support values (>70%) are reported above branches.

in particular, differed from that in the concatenated data set but these relationships were not supported. The position of *S. dendroides* was perhaps the most disparate between 28S and mitochondrial 12S: in the 28S data set, *S. dendroides* was recovered as sister to *Pannariaceae*, while in the mitochondrial 12S data set it came out as sister to *Koerberiaceae*, though again in both cases lacked support (Fig. 2).

Discussion

Our assessment of *Spilonema* paints a diverging picture of the phylogenetic position of the species studied. The core species of *Spilonema*, including *S. paradoxum* (the type species) and *S. revertens*, are paraphyletic to *Spilonemella*, a genus that differs from *Spilonema* in photobiont, cortex and ascomatal development (Henssen & Tønsberg 2000). *Spilonema paradoxum*, for which we have only a single mitochondrial 12S sequence, is however so close to *Spilonemella* as to form a strongly supported sister group relationship, with *S. revertens* more distantly related. Taken together, *Spilonema* and *Spilonemella* form a strongly supported monophyletic group. While we did not test the hypothesis using an ancestral state reconstruction, it is possible that the switch to *Scytonema* as a photobiont in *Spilonemella americana* is no more than a reversion to the ancestral state in *Coccocarpia*; this is in fact likely owing to the absence of *Stigonema* as a photobiont throughout the rest of the known *Peltigerales*. Finally, at the next higher level, the close relationship of the morphologically disparate genera *Spilonema* and *Coccocarpia*, first postulated by Henssen (1963) based on ascomatal ontogeny alone, is resoundingly confirmed by our analysis.

The relationships of *Spilonema dendroides*, by contrast, are rather more problematic. In the three-locus analysis, *S. dendroides* comes out with weak support in the *Peltigerineae*. Interestingly, the inclusion of *S. dendroides* in the phylogeny of the *Peltigerales* affects two of the main upper nodes that have been recovered as strongly supported in previous studies. The higher level relationships within *Collematineae* (mostly involving *Collemataceae* / *Placynthiaceae* on the one hand, and *Pannariaceae* on the other) are weakened by its inclusion, as is the monophyly of the *Collematineae* itself. In the *Peltigerineae*, which were recovered as monophyletic in previous analyses, the sister group relationship of *Koerberiaceae* to the remaining families (*Vahliellaceae*, *Massalongiaceae*, *Peltigeraceae*, *Nephromataceae* and *Lobariaceae*), supported in a previous Bayesian analysis (Spribille & Muggia 2013), is compromised with the inclusion of our new sequence data. Interestingly, these backbone-weakening effects are reversed if *S. dendroides* is selectively deleted from the data set and the phylogeny is run with the remaining taxa and *Spilonema* s. str., using exactly the same parameters as the whole data set (data not shown).

The underlying reason for the ambiguity in placement of *S. dendroides* is not yet clear. There is broad concordance between the 28S and mitochondrial 12S topologies and it is easy to see how combination of the data sets leads to robust support for, for example, the monophyly of the *Vahliellaceae* / *Peltigeraceae* / *Massalongiaceae* / *Nephromataceae* / *Lobariaceae* clade (the core of *Peltigerineae*) as well as the *Collemataceae* / *Placynthiaceae* / *Coccocarpiaceae* / *Pannariaceae* clade (the core of *Collematineae*). These relationships have been recovered repeatedly in numerous studies, including those using additional loci (Spribille & Muggia 2013). The most uncertainty appears to be associated with the sister group relationships of *Koerberiaceae* and *S. dendroides*, respectively. Although the position of *S. dendroides* in the single locus phylogenies is not supported in any locus, one hypothetical explanation for its ambiguous placement in the combined analysis could be an underlying incongruence between the *S. dendroides* 28S and mitochondrial 12S gene genealogies and those of other sampled *Peltigerales*. The poor resolution (and almost complete lack of topological support) achieved in 18S extends so far that *Lecideaceae* and *Cladia* are not separated from *Peltigerales*. That 18S has the lowest level of polymorphisms of the three loci studied probably does not play a

major role in the overall topological instability of the concatenated data set. However, the single locus analysis does suggest that 18S, which has been used in phylogenetic analyses of *Peltigerales* by Miądlikowska & Lutzoni (2004), Muggia *et al.* (2011) and Spribille & Muggia (2013), may not be especially informative for elucidating phylogenetic relationships in this order.

The evolutionary relationships of *Spilonema dendroides* are not likely to be elucidated without analysis of additional loci. That said, none of the sequence reads obtained from multiple individuals and not one of our phylogenetic reconstructions place it close to *Spilonema* s. str., and it can safely be excluded from that genus. It is not clear why *S. dendroides* should have diverging nuclear and mitochondrial sequence data compared to the rest of *Peltigerales*. The co-occurrence of motifs from both *Peltigerineae* and *Collematineae* may suggest the divergence of *S. dendroides* from a pan-Peltigeralean ancestor around the time of divergence of *Peltigerineae* and *Collematineae*; the association of *S. dendroides* with the rare photobiont *Hyphomorpha*, otherwise lacking in *Peltigerales*, may be further evidence of evolutionary isolation. This is the most striking case of locus incongruence known to us from several different taxon and locus samplings within the *Peltigerales*.

Even after our phylogenetic analysis, *Coccocarpiaceae* continue to represent a concentration of body plan and photobiont diversity with few parallels in *Lecanoromycetidae*, with a range from dorsiventral to minutely branched caespitose thalli and with three cyanobacterial families represented as photobionts. The reason for evolution of thread-like filaments and cushions, and the path by which this body plan evolves from foliose ancestors in *Polychidium*, *Leptogidium* (Muggia *et al.* 2011) and likely *Spilonema*, is not known. The increase in surface area in a filamentose body plan compared to that of a foliose lichen may allow increased control over the wetting-drying process (e.g., Kershaw 1985), probably of importance to species that, like *Spilonema*, grow in close association with bryophytes in rainforests and on seepage tracks. The frequency of photobiont and body plan transitions within the *Coccocarpiaceae* clade, compared to other clades of Lecanoromycetes where this is virtually unheard-of, suggests a lineage-specific relaxation of body plan conservatism in *Coccocarpiaceae*. This could be a promising target for evolutionary developmental studies in lichen symbiotic interactions.

The full range of photobiont and body plan diversity in *Coccocarpiaceae* is still not known. In particular, the subantarctic genus *Peltularia* has yet to be sampled for a molecular phylogeny and fresh material is not available (D. Galloway, pers. comm.). Further sequence diversity can be anticipated within *Coccocarpia* and more, yet unsampled genera may still be recognized as belonging to *Coccocarpiaceae*.

It is clear from sequence motifs already at the DNA alignment stage that *Spilonema dendroides* cannot be closely related to *Coccocarpiaceae*. In addition, it differs from all currently accepted species of *Spilonema* in its habit of producing secondary and tertiary branching, the differentiated coloration of light primary 'trunks' and dark outer branches (in *S. dendroides*), and its photobiont *Hyphomorpha* (Henssen 1981). The dense, caespitose habit is grossly similar to *Spilonema revertens*, but in that species the cushion interior is comprised of a dense pillow of 'rhizines' giving rise to a continuously regenerating outer layer of lichenized primary branches; a dendroid growth habit as in Fig. 3 is never achieved. *Spilonema dendroides* and the likely closely related *S. schmidtii* cannot be accommodated in *Spilonema* even in the broadest sense, and accordingly we propose here the establishment of a new genus for these species.

Taxonomy

Erinacellus T. Sprib., Muggia & Tønsberg gen. nov.

MycoBank No.: MB 803465

Ascomycetes cyanobacteriis generis *Hyphomorpha* lichenisati. Thallus pulvinatus, ramis filiformibus minutis suberectis compositus. Rami ipsi dichotome quasi isotome ramosi, ramis primariis pallide cinereis vel fuscis, et

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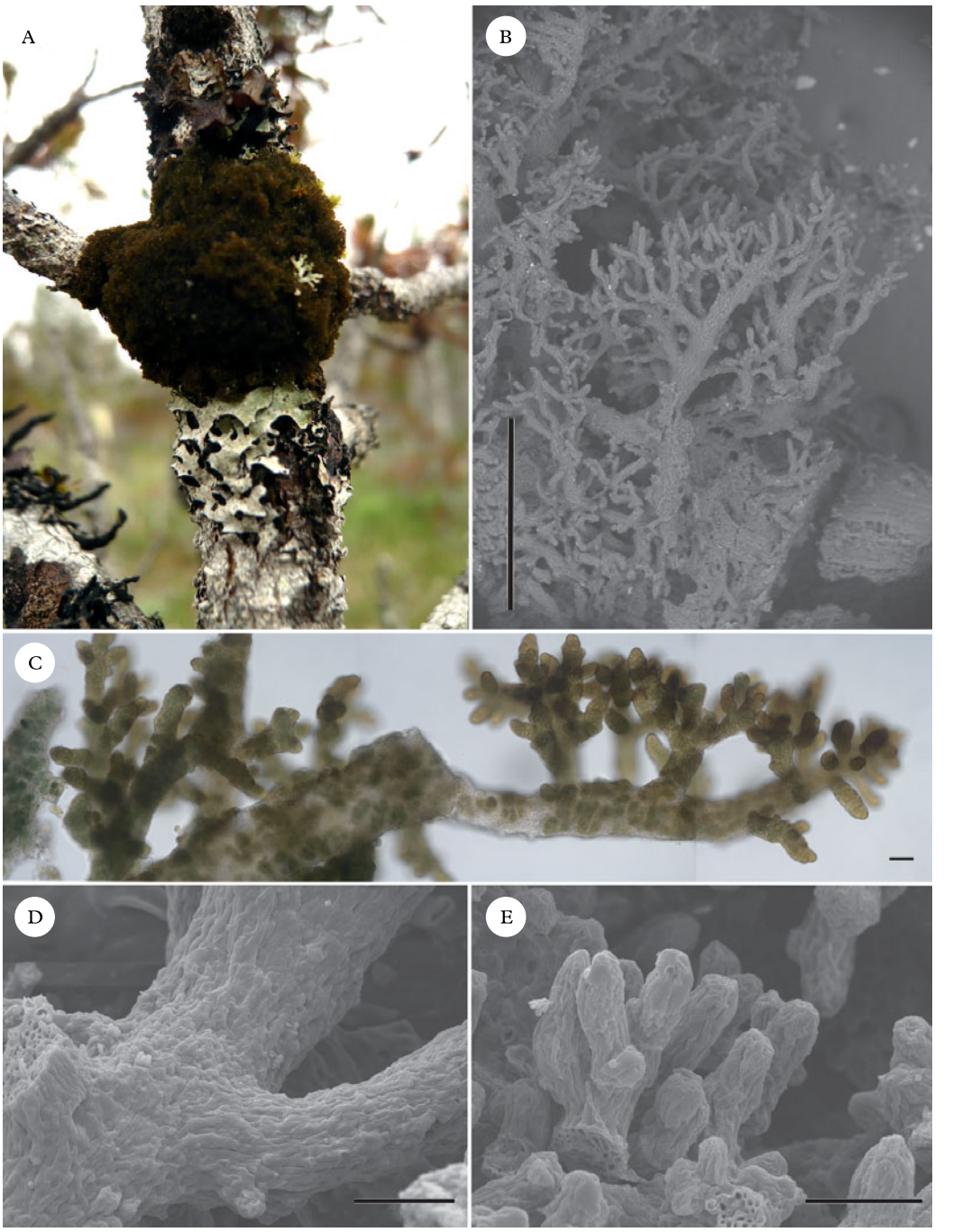

FIG. 3. *Erinacellus dendroides*. A, habit, on a branch of *Pinus contorta* subsp. *contorta*, Glacier Bay National Park, Alaska, July 2012; B, habit of dried, broken-open cushion in environmental SEM; C, branching (in H_2O, light microscope); D & E, architecture and surficial properties (dry in SEM following gold sputtering). B–E from *Spribille* 36301 (GZU). Scales: B = 500 μm; C, D & E = 50 μm. In colour online.

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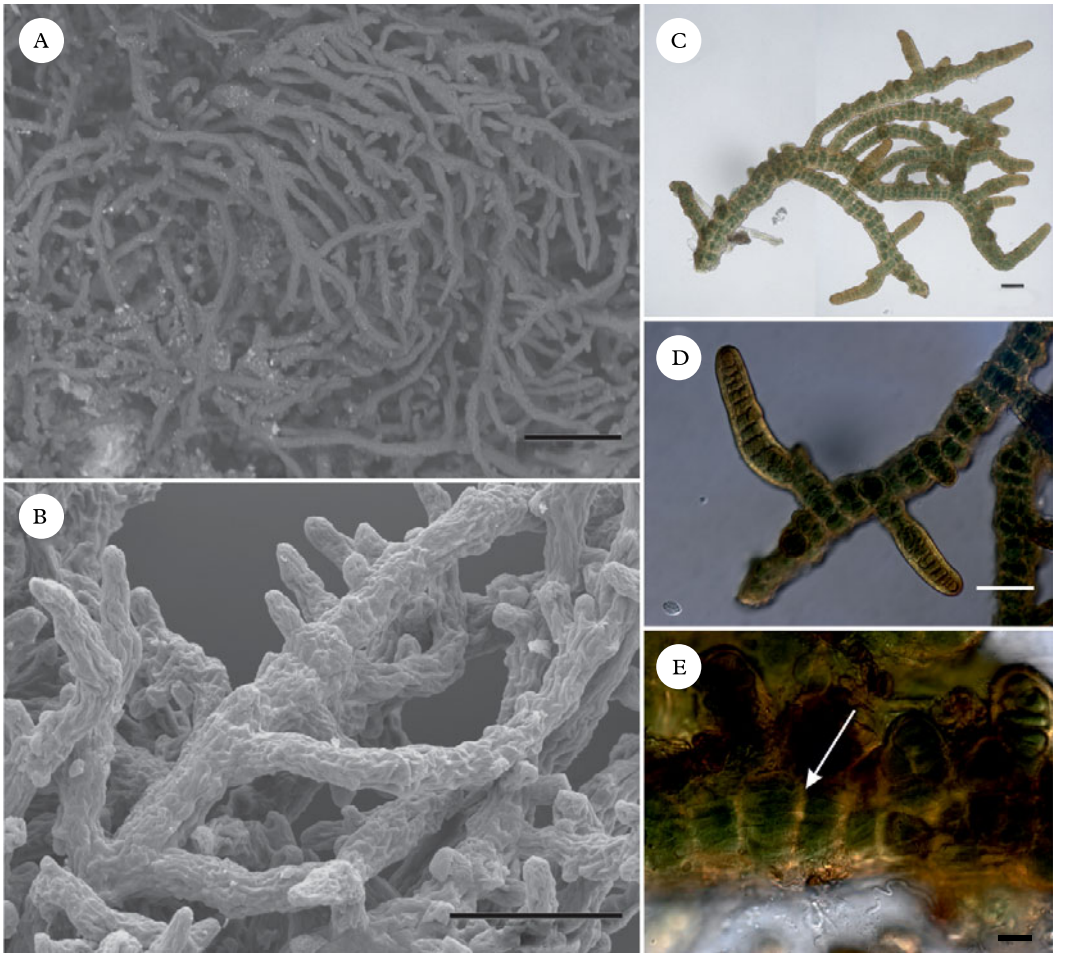

Fig. 4. *Spilonema paradoxum* (*Henssen* 22509, GZU). A & B, habit, A in environmental SEM, B SEM after sputter-coating in gold; C & D, anatomy of branches in H_2O (light microscope); E, cortical fungal cells (arrow). Scales: A = 200 μm; B = 100 μm; C & D = 50 μm; E = 10 μm. In colour online.

ramis secundariis tertiariisque obscure fuscis. Hyphae circum filamentos photobionti vaginas continuas sed non corticem cellulosum formantes. Ascomata matura et pycnidia adhuc ignota.

Typus generis: *Erinacellus dendroides* (Henssen) T. Sprib., Muggia & Tønsberg.

Ascomycetes lichenized with the cyanobacterial genus *Hyphomorpha*. Thallus comprised of a dense cushion of erect, thread-like branches, differentiated into primary branches, which are light grey or dark brown, and secondary and tertiary branches which are dark brown. Terminal branching nearly isotomic dichotomous. Fungal hyphae enclosing photobiont in continuous sheath, the sheathing fungal cells rectangular. Ripe ascomata and pycnidia unknown.

Etymology. Diminutive of *Erinaceus*, the genus of Eurasian hedgehogs; from a fancied resemblance to the dark, cushion-forming thalli.

The relationships of *Erinacellus* within the *Peltigerales* are unclear. Multiple Bayesian and maximum likelihood analyses with different combinations of taxa have recovered it in the *Peltigerineae* as sister to the *Koerberiaceae*, or

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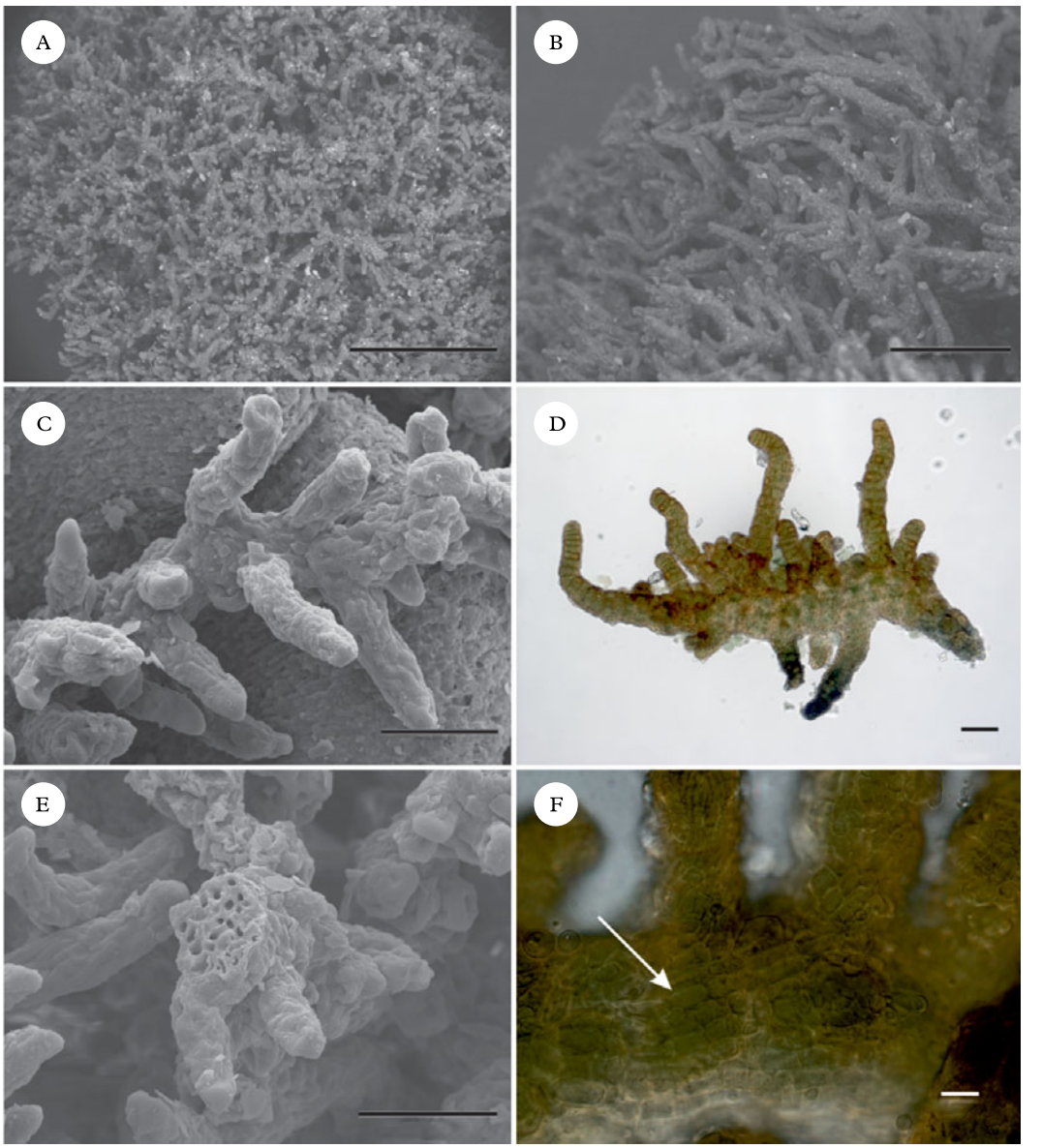

FIG. 5. *Spilonema revertens* (*Spribille* 27944, GZU). A–C, habit, dry in environmental SEM (A & B) and after gold sputtering (C); D, anatomy of branches in H_2O (light microscope); E, architecture and broken branch in SEM; F, cortical fungal cells (arrow). Scales: A = 500 μm; B = 200 μm; C, D & E = 50 μm; F = 10 μm.In colour online.

unresolved in a polytomy with *Koerberiaceae* and the clade that includes *Vahliellaceae*, *Massalongiaceae* and *Lobariaceae* (data not shown). The consensus tree we use in Fig. 1 shows it sister to *Koerberiaceae* but with low support and, as noted above, its inclusion affects the relationship of *Koerberiaceae* to the rest of the *Peltigerineae*. We think the genus should be treated *ad interim* as *Peltigerales incertae sedis*. Acquisition of genetic material

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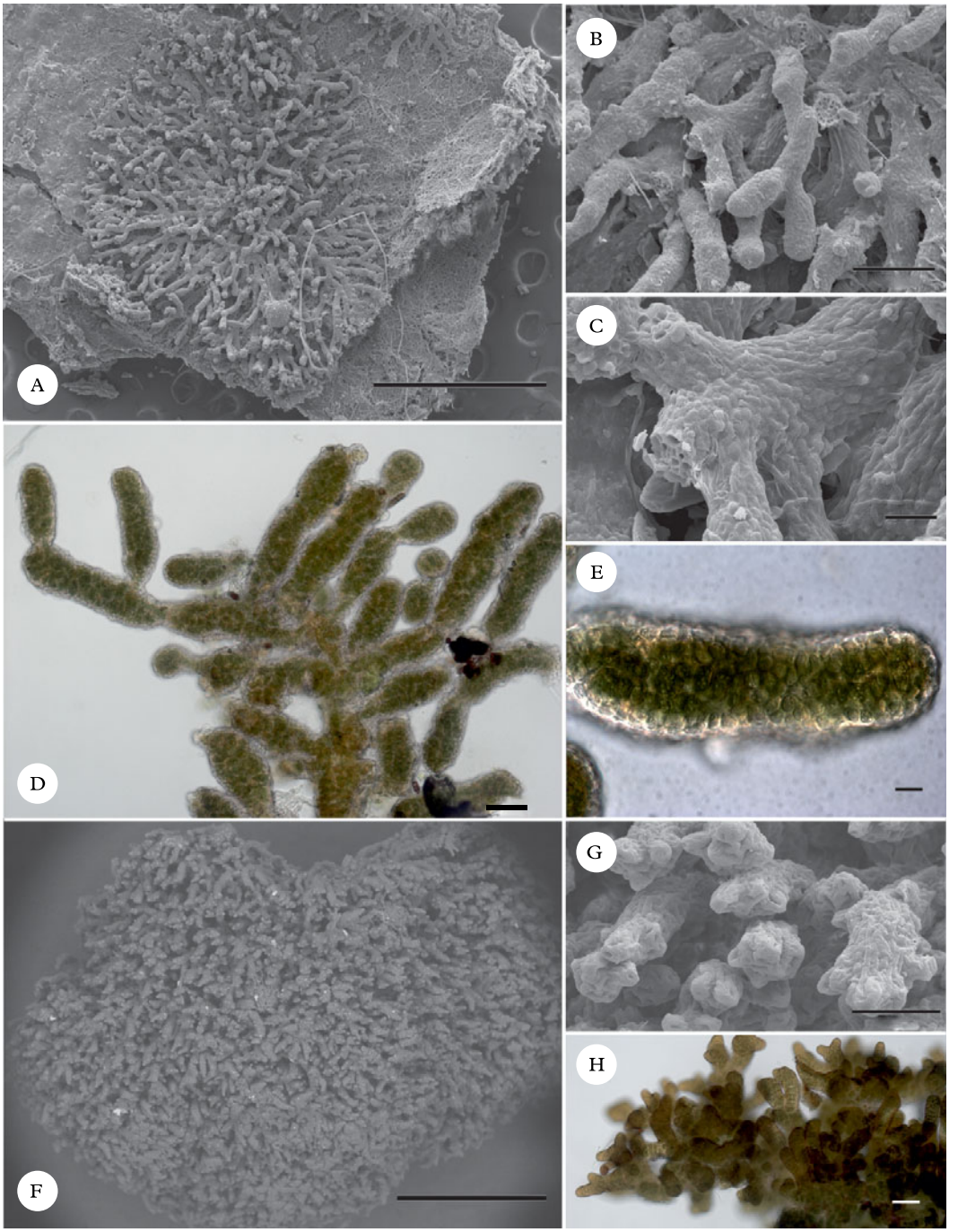

FIG. 6. A–E, *Spilonema americanum* (*Spribille* 27038, GZU). A–C, habit; D & E, cellular anatomy, in H_2O (light microscope). F–H, *Spilonema* sp. 1 (*Björk* 19757, UBC). F & G, habit; H, branching pattern in H_2O. All SEM images taken after sputter-coating. Scales: A = 1000 μm; B = 100 μm; C = 20 μm; D, G & H = 50 μm; E = 10 μm; F = 500 μm. In colour online.

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from the second *Hyphomorpha*-containing species (*Spilonema schmidtii*, here transferred to *Erinacellus*) may help clarify the evolutionary relationships.

Erinacellus dendroides (Henssen) T. Sprib., Muggia & Tønsberg comb. nov.

MycoBank No.: MB 803466

Spilonema dendroides Henssen, *Symb. Bot. Upsal.* **18**(1): 97 (1963); type: New Zealand, Stewart Island, 1927, *Du Rietz* (UPS—holotypus!).

(Fig. 3)

Erinacellus dendroides is known from only a few sites worldwide. In addition to New Zealand, the species was first reported from Alaska by Henssen (1981) and from British Columbia by Brodo & Tønsberg (1994). It is locally common on shore pines (*Pinus contorta* subsp. *contorta*) in blanket bogs, locally known as muskegs, in south-eastern Alaska (T. Spribille, pers. obs.). This species was referred to as *Spilonema* sp. 1 by Goward (1999).

Erinacellus schmidtii (Vain.) T. Sprib., Muggia & Tønsberg comb. nov.

MycoBank No.: MB 803467

Leptodendriscum schmidtii Vain., *Ann. Acad. Scient. Fenn. Ser. A* **15:** 34 (1920); type: "Siam" [=Thailand], Insel Koh Chang, 1900, *Schmidt* 24 (TUR, seen by Henssen 1963).

Erinacellus schmidtii is a palaeotropical species known from Thailand and Sri Lanka (Henssen 1981). Henssen considered it to differ from *E. dendroides* in the dark brown (as opposed to light silvery grey) base of its primary branches.

Spilonema Born.

Mémoires de la Société impériale des sciences naturelles de Cherbourg **4:** 226 (1856) ; typus generis: *Spilonema paradoxum* Born., *ibidem* **4:** 226 (1856).

Spilonemella Henssen & Tønsberg, *Bryologist* **103:** 108 (2000), **syn. nov.**; typus generis: *Spilonemella americana* Henssen & Tønsberg, *Bryologist* **103:** 113 (2000).

The two core species of *Spilonema* were described from Europe in the 19th century. The type species, *S. paradoxum* Born. (Fig. 4), is a loosely appressed species with sprawling branches and resembles the unrelated *Ephebe* (*Lichinaceae*). A detailed morphological analysis was provided by Henssen (1963). The only other widely distributed species is *S. revertens* Nyl., a dense cushion-forming species (Fig. 5). A further species, termed here "*Spilonema* sp. 1" has been found in coastal British Columbia (*Björk* 19757, UBC; isolate L944 in Fig. 1). It was originally thought to belong to *S. revertens*, but was found to have distinct DNA sequences in our analysis. It is broadly similar to *S. revertens* but consists of minute cushions only a few millimetres across (Fig. 6F–H), smaller than typically seen in *S. revertens*. The terminal branches are shaped like studded clubs (Fig. 6G) but the material in most other respects appears similar to *S. revertens*. We include photographs of the sample here in the hope that more collections can be made and a morphological and ecological characterization is eventually developed for this species.

Spilonema americanum (Henssen & Tønsberg) T. Sprib., Muggia & Tønsberg comb. nov.

MycoBank No.: MB 803468

Spilonemella americana Henssen & Tønsberg, *Bryologist* **103:** 113 (2000); type: USA, Washington, Jefferson Co., SE of Hwy 101, 3·3 km (along road) S of Hoh River bridge, 47°47·6′N, 124°15·1′W, alt. 60 m, corticolous on trunk of *Alnus rubra*, 31 March 1998, *T. Tønsberg* 25758 [holotypus—BG; isotypi—FH, H (from hb. Henssen), TNS, WTU].

(Fig. 6)

A detailed anatomical study of this species was provided by Henssen & Tønsberg (2000).

Spilonema japonicum (Henssen & Tønsberg) T. Sprib., Muggia & Tønsberg comb. nov.

MycoBank No.: MB 803469

Spilonemella japonica Henssen & Tønsberg, *Bryologist* **103:** 116 (2000); type: Japan, central Japan, [Honshu,] Prov. Sagami, Hakone, 1931, *Sato* [holotypus—TNS; isotypi—H (from hb. Henssen)].

We have not seen fresh material of *Spilonema japonicum* and thus cannot vouch with certainty for its phylogenetic position. However, it appears to be close to *S. americanum* and in any case the genus *Spilonemella* is no longer available to house it.

We thank Curtis Björk, Trevor Goward, Peter Nelson, Jay Scelza and Tim Wheeler for providing material for sequencing, and the curator of UPS for the loan of type material. Josef Hafellner translated the diagnosis into Latin. Support for laboratory work came from the University of Bergen, Norway. The management and staff of Klondike Gold Rush National Historical Park and Glacier Bay National Park and Preserve, Alaska, supported lichenological fieldwork during which *Erinacellus* was collected.

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