

When morphology and molecules tell us different stories: a case-in-point with *Leptodon corsicus*, a new and unique endemic moss species from Corsica

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SUMMARY

Leptodon corsicus (Neckeraceae) is described as the first endemic moss species from Corsica. It strikingly differs from the other species of the genus by the lack of a dense and pinnate to bipinnate mode of branching; about 10 times smaller shoots that do not inroll upon drying; the lack of paraphyllia; and few, occasional small pseudoparaphyllia. Due to its small size and several leaf characters, *L. corsicus* shares at first glance more similarities with *Homalia webbiana* and *Neckera besseri* than with *Leptodon*. Yet, phylogenetic analysis of chloroplast and nuclear DNA sequences unambiguously shows that *L. corsicus* is deeply nested within *L. smithii*. The numerous morphological characters that distinguish *L. corsicus* from *L. smithii* cannot be attributed to plasticity. Consequently, we interpret the phylogenetic position of *L. corsicus* as the result of a recent speciation process, involving mutations at one or a few coding loci or differences in gene expression, which have tremendous consequences for phenotypic appearance, and retention of ancestral polymorphism in the non-coding sequences used for phylogenetic reconstruction. Such an explanation might also apply to other species of mosses, which exhibit a striking morphology, and yet share identical non-coding sequences with the common species they derive from. The notion of species in mosses is discussed in this context.

KEYWORDS: *Leptodon*, *Homalia webbiana*, *Neckera besseri*, Corsica, endemism, species concept, incongruence, morphology, phylogeny.

INTRODUCTION

By comparison with angiosperms, bryophytes are noteworthy for their extremely low levels of island endemism. In the Galapagos for example, Gradstein & Weber (1982) documented 16% of endemic liverworts and 6% of endemic mosses against about 50% in the native angiosperm flora. In the Canary Islands, the 1.5% of species endemism pale by comparison with the 40% of endemism rates observed in angiosperms (González-Mancebo *et al.*, 2008). In Hawaii, one of the world's richest hot-spots of endemism, 29.4 and 48.6% of the moss and liverwort species, respectively, are endemic to the archipelago (Staples *et al.*, 2004; Staples & Imada, 2006), whereas the rate of species endemism in angiosperms reaches 90% (Sakai, Wagner & Mehroff, 2002). In the western Mediterranean, Corsica and Sardinia, which harbour nearly 10% species endemism in angiosperms, no endemic bryophyte species at all had been

described (Sotiaux *et al.*, 2007) until we found an as yet undescribed pleurocarpous moss in Corsica. The plant bears strong resemblance to *Homalia webbiana* (Mont.) Schimp. and *Neckera besseri* (Lob.) Jur., and a cladistic analysis of gametophytic characters confirmed that it is morphologically related to these two species. Yet, molecular data soon revealed that the unknown Corsican species actually belongs to *Leptodon*. Although it differs from the other species of the genus by a much smaller size, leaves that remain unmodified after desiccation, absence of paraphyllia, and pseudoparaphyllia that are small and only occasionally present in limited number, the phylogenetic analysis of the molecular dataset showed that the undescribed Corsican species is deeply nested within *L. smithii* (Hedw.) F. Weber & D. Mohr.

In this paper, we present a phylogeny of *Leptodon* and related genera of the Neckeraceae based on morphology and molecules. We show that the two data sets are

incongruent, contrast several hypotheses to explain the discrepancy among data sets, and discuss the notion of species in mosses in this context.

MATERIAL AND METHODS

Taxon sampling and morphological character scoring

With approximately 200 species, the Neckeraceae are one of most diverse families of pleurocarps (Enroth, 1994; Olsson *et al.*, 2009c). Therefore, two different sampling strategies were applied. We first determined the position of the unknown Corsican species in the context of a broad phylogenetic analysis of the Neckeraceae (Olsson *et al.*, 2009a). We identified that the Corsican species was nested within *Leptodon* as part of a larger clade comprised of *Alsia*, *Forsstroemia*, and *Neckera* (data not shown). Our sampling thus included representative species of those genera, with a special emphasis on *Leptodon* (Table 1). The latter includes *L. smithii*, *L. fuciformis* (Brid.) Enroth, (*Cryptoleptodon acuminatus* M. Fleisch., *L. longisetus* Mont., *L. rigidulus* (Wilson ex Mitt.) A. Jaeger, and *L. pluvini* (Brid.) A. Jaeger (Olsson *et al.*, 2009a). Except for *L. fuciformis*, which is endemic to La Réunion and

apparently only known from the type material, which is more than 100 years old, and *L. rigidulus* and *C. acuminatus* from the Himalayan region, for which sufficiently recent material for DNA studies is not available, several accessions of each *Leptodon* species were sampled in order to cover their distribution range. *Homalia webbiana*, which is morphologically very similar to the Corsican species but is unrelated to the *Leptodon-Alsia-Forsstroemia-Neckera* clade, as revealed by recent phylogenetic reconstructions of the Neckeraceae (Olsson *et al.*, 2009a), was used as outgroup.

In the absence of sporophytes in the Corsican material, 14 morphological characters that are variable in the group of mosses under study were scored from the gametophytes of each of the accessions used in the molecular analyses (Appendix).

Molecular protocols and phylogenetic analyses

Sequencing was performed for the internal transcribed spacer of nuclear ribosomal DNA (ITS1 & 2), including the 5.8S gene, the plastidal *rpl16* group II intron, as well as the *rps4-trnT-trnL-trnF* cluster, including three tRNAs [*trnT*, *trnL*, *trnF* (partial)], a fast evolving gene (*rps4*, partial),

Table 1. Taxon sampling, voucher information (collection# and herbarium), and GenBank accession numbers for the *rps4-trnF*, *rpl16*, and ITS regions in *Leptodon* and related genera

Taxon and label	Voucher	Origin	GenBank accession no.		
			<i>rps4-trnF</i>	<i>rpl16</i>	ITS
<i>Alsia californica</i> (Hook. & Arn.) Sull.	Düill 365.14a (B)	USA, CA	FM210280	FM160946	FM161073
<i>Forsstroemia producta</i> (Hornsch.) Paris	Koponen 46545 (H)	China	FM201504	FM160967	FM161102
<i>F. trichomitria</i> (Hedw.) Lindb.	H. Streimann & T. Pocs 65120A (Buchbender)	Australia	AM990365	FM160968	FM161103
<i>Homalia webbiana</i> (Mont.) Schimp. (Canary Isl.)	F. Müller K68 (DR)	Canary Isl.	AM990387	FM160982	FM161127
<i>H. webbiana</i> (Azores)	Hedenäs & Bisang. s.n. (S)	Azores	AM990386	FM160981	FM161128
<i>Leptodon longisetus</i> Mont. 1	Vanderpoorten PALM1466 (LG)	Canary Isl.	FN252900	FN256357	FN256357
<i>L. longisetus</i> 2	Schäfer-Verwimp & Verwimp 4228 (H)	Canary Isl.	AM990356	FM160957	FM161091
<i>Leptodon pluvini</i> (Brid.) A. Jaeger	Huttunen s.n. (Huttunen)	China	FM210284	FM160958	FM161092
<i>Leptodon smithii</i> (Hedw.) F. Weber & D. Mohr (South Africa)	Vanderpoorten 314 (LG)	South Africa	FN252315	FN252892	FN256358
<i>L. smithii</i> (Yemen)	Kürschner 02-38 (B)	Yemen	FN252316	FN252893	FN256359
<i>L. smithii</i> (Canary Isl. 1)	Vanderpoorten PALM1561 (LG)	Canary Isl.	FN252317	FN252894	FN256360
<i>L. smithii</i> (Canary Isl. 2)	Vanderpoorten PALM1477 (LG)	Canary Isl.	FN252318	FN252895	FN256361
<i>L. smithii</i> (France)	De Sloover 44851 (B)	France	AM990403	FM160997	FM161147
<i>L. smithii</i> (Corsica)	Vanderpoorten 415 (LG)	Corsica	FN252319	FN252896	FN256362
<i>L. corsicus</i> 1	Sotiaux W178 (LG)	Corsica	FN252320	FN252897	FN256363
<i>L. corsicus</i> 2	Pioli s.n. (LG)	Corsica	FN252901	FN252898	FN256364
<i>L. smithii</i> (Italy)	Vanderpoorten 556 (LG)	Italy	FN252321	FN252899	FN256365
<i>Neckera besseri</i> (Loborzewski) Jur.	Olsson 107 (Quandt)	Hungary	FM210294	FM161003	FM161156
<i>N. brownii</i> Dixon	Tangney 2330 (H)	New Zealand	FM210295	FM161004	FM161157
<i>N. complanata</i> (Hedw.) Huebener (Germany)	Buchbender 204 (Buchbender)	Germany	AM990413	FM161005	FM161158
<i>N. complanata</i> (South Africa)	Hedderson 15065 (Enroth)	South Africa	FM210296	FM200842	FM201492
<i>N. hymenodonta</i> Müll. Hal.	Streimann 65307 (H)	Australia	FM210302	FM161011	FM161164
<i>N. jamesonii</i> Taylor	Lewis 88-1880 d-3 (B)	Bolivia	FM210304	FM161013	FM161166
<i>N. remota</i> Bruch & Schimp. ex Müll. Hal.	Hedenäs s.n. (S)	Tanzania	AM990415	FM161018	FM161171
<i>N. scabridens</i> Müll. Hal.	Kürschner <i>et al.</i> 95-498 (B)	Peru	FM210308	FM161019	FM161172
<i>N. urnigera</i> Müll. Hal.	Hedenäs s.n. (S)	Mexico	AM990416	FM161021	FM161174

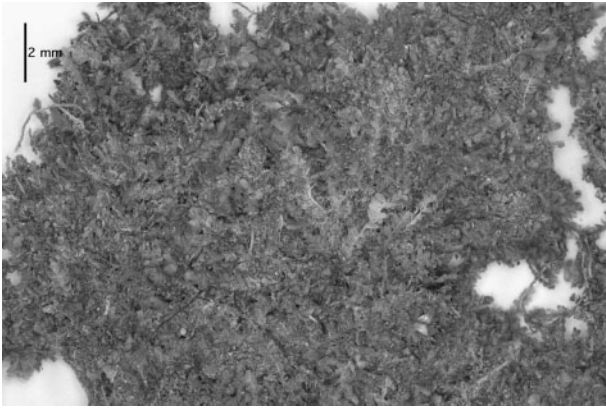


Figure 2. General habit of *Leptodon corsicus*, dry state. The plant is not modified by desiccation (Photo M. Lehtonen from holotype *Sotiaux Corse 178*).

for the binary indel partition. To allow for possible deviating substitution models (i.e. plastidal versus nuclear), the sequence data was divided into two partitions (partition 1: plastidal DNA; partition 2: nuclear DNA). Posterior probability (PP) distributions of trees were created using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method. Ten runs with four chains (10^6 generations each) were run simultaneously, with a tree sampling every 1000 generations. Stationary was reached well before generation 25 000 and thus set as burn-in point. Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph (Müller & Müller, 2004).

Ancestral character state reconstruction for one characteristic indel was performed by maximum likelihood with a model implementing a single transition rate as implemented by Mesquite 2.6 (Maddison & Maddison, 2009) on one of the trees sampled at random from the posterior probability distribution.

The morphological dataset was analysed by MP using heuristic searches with 1000 random addition replicates and TBR branch swapping, as implemented by PAUP. Bootstrap analyses were conducted with 300 replicates using simple taxon addition.

Congruence between the morphological and molecular partitions was assessed by the Incongruence Length Difference test with 1000 replicates as implemented by PAUP. Non-informative characters were excluded from the analysis as recommended by Lee (2001)

RESULTS

For reasons discussed below, the unknown Corsican pleurocarpous moss is described as a new species of *Leptodon*:

Leptodon corsicus Enroth, Sotiaux, D. Quandt & Vanderpoorten, *sp. nov.* (Figs 2, 3, 4a, e)

Muscus pleurocarpus familiae Neckeracearum, statu complanato et foliis asymmetricis, nervo simplici munitis. Ab omnibus aliis speciebus generis Leptodontis differt statura

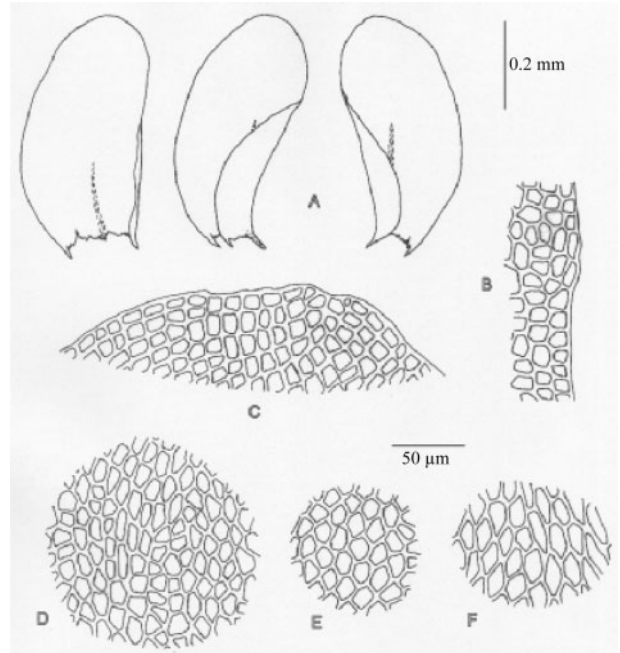


Figure 3. *Leptodon corsicus*. (A) three stem leaves; (B) leaf margin at mid-leaf; (C) leaf apex; (D) median laminal cells; (E) basal laminal cells in acroscopic part of leaf; (F) basal laminal cells in basiscopic part of leaf. Drawn from the holotype.

multo minore, caulibus haud ramosis et in sicco immutatis, paraphylliis carentibus, pseudoparaphylliis quam in aliis speciebus pusillioribus interdum praesentibus.

Type: Corsica. Corse du Sud, Baie de Figari, tour de Caldarello, 0 m, 15 May 2003, *Sotiaux Corse 178* (holotype: H; isotypes: H, LG, herb. A. Sotiaux).

Plants small, pure green to yellowish green, dull, forming thin, loose wefts on substrate, young, green shoots mixed with older, withered and brownish, partly leafless ones. *Stems* creeping, lax, very irregularly and loosely branched, leaves mostly eroded off, ca 130–150 μm diameter, in cross-section with 3–4 layers of relatively small, thick-walled cortical cells and a medulla of larger, thinner-walled cells, central strand none. *Rhizoids* in tufts just below leaf insertions, unbranched or sparsely branched, pale brownish orange, smooth. *Leafy shoots* ca 3–7 mm long, 1 mm wide with leaves, simple or sparsely branched. *Leaves* imbricate, complanate and spreading, often slightly arcuate, asymmetric, ovate to oblong-elliptic, not or shortly and narrowly decurrent, to 0.5–0.9 mm long and 0.30–0.65 mm wide; margins entire but often slightly sinuous near leaf apex, plane, basiscopic margin mostly inflexed near leaf base; *costa* weak, single, reaching to mid-leaf at most; *laminal cells* smooth, walls firm and solid throughout; apical cells mostly rhombic, some subquadrate to near rounded, 10–15 \times 7–10 μm or isodiametric; median cells rhombic to near hexagonal and especially in basiscopic lamina longer than apical cells, in acroscopic lamina similar to apical cells or isodiametric; marginal cells somewhat smaller than laminal cells and mostly subquadrate throughout, ca 6–8 μm across, towards alar regions often transversely elongate;

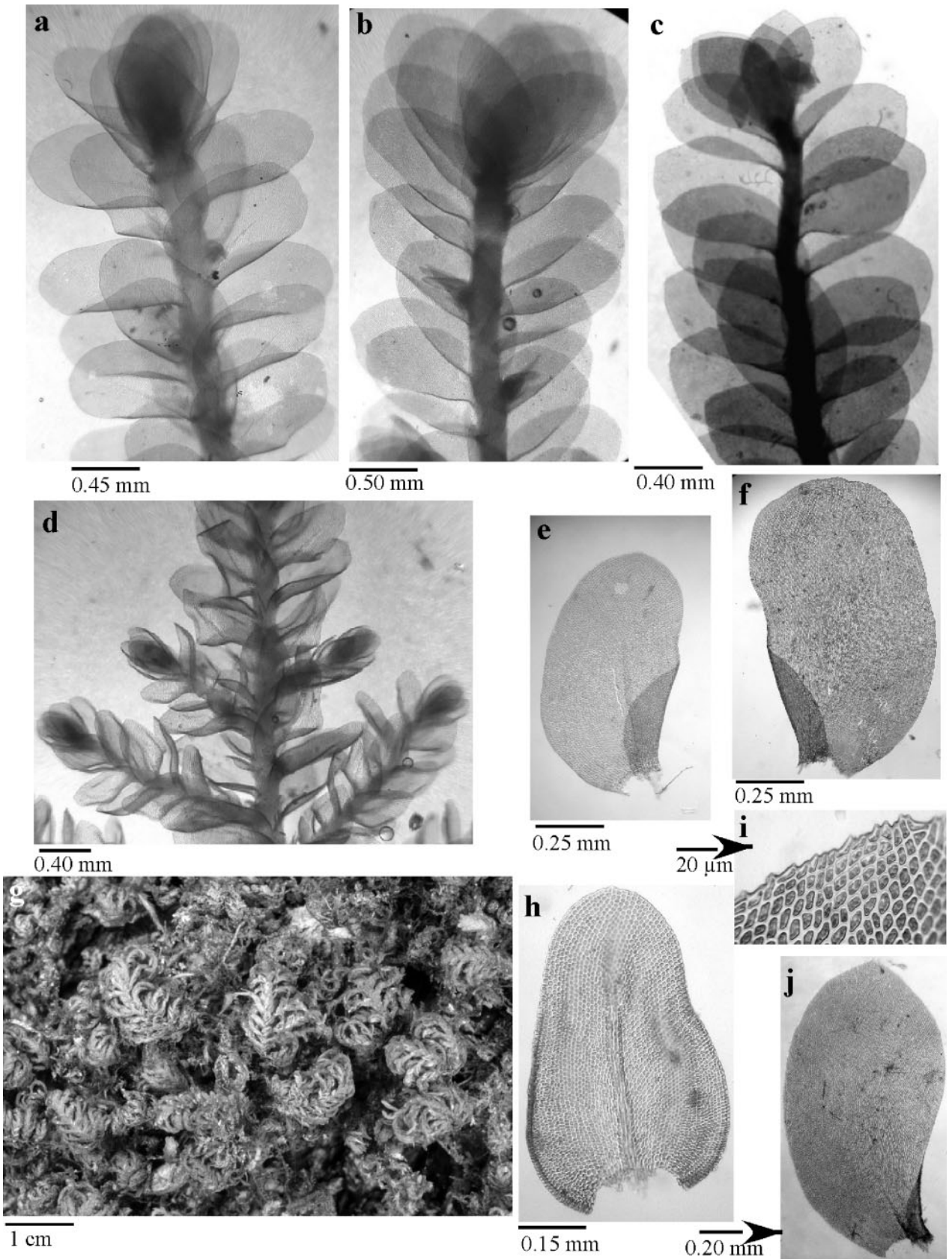


Figure 4. Comparison of the shoots and leaves of *Leptodon corsicus* (a, wet habit; e, leaf shape of *Sotiaux Corse 178*) with those of similar Neckeraceae: *Neckera besseri* (b, wet habit; f, leaf shape from *Sotiaux Corse 750*), *Leptodon smithii* ... (d, wet shoot; g, dry shoot (photo: J.-P. Frahm); h, leaf shape from *Sotiaux Corse 243*) and *Homalia webbiana* (c, wet habit; i, leaf apex; j, leaf shape from *Vanderpoorten A065*).

alar cells indistinct. *Paraphyllia* none. *Pseudoparaphyllia* none or very few per branch primordium, filiform or narrow-lanceolate, to ca 80 µm long. *Perigonia*, *perichaetia* and *sporophytes* unknown.

The MP analysis of 14 variable gametophytic characters (Appendix, Table 2) produced 27 equally parsimonious trees of L=28, CI=0.53, RI=0.83, whose strict consensus is presented in Fig. 5. Support for most clades is weak, but *L. corsicus* appears at a polytomy including: (1) all accessions of *L. smithii*, which form a clade supported at 90%; (2) *Alsia* and *Forsstroemia* spp.; (3) *Leptodon longisetus*; (4) *L. pluvinii*; and (5) *Neckera brownii*.

The molecular dataset assembled to determine the phylogenetic position of *L. corsicus* included 242 variable positions out of the 3012 alignment sites, and 129 parsimony-informative (pi) characters. The MP analysis produced three competing trees of L=311, CI=0.839, RI=0.877. The simple indel coding approach yielded another 124 sites with 69 pi-characters. The inclusion of the indel matrix resulted in a congruent consensus tree with similar or higher support values (see below). Phylogenies obtained via Bayesian inference, with or without the inclusion of the indel matrix, were congruent to parsimony analyses, but reached substantial support for several clades that were only poorly supported using parsimony. One of the trees sampled from the posterior probability distribution is presented in Fig. 6 with the posterior probabilities (PP) plotted above the branches, whereas bootstrap support (BS) from the parsimony analyses is shown below.

Table 2. Morphological data matrix of 14 gametophytic characters in *Leptodon* and related genera. The specimens examined correspond to those listed in Table 1 and the characters are presented in the same order as in Appendix

Homalia webbiana 1	1	0	0	1	N	0	1	1	0	0	1	0	0	0
Homalia webbiana 2	1	0	0	1	N	0	1	1	0	0	1	0	0	0
Forsstroemia trichomitria	0	0	0	1	1	1	0	0	0	2	1	0	1	0
Alsia californica	0	1	0	1	1	2	1	0	1	0	1	1	0	1
Forsstroemia producta	0	0	0	1	1	1	0	0	0	1	1	0	1	0
Neckera besseri	1	0	0	1	0	1	1	0	1	1	0	0	0	0
Neckera urnigera	1	1	0	1	1	0	1	0	1	0	2	1	1	0
Neckera hymenodonta	1	1	0	1	1	1	1	0	1	0	2	0	1	0
Neckera brownii	1	0	0	1	1	1	0	0	0	2	0	0	0	0
Neckera remota	1	1	0	1	0	1	0	1	0	2	1	1	0	0
Neckera jamesonii	1	1	0	1	0	1	0	1	1	2	0	1	0	0
Neckera scabridens	1	1	0	1	0	1	0	1	0	2	0	1	0	0
Neckera complanata (Germany)	1	1	0	1	0	1	0	0	1	2	0	0	0	0
Neckera complanata (South Africa)	1	1	0	1	0	1	0	0	1	2	0	0	0	0
Leptodon pluvinii	1	0	0	1	1	1	0	1	0	0	1	0	0	0
Leptodon longisetus (Canary Isl. 1)	1	1	0	1	1	1	1	0	1	0	0	1	0	0
Leptodon longisetus (Canary Isl. 2)	1	1	0	1	1	1	1	0	1	0	0	1	0	0
Leptodon smithii (South Africa)	1	0	1	1	1	1	0	1	1	0	1	0	0	0
Leptodon smithii (Yemen)	1	0	1	1	1	1	0	1	1	0	1	0	0	0
Leptodon smithii (Canary Isl. 1)	1	0	1	1	1	1	0	1	1	0	1	0	0	0
Leptodon smithii (Canary Isl. 2)	1	0	1	1	1	1	0	1	1	0	1	0	0	0
Leptodon smithii (Corsica)	1	0	1	1	1	1	0	1	1	0	1	0	0	0
Leptodon smithii (France)	1	0	1	1	1	1	0	1	1	0	1	0	0	0
Leptodon smithii (Italy)	1	0	1	1	1	1	0	1	1	0	1	0	0	0
Leptodon corsicus 1	1	0	0	1	1	0	N	0	0	0	0	0	0	0
Leptodon corsicus 2	1	0	0	1	1	0	N	0	0	0	0	0	0	0

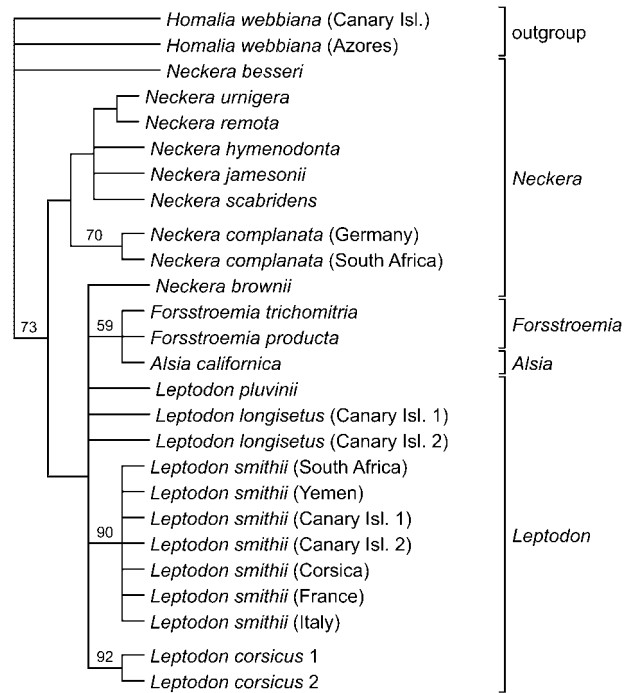


Figure 5. Strict consensus of 27 equally parsimonious trees resulting from the MP analysis of 14 variable morphological gametophytic characters for a sample of *Leptodon* and related genera. Numbers above the branches are the bootstrap proportions >50%.

Leptodon corsicus is deeply nested within *Leptodon*, and more precisely within a strongly supported clade (PP 1, BS 92) comprised of all the accessions of *L. smithii*. Within the *L. smithii* clade, a clear biogeographic structure emerges, with *L. corsicus* embedded within a Mediterranean group of accessions supported at 63% (BS, if indels are taken into account the BS increases to 86) and a PP of 0.99. The Mediterranean group is resolved as sister to a clade including the two accessions from the Canary Islands. This relationship between the Canarian and Mediterranean accessions receives high statistical support (PP 99, BS 73, with indel coding BS increases to 97). The Canarian-Mediterranean group is sister to a clade comprised of the Yemen and South African accessions (PP 1, BS 92). The strong geographic pattern among *L. smithii* accessions is, in addition, supported by: (1) various simple sequence repeats that occur on population level, as e.g. illustrated in Fig. 1C; and (2) the *trnL-F* inversion that changes conformation on population level as well. In fact, while most *L. smithii* accessions display the TC-type, the accessions from South Africa and Yemen have the GA-type of inversion. In addition, ancestral state reconstructions reveal that these samples lost a 20 nt SSR in the *rp116* intron (Fig. 1C). The Mediterranean accessions of *L. smithii* and *L. corsicus* show identical sequences in the *rps4-trnF* region and in the ITS. They further share a second 20 nt SSR (Fig. 1C), but differ by one substitution in the *rp116* intron.

The topologies displayed by the morphological and molecular analyses thus appear conflicting. In fact, the sum of the length of the trees obtained by a separate MP

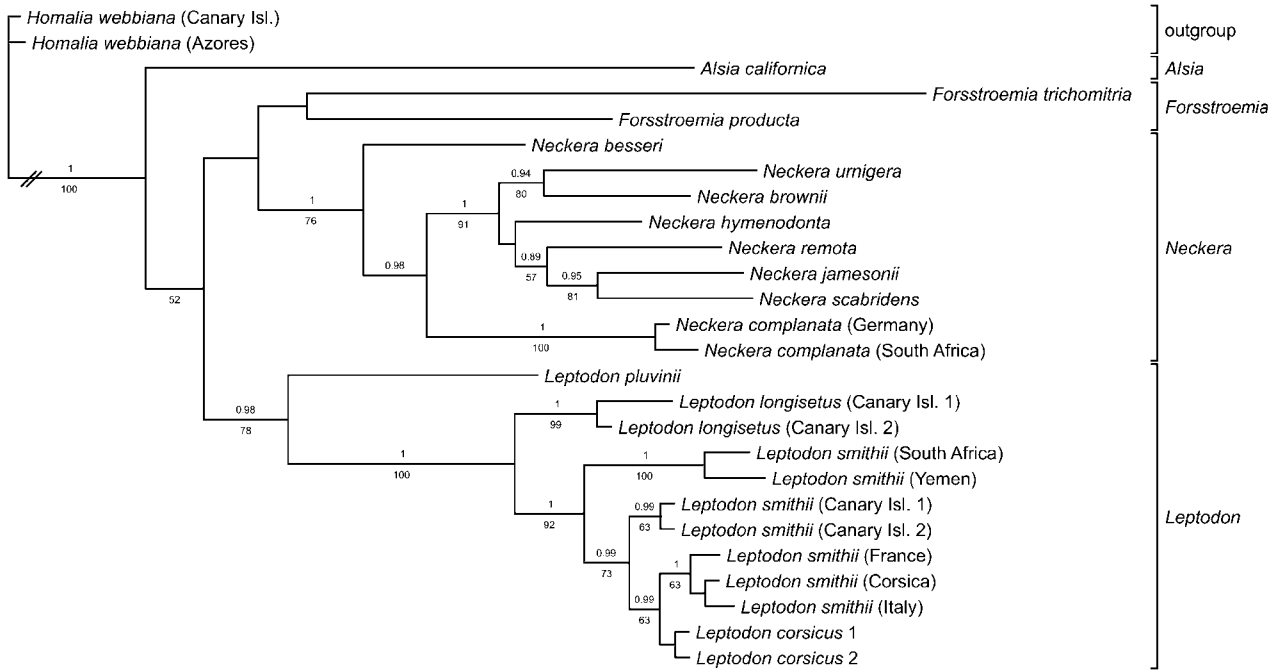


Figure 6. Phylogram of one of the trees sampled from the posterior probability distribution derived from a Bayesian analysis of *rpl16*, *rps4-trnF*, and ITS sequences (without indels) for a sample of *Leptodon* and related genera. The tree is rooted with *Homalia webbiana*. Posterior probabilities (PP) are plotted above the branches, whereas bootstrap support (BS) values from the parsimony analyses are shown below. // indicates that the long branch has been rescaled for graphical purpose.

analysis was significantly lower than that of the trees obtained after the analysis of 1000 replicates of a combined dataset of the same size ($p < 0.001$).

DISCUSSION

The results of the molecular phylogenetic analysis unambiguously show that the unknown Corsican species described as *Leptodon corsicus* is deeply nested within *L. smithii*. In contrast with all other *Leptodon* species, its shoots are about ten times smaller and are not at all frondose. They lack the dense and mostly pinnate to bipinnate mode of branching characteristic of *L. smithii* and *L. fuciformis* (Enroth, 1992) and, unlike the latter, are not inrolled when dry (contrast Figs 2 and 4g). *Leptodon corsicus* also lacks paraphyllia and its pseudoparaphyllia are small and only occasionally present in limited number, whereas these two structures are abundant and large in the other species of the genus. Due to its small size as well as leaf shape and some other cell characters, *L. corsicus* shares at first glance more similarities with *Homalia webbiana* and *Neckera bessi* than with *Leptodon* (Table 3). In fact, a cladistic analysis of the gametophytic traits of *Leptodon* and related genera shows that *L. corsicus* is not nested within *L. smithii*, which causes a significant conflict between the morphological and molecular data sets.

Incongruence between different data sets, and especially between morphology and molecules, remains one of the central issues in systematics (Lee, 2001). Among the mechanisms that may account for the paraphyly of *L.*

smithii in the molecular tree, mismatch between the gene tree and the species tree due to the sampling of paralogous ITS loci or hybridization are unlikely because of the congruence in the phylogenetic signal displayed by all the chloroplast and nuclear loci investigated.

An alternative explanation is that the morphological tree does not reflect the actual evolutionary history, and one of the most obvious reasons for it is morphological plasticity. Since many moss species are defined based on gametophytic characters, i.e., based on characters of the generation that is directly in contact with the substrate and exposed to a changing environment, many moss species were therefore, perhaps, defined on more plastic traits than are, for example, angiosperm species, and might hence be more vulnerable to phylogenetic testing (Vanderpoorten & Goffinet, 2006). This is typically the case for many aquatic species, which were defined based on plastic gametophytic traits, for example in the genera *Fontinalis* (Shaw & Allen, 2000) and *Hygroamblystegium* (Vanderpoorten & Jacquemart, 2004). Plasticity, however, can hardly be invoked in the case of *L. corsicus*. In fact, the latter differs from *L. smithii* by a series of traits including the absence of paraphyllia; the absence of modification upon drying; the lack of a dense and mostly pinnate to bipinnate mode of branching; and the scarcity of pseudoparaphyllia, which, when present, are exceedingly small. All these characters are almost certainly not influenced by local environmental conditions. In its only known locality, *L. corsicus* is abundant on shaded granitic boulders at sea level, where it forms pure colonies on several square meters. In addition, the dense mode of branching, paraphyllia and inrolled

Table 3. Comparison of diagnostic gametophytic traits between *Leptodon corsicus*, *L. smithii*, *Neckera bessi*, and *Homalia webbiana*.

	<i>L. corsicus</i>	<i>L. smithii</i>	<i>N. bessi</i>	<i>H. webbiana</i>
Leaf shape, margins	Ovate to oblong-elliptic; margins entire but often slightly sinuous near leaf apex	Ovate; margins entire	Obovate-lanceolate with smooth to minutely denticulate margins	Obovate or oblong-spathulate with denticulate margins
Costa	Weak, single, reaching to mid-leaf at most	Fairly strong, single, often reaching above mid-leaf	Very weak and short, often double, or absent	Short and often double
Paraphyllia	Absent	Abundant	Absent	Absent
Pseudoparaphyllia	Absent or few, up to 80 μm	Numerous, ca 100 μm	Few, 65–348 μm^*	Numerous, ca 100 μm
Gemmae	Absent	Absent	Often present	Absent
Habit	Not frondose, very irregularly and loosely branched	Frondose, densely and regularly pinnate to bipinnate	\pm frondose, regularly branched	Not frondose, irregularly branched
Modification after desiccation	No	Yes	No	No
Length and width of leafy shoots	3–7 \times 1 mm	To ca. 40 \times 1.5 mm (main stems of fronds)	To 80 \times 1.5 mm	To 75 \times 1–2(–2.5) mm

*Data from Cubero, Marcos-Samaniego & Enroth (2006).

fronds are already present in juvenile specimens of *L. smithii* (J. Enroth, pers. obs.), so that the idea that the specimens collected represent an immature developmental stage of *L. smithii* can also be ruled out.

As opposed to ‘cryptic’ species of bryophytes, which are characterized by substantial genetic divergence within morphologically homogeneous entities (Shaw, 2009), *L. corsicus* is morphologically strikingly different from *L. smithii*, and yet, is phylogenetically nested within it. In fact, many differences in complex morphological traits are not the result of the presence or absence of particular genes or extensive mutations, but are based on single or few point mutation, or even to changes in the mechanisms of gene regulation affecting when and where a gene is expressed (Brakefield, 2006). In the beach mouse for example, a single amino acid mutation contributes to adaptive color pattern (Hoekstra *et al.*, 2006). Hedenäs & Eldenäs (2008) similarly evoked the possibility that a single or a few genes may be responsible for dramatic morphological modifications in mosses. Such an interpretation definitely contrasts with the view that rates of molecular and morphological evolution are correlated (see Stenoien, 2008, for review). Fast and dramatic morphological changes owing to single or few genetic mutations, or changes in gene expression, would explain why characters can shift states so easily along moss phylogenies (Vanderpoorten *et al.*, 2002).

While a few mutations or changes in gene expression may account for the dramatic morphological divergence between *L. corsicus* and *L. smithii*, the bulk of the genome, and especially the non-coding sequences used here, may not yet have had the time to sort out. The idea, that a new species renders its parental species paraphyletic because the time since speciation was not long enough for the sorting of alleles to be complete, is at the basis of a speciation mechanism called ‘budding’ speciation (see Funk & Omland, 2003, for review). Such a situation has been recently described in other bryophyte species that may (e.g., *Homalothecium lutescens* (Hedw.) H. Rob. and *H. sericeum* (Hedw.) Schimp.; Hedderson & Nowell, 2006; but see Huttunen *et al.*, 2008; *Ditrichum plumbicola* Crundw. and *D. lineare* (Sw.) Lindb.; Frahm, Sabovlevic & Nokhbehssaim, 2008; *Scorpidium cossonii* (Schimp.) Hedenäs and *S. scorpioides* (Hedw.) Limpr.; Hedenäs & Eldenäs, 2008), or may not (e.g., *Leptoscyphus azoricus* Grolle and *L. porphyrius* (Nees) Grolle; Vanderpoorten & Long, 2006), be already morphologically well differentiated.

The interpretation that *L. corsicus* started to diverge from parental populations of *L. smithii* recently enough, so that the non-coding DNA sequences used for phylogenetic reconstruction had no time to sort yet, might also apply to other species of mosses, which exhibit a striking morphology, and yet share identical non-coding sequences with the common species they derive from (e.g., *Platyhypnidium mutatum*; Stech & Frahm, 1999; *P. torrenticola*; Werner *et al.*, 2007; *Thamnobryum angustifolium*; Olsson *et al.*, 2009d). The question of whether such taxa should be

recognized at the species level remains, of course, open, and rejecting the monophyletic species concept leaves the door open to the acceptance of poorly defined species based on vague combinations of morphological characters. In the context of the monophyletic species concept, the only way to give the species status to *L. corsicus* is to raise the African and Canarian population of *L. smithii* at the rank of 'cryptic' species. Each of these two clades is characterized by many synapomorphic substitutions. The branches leading to these clades are, in fact, much comparable to those separating well-recognized species of *Leptodon*, such as *L. longisetus* and *L. smithii*, or of other genera like *Neckera*. Although thorough molecular and morphological investigation, currently underway (I. Draper, pers. comm.), may ultimately reveal morphological differentiation among those lineages, nobody welcomes, however, the prospect of more floods of new 'species' swamping the literature and herbaria and recognizable only using cytological or molecular techniques (Rowley, 2007). As a matter of fact, budding speciation inevitably results in paraphyletic relationships among species until the sorting of alleles by drift is complete so that a monophyletic species concept is not applicable. In those circumstances, forcing taxonomy to reflect gene tree monophyly by synonymizing the nested and parent species ignores the distinctive nature of the nested lineage (Funk & Omland, 2003) so that a classification, wherein both similarity and common descent are used as criteria, better reflects the evolutionary patterns increasingly revealed by molecular studies (Hedenäs & Eldenäs, 2008).

One puzzling remaining question is why, if *L. corsicus* is indeed a new species that started to diverge recently from mutations or expression in a single or few genes, did it evolve in the direction of already existing, completely unrelated species such as *Homalia webbiana* or *Neckera bessi*? Such a gametophytic similarity among unrelated moss lineages has long been acknowledged, for example between genera of the Orthotrichaceae and the Pottiaceae (Zander & Vitt, 1979) and suggests that the trajectory of phenotypic evolution is constrained in the short term by genetic correlations among traits. In other words, all the space of morphologies cannot be explored because there exists evolutionary constraints in character evolution. In fact, absolute or strict constraints account for some boundaries in patterns of occupancy of morphological space. Some morphologies are, hence, more readily generated than others, and developmental mechanisms can limit or channel evolutionary change, which can result in the clustering of populations or species along 'lines of least resistance', rendering alternative directions of evolution less likely (Beldade, Koops & Brakefield, 2002). In mosses, although McDaniel (2005) found that genetic correlations do not impose a lasting constraint on the evolution of life-history variation in *Ceratodon purpureus*, some characters were found to evolve in a parallel, correlated fashion among phylogenetically independent lineages of the aquatic moss *Hygroamblystegium* (Vanderpoorten & Jacquemart, 2004).

As emphasised by Hedenäs & Eldenäs (2008), we still know little about the mechanisms of morphogenesis in mosses. With the advances of population genomics and quantitative genetics, we now have the toolkit to discover how such bizarre morphologies evolved and to find the genes underlying ecologically important traits.

APPENDIX

List of 14 variable characters scored on *Leptodon* and related genera

1. stem leaf: asymmetric (1) or not (0)
2. stem leaf apex: entire to slightly denticulate (0) or denticulate (1)
3. stem leaves: similar in shape wet or dry (0) or highly modified by desiccation (1)
4. stem leaves: not or very shortly decurrent (0) or distinctly decurrent (1)
5. stem leaves: length to width ratio <1.5 (0) or >1.5 (1)
6. costa: absent or short and double (0), present (1), or both (2)
7. pseudoparaphyllia: absent (0) or present (1)
8. pseudoparaphyllia shape: foliose (0) or both filamentous and foliose (1)
9. paraphyllia: absent (0) or present (1)
10. specialized vegetative gemmae: absent (0) or present (1)
11. mid-leaf cell length to width ratio: 1:1 (0), 2-3:1 (1), or >3:1 (2)
12. stem leaf margin: plane (0) or recurved (1)
13. stem leaves: plane (0) or undulate (1)
14. subapical leaf cell walls: porose (1) or solid (0)

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TAXONOMIC ADDITIONS AND CHANGES: *Leptodon corsicus* Enroth, Sotiaux, D. Quandt & Vanderpoorten, sp. nov.

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