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Phylogenetic inference in *Leucodon* Schwägr. subg. *Leucodon* (Leucodontaceae, Bryophyta) in the North Atlantic region

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Abstract Systematic and biogeographic relationships of mainly European and North American taxa of the moss genus *Leucodon* subg. *Leucodon* are inferred by phylogenetic reconstructions and haplotype analyses, based on sequence data from three plastid regions and nrITS. The two Macaronesian endemic species *L. canariensis* and *L. treleasei* are clearly separated from each other and from *L. sciurooides*, which is widespread in Eurasia including Macaronesia. A well-supported sister-group relationship with the American species *L. curvirostris* and *L. julaceus* indicates a New World ancestor of *L. treleasei*, which is probably a neoendemic species that colonized the Macaronesian islands after (recent) dispersal. The position of *L. canariensis* sister to the clade of *L. treleasei* and the American species is less well-supported, leaving its evolutionary history ambiguous. The Eastern North American *L. andrewsianus* is neither molecularly nor morphologically unambiguously distinguishable from *L. sciurooides*. Synonymisation of *L. andrewsianus* with *L. sciurooides* solves the long-debated question whether the latter species occurs in North America, and results in a Holarctic instead of Palaearctic distribution pattern of *L. sciurooides*. The Afrotropical *L. dracaenae* cannot be clearly separated from *L. sciurooides* as well, whereas the Eastern Mediterranean to Central Asian *L. immersus* differs from *L. sciurooides* by few substitutions and indels as well as morphologically by the short seta and immersed capsule. Further analyses are necessary before taxonomic conclusions should be inferred for *L. dracaenae* and *L. immersus*. Intraspecific diversity in *L. sciurooides* does not support recognition of the mainly Mediterranean var. *morensis*. Instead, a basic separation of Mediterranean (plus Macaronesian) versus non-Mediterranean haplotypes is indicated. The higher haplotype diversity in the Mediterranean (and Macaronesia) in contrast to the other parts of Europe is in accordance with genetic depletion in formerly glaciated areas.

Keywords *atpB-rbcL* spacer; haplotype analysis; *Leucodon*; Macaronesia; molecular phylogeny; North Atlantic region; nrITS; *trnG_{GGA}* intron; *trnT_{ACC}-trnE_{GAA}* spacer

■ INTRODUCTION

Leucodon Schwägr. is a genus of pleurocarpous mosses (Hypnales, Leucodontaceae) that comprises 37 species (Frey & Stech, 2009). It is characterized by robust plants growing on rock or trees, with creeping stems and simple upright branches that are often curved when dry (‘squirrel-tail moss’) as well as usually plicate leaves without a nerve, and a white peristome.

The genus has a subcosmopolitan distribution mainly in temperate regions, with a centre of diversity in East Asia where 20 species occur (Akiyama, 1988). Six and five species, respectively, are reported for the areas dealt with in the present study, viz., Europe (Frey & al., 2006; Hill & al., 2006) and the

Americas (Reese & Anderson, 1997; Gradstein & al., 2001). Of the European species, *Leucodon sciurooides* (Hedw.) Schwägr. is widespread in the Palaearctic, including Macaronesia; the other five species are more narrowly distributed. *Leucodon canariensis* (Brid.) Schwägr. and *L. treleasei* (Cardot) Paris are endemic to Macaronesia, *L. flagellaris* Broth. and *L. immersus* Lindb. extend from the Caucasus to the East Mediterranean, and the Asian species *L. pendulus* Lindb. reaches westwards to the European part of Russia (Ignatov & Ignatova, 2004). Two of the American species, *L. brachypus* Brid. and *L. andrewsianus* (H.A. Crum & L.E. Anderson) W.D. Reese & L.E. Anderson, are restricted to North America (Reese & Anderson, 1997), one is endemic to Mexico (*L. cryptotheca* Hampe), and the

remaining two, *L. curvirostris* Hampe and *L. julaceus* (Hedw.) Sull., reach southwards to the Neotropics (*L. curvirostris* to Mexico, Colombia and Peru; *L. julaceus* to Mexico and the Dominican Republic; cf. Gradstein & al., 2001).

Leucodon has been considered one of the most difficult moss genera to classify (Akiyama, 1988). Intrageneric classification as well as delimitation and identification of *Leucodon* species mainly rely on sporophytic characters such as peristome and spore structure, as the differences in gametophytic characters are limited. Consequently, species identification, which is usually based on sterile material, is often difficult. Up to now, only one study employed DNA data, namely plastid *rbcl* sequences, to study suprageneric and family relationships within the former suborder Leucodontineae of Hypnales (Maeda & al., 2000). No molecular data are available yet to test the intrageneric classification of *Leucodon* by Akiyama (1988; 1994) into three subgenera, *Leucodontella*, *Cryptotheca*, and *Leucodon*, the latter with two sections, *Macrosporiella* and *Leucodon*, and to assess the taxonomic status and systematic relationships of *Leucodon* species.

The present study aims at clarifying systematic and biogeographic relationships of selected species of *Leucodon* subg. *Leucodon* in the North Atlantic region (Europe, Macaronesia, America), based on molecular phylogenetic reconstructions and haplotype networks from plastid *atpB-rbcL* spacer, *trn-T_{ACC}-trnE_{GAA}* spacer, and *trnG_{GGA}* intron, as well as nuclear ribosomal ITS sequences. The selected species (all from sect. *Leucodon*) include *L. canariensis*, *L. immersus*, *L. sciuroides* (including var. *morensis*) and *L. treleasei*, as well as *L. andrewsianus*, *L. curvirostris* and *L. julaceus*. *Leucodon dracaenae* Solms ex Venturi, an Afrotropical species (cf. O'Shea, 2006; Kürschner, 2008) also from *L. sect. Leucodon*, and one species from East Asia, *L. sapporensis* Besch. (*L. sect. Macrosporiella*), are included as well. The main systematic problem to be tackled is the morphological distinction of the two Macaronesian endemics, *L. immersus*, *L. andrewsianus*, and *L. dracaenae* from the widespread *L. sciuroides*. The diagnostic morphological characters and taxonomic status of these taxa will be evaluated based on the molecular phylogenetic reconstructions. Intraspecific variation in *L. sciuroides*, including the status of var. *morensis* (Schwägr.) De Not., a more robust mainly Mediterranean and Macaronesian taxon (rare northwards to southern Scandinavia) whose taxonomic status is controversial (Smith, 2004), will be tested as well.

Biogeographic inference will focus on (1) molecular divergence of *Leucodon sciuroides* and allied species across the North Atlantic, in comparison with recent analyses of intercontinental distribution patterns of other moss taxa (see discussion in Huttunen & al., 2008); (2) potential geographic structuring of genetic diversity in *L. sciuroides* in Europe, to test Cronberg's (2000) clustering of (largely) Mediterranean versus non-Mediterranean populations based on isozymes; (3) possible origins of the two Macaronesian endemic species. In contrast to the well-known examples of angiosperm radiations in Macaronesia (e.g., Kim & al., 2008 and references therein), bryophyte genera with more than one Macaronesian endemic species are rather rare. *Leucodon* is thus, similar to another

recently studied moss genus, *Rhynchostegiella* (Aigoïn & al., 2009), a good model to test hypotheses on the origin of the Macaronesian bryoflora. Macaronesian endemics have originally been interpreted as relicts of vegetation types (in particular the laurel forest) that were widespread throughout the Mediterranean region in the Tertiary, but survived only on the Atlantic islands (starting with Engler, 1879; cf. Vanderpoorten & al., 2007, for review). On the contrary, endemic species may have been evolved more recently from ancestors that reached Macaronesia by dispersal of diaspores from continental areas. In bryophytes, few taxa may in fact be palaeo-endemics, such as *Hedenasiastrum* (Aigoïn & al., 2009) or *Echinodium* (Stech & al., 2008). However, there is accumulating molecular evidence that the Macaronesian bryoflora comprises a considerable number of recently evolved taxa as well, which reached the Atlantic islands by dispersal from continental areas in the New and Old World such as the Neotropics, Palearctica, or Europe (see discussion). Plausible hypotheses to be tested for *L. canariensis* and *L. treleasei* are thus that they either share a common ancestor with the widespread *L. sciuroides*, or show affinities with the American *Leucodon* species.

■ MATERIALS AND METHODS

Plant material. — The present dataset comprised fifty-two specimens of *Leucodon*, seven specimens of other genera previously included in Leucodontaceae based on morphological characters (*Antitrichia californica* Sull., *A. curtispindula* (Timm ex Hedw.) Brid., *Forsstroemia producta* (Hornsch.) Paris, *F. trichomitria* (Hedw.) Lindb., *Pterogonium gracile* (Hedw.) Sm.), five species of other families of Hypnales (Amblystegiaceae: *Drepanocladus aduncus* (Hedw.) Warnst. [cf. Stech & Frahm (2001), as *Cratoneuroopsis relaxa* (Hook. f. & Wils.) Fleisch.], *Hypnobartlettia fontana* Ochyra; Brachytheciaceae: *Platyhypnidium riparioides* (Hedw.) Dixon; Echinodiaceae: *Echinodium spinosum* (Mitt.) Jur.; Neckeraceae: *Thamnobryum pandum* (Hook. f. & Wilson) I.G. Stone & G.A.M. Scott), and two species of Hookeriales (*Hookeria lucens* (Hedw.) Sm., *Hypopterygium tamarisci* (Sw.) Brid. ex Müll. Hal.) as outgroup representatives. Outgroup selection was based on the sister-group relationship of Hypnales and Hookeriales in previous molecular phylogenetic reconstructions (e.g., Bell & al., 2007; Stech & Frey, 2008). All *Leucodon* samples were newly analyzed for the present study and taken from specimens hosted in herbaria B, DUKE, E, HYO, L, LISU, MUB, S, TFC, and VIT as well as the personal herbarium of H. Kürschner. Sequences of specimens from the other genera were either newly generated or taken from own earlier studies. Voucher information and GenBank accession numbers are shown in the Appendix.

DNA extraction, PCR, and sequencing. — Plant material was thoroughly cleaned with distilled water and ultrasonic treatment. Total DNA was extracted from dry material using the NaOH extraction method as explained in Werner & al. (2002). The chloroplast *trnG_{UCC}* intron was amplified in 50 µl final volume with primers trnGF-Leu (GGC TAA GGG

TTA TAG TCG GC; Werner & al., 2009) and *trnGR* (GCG GGT ATA GTT TAG TGG; Pacak & Szweykowska-Kulińska, 2000). The *atpB-rbcL* spacer was initially amplified with primers *atpB-1* (ACA TCK ART ACK GGA CCA ATA A) and *rbcL-1* (AAC ACC AGC TTT RAA TCC AA) of Chiang & al. (1998). In several cases we were unable to amplify this region with the original primers and used the redesigned *atpB-2* (AAT AAG TGT TGA AGT CCC) and *rbcL-2* (CCC TCC CTA CAA CTC A) of Vanderpoorten & Long (2006) instead. The *trnT_{ACC}-trnE_{GAA}* spacer was amplified with primers *trnE* (GCC TCC TTG AAA GAG AGA TG; Doyle & al., 1992) and *trnT(P*)* (CTA CCA CTG AGT TAA AAG GG; Demesure & al., 1995). ITS1 and ITS2 were amplified in separate reactions due to problems with amplifying the complete ITS1-5.8S-ITS2 especially from older herbarium samples. The primers used were 18F (GGA AAG AGA AGT CGT AAC AAG G) and 5.8SR (GCT GCG TTC TTC ATC GTT GC) for ITS1 and 5.8F (GCA ACG ATG AAG AAC GCA GC) and 25R (TCC TCC GCT TAG TGA TAT GC) for ITS2 (Stech & Frahm, 1999). For all amplification reactions 4 µl of stock DNA were added as template. 200 µM of each dNTP, 2 mM MgCl₂, 2 units Taq polymerase (Appligene Oncor, Illkirch, France), 1 µl BLOTTO (10% skimmed milk powder and 0.2% NaN₃ in water) and the buffer provided by the enzyme supplier were added. BLOTTO attenuates PCR inhibition caused by plant compounds (De Boer & al., 1995). The amplification conditions were as follows: 3 min at 94°C, 35 cycles with 30 s at 94°C, 30 s at 50°C and 1 min at 72°C, and a final 7 min extension step at 72°C. Amplification products were cleaned with the GenElute PCR Clean-Up Kit (Sigma-Aldrich, St. Louis, Missouri, U.S.A.). Cycle sequencing was performed with the Big Dyes Sequencing Kit (Perkin Elmer, Waltham, Massachusetts, U.S.A.) using a standard protocol and the amplification primers. The annealing temperatures were set at 50°C. The reaction products were separated on an ABI Prism 3700 automatic sequencer (Perkin Elmer).

Phylogenetic analysis. — Based on the criteria laid out in Kelchner (2000) and Quandt & Stech (2005), DNA sequences were manually aligned in PhyDE[®] v.0.995 (Müller & al., 2006).

Phylogenetic reconstructions were based on combined *atpB-rbcL*, *trnT-trnE*, *trnG*, and ITS sequences. In addition, the four regions were analyzed separately to detect possible incongruence between the markers, as inferred from the potential occurrence of conflicting well-supported clades. Tree calculations according to the maximum parsimony (MP) and maximum likelihood (ML) optimality criteria were performed using PAUP* v.4.0b10 (Swofford, 2002). Heuristic searches under parsimony were implemented using random sequence addition with 1000 replicates and employing the default settings otherwise. Gaps were either treated as missing data or coded as informative by a simple indel coding (SIC) strategy (Simmons & Ochoterena, 2000) as implemented in SeqState (Müller, 2004). Heuristic bootstrap searches under parsimony were performed with 1000 replicates and 10 random addition cycles per bootstrap replicate with the same options in effect.

Maximum likelihood analyses were executed assuming a general time reversible (GTR) model and a rate variation among

sites following a gamma distribution. GTR+ Γ +I was chosen as the model that best fits the data according to the hLRT and AIC criteria as evaluated by MrModeltest v.2.3 (Nylander, 2004) employing MrMTgui (Nuin, 2005). The proposed settings by MrModeltest were executed in PAUP*: Basefreq = (0.3043 0.1830 0.1834), Nst = 6, Rmat = (0.8711 2.7382 0.3131 1.0894 3.7361), Shape = 0.7731, Pinvar = 0.3775. Heuristic likelihood bootstrap searches were performed with 100 replicates.

For further measurement of support, posterior probabilities were calculated using MrBayes v.3.1 (Huelsenbeck & Ronquist, 2001). As in the maximum likelihood analysis, the GTR model of nucleotide substitution was employed, assuming site-specific rate categories following a gamma distribution. In a second Bayesian analysis the indels coded by SIC were included, with sequence and indel data treated as separate and unlinked partitions, employing the restriction site model ('F81') for the indel matrix. The a priori probabilities supplied were those specified in the default settings of the program. Posterior probability (PP) distributions of trees were created using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method. Four runs with four chains (10⁶ generations each) were run simultaneously, with the temperature of the single heated chain set to 0.2. Chains were sampled every 10 generations and the respective trees written to a tree file. Consensus trees and posterior probabilities of clades were calculated by combining the four runs and using the trees sampled after the chains converged. Trace plots generated in Tracer v.1.5 (Rambaut & Drummond, 2007) were used to check for convergence of the runs (plateaus of all runs at comparable likelihoods) and to infer the burn-in, which approximately ranged between the first 150,000 and 200,000 generations (first 15,000–20,000 sampled trees). Consequently, the first 25,000 trees (25%) were deleted by default to be sure that only trees of the stationary phase were included.

Haplotype analysis. — Relationships among plastid and ITS haplotypes in the clade of *Leucodon sciurooides* and allies were evaluated based on statistical parsimony, using TCS v.1.21 (Clement & al., 2000). Haplotype networks were constructed separately for the combined plastid markers and for ITS, with only complete sequences without any ambiguous positions included. Analyses were performed with gaps coded as missing data or with indels included as a fifth character state (gaps = 5th state), as it is currently not possible to include indels coded as additional characters, e.g., by a SIC approach, in TCS. This may lead to overestimation of patterns supported by longer indels, which needs to be taken into account when comparing and discussing haplotype networks with and without indels included.

■ RESULTS

Sequence and alignment characterization. — Sequence lengths were 554–574 nucleotides (nt) in *Leucodon* (476–574 nt in the other included taxa) for *atpB-rbcL* spacer, 253–257 nt (254–264) for *trnT_{ACC}-trnE_{GAA}* spacer, 581–598 nt (569–589) for *trnG_{GGA}* intron, and 704–776 nt (525–697) for the ITS1-5.8S-ITS2 region. Sequence data were missing for *trnT-trnE* of *Leucodon curvirostris* as well as ITS1 of *L. dracaenae* and

Forsstroemia trichomitria, as no PCR products could be obtained. The combined alignment comprised 2737 positions (*atpB-rbcL* 651, *trnT-trnE* 273, *trnG* 626, ITS 1187 positions). The 5.8S gene, 781 positions of the ITS with ambiguous alignment across the Hypnales and outgroup representatives, and 34 incompletely sequenced positions at the end of the *atpB-rbcL* spacer were excluded from all analyses. Of the remaining 1956 included positions, 429 were variable, and 221 of the variable positions were parsimony-informative (*atpB-rbcL* 44, *trnT-trnE* 31, *trnG* 62, ITS 84 parsimony-informative positions). The SIC approach yielded an additional 147 parsimony-informative indel characters, resulting in a total of 368 parsimony-informative characters.

Phylogenetic reconstructions. — Separate analyses of *atpB-rbcL*, *trnT-trnE*, *trnG*, and ITS, respectively, resulted in differently resolved (consensus) trees, due to different numbers of parsimony-informative characters (see above) and different degrees of homoplasy, as inferred from the respective consistency indices. However, no incongruence between the different markers could be inferred, as no conflicting well-supported clades were found by visual comparison of the respective tree topologies (data not shown). The combined four-marker MP analysis retained four most parsimonious trees (length 639, consistency index, CI, 0.775, retention index, RI, 0.875) with indels excluded and 10,000 most parsimonious trees (length 1042, CI 0.790, RI 0.883) with indels included. In the ML analysis a single optimal tree was found (lnL = -6116.53984). The ML tree is shown in Fig. 1, with bootstrap support (BS) values >70% from MP, MP-SIC and ML analyses, and significant (>95) posterior probabilities (PP) from Bayesian analyses without and with indels indicated above the branches. In this tree, all *Leucodon* specimens form a clade with 97%–100% BS and PPs of 100. Within *Leucodon*, two main groups are observed. The first group comprises all specimens of *L. sciuroides*, *L. andrewsianus*, *L. dracaenae* and *L. immersus* (77%–94% BS, PP 99–100) as sister to *L. sapporensis* (no significant BS, PP 98–99). Relationships within the *L. sciuroides* and allies clade are poorly resolved and supported; only two clades of *L. sciuroides* specimens from Cape Verde, Canary Islands, mainland Spain and Italy receive significant PPs of 96–99. The second main group (MP: 80% BS, PP 96–97) includes the specimens of the Macaronesian endemic *L. canariensis* (99%–100% BS, PP 100) and *L. treleasei* (99%–100% BS, PP 100), as well the American *L. curvirostris* and *L. julaceus* (maximal support in all calculations). The latter two are sister to *L. treleasei* (87%–98% BS, PP 100).

Haplotype analysis. — The statistical parsimony networks of the plastid and ITS haplotypes in the clade of *Leucodon sciuroides* and allies are shown in Fig. 2. Both the plastid and ITS data indicate the existence of two main haplotypes that are present in the majority of the included specimens, with four to seven additional haplotypes being represented by one to four specimens each. The largest plastid haplotype in the network based on substitutions only (Fig. 2A) includes the specimens of *L. andrewsianus*, *L. dracaenae* and *L. immersus*, one specimen of *L. sciuroides* var. *morensis*, plus exclusively non-Mediterranean specimens of *L. sciuroides* s.str. (specimen 2027 originates from Central France, 2047 from the Alpine part

of Italy). The Mediterranean and Macaronesian *L. sciuroides* specimens (plus var. *morensis* from U.K.; 2022 originates from Umbria, i.e., Mediterranean Central Italy) are split into two groups of two haplotypes each (to the left and right of the largest plastid haplotype; surrounded by boxes with dotted lines). The two haplotypes on the left correspond to the clades with significant posterior probabilities in Fig. 1. The respective plastid haplotype network including indel characters (Fig. 2B) is largely congruent, except for two additional haplotypes based on one indel each (four and 14 nt, respectively), which separate *L. immersus* as well as two Western European samples of *L. sciuroides* (2019, 2027) from the largest haplotype. The high number of 3 or 13, respectively, hypothetical intermediate haplotypes leading to these two additional haplotypes is probably caused by the indels being coded as fifth character state.

In the ITS network without indels (Fig. 2C), the five haplotypes on the left comprise all Mediterranean/Macaronesian specimens (except one var. *morensis* from Spain, plus var. *morensis* from U.K.), whereas the three haplotypes on the right include the non-Mediterranean specimens of *L. sciuroides* s.str. plus those of the other *Leucodon* species. *Leucodon dracaenae* is not included as the ITS region could not be sequenced completely. The same haplotypes comprising the same samples were recovered in the respective analysis including indels (network not shown). Hypothetical intermediate haplotypes were indicated based on indel characters, namely one between the two main haplotypes and two between the largest haplotype and *L. immersus*, probably due to the indels being coded as fifth character state.

■ DISCUSSION

The species of *Leucodon* subg. *Leucodon* analyzed in the present study clearly form a monophyletic group in the molecular phylogenetic reconstructions (Fig. 1), which supports the only previous molecular study by Maeda & al. (2000) based on *rbcL* sequences, now with significant statistical support. The present molecular data furthermore indicate that *Leucodon* is not closely related to the other included genera of the traditional Leucodontaceae, namely *Antitrichia* and *Pterogonium*, nor to *Forsstroemia*. The systematic placement of the latter either in Cryphaeaceae, Leptodontaceae, or Leucodontaceae, has long been debated (e.g., Buck, 1980; Akiyama, 1994; Ignatov & Czerdantseva, 1995). Most recently Leptodontaceae, including *Forsstroemia*, has been included in Neckeraceae based on molecular results (Olsson & al., 2009), which is supported by the close relationship of *Forsstroemia* and *Thamnobryum* in the present study. *Antitrichia* was recently segregated in its own family Antitrichiaceae by Ignatov & Ignatova (2004). For *Pterogonium*, in contrast, this is the first indication that it may be better placed outside Leucodontaceae, but molecular phylogenetic analyses of a larger taxon sampling of Hypnales are necessary before its systematic position can be established.

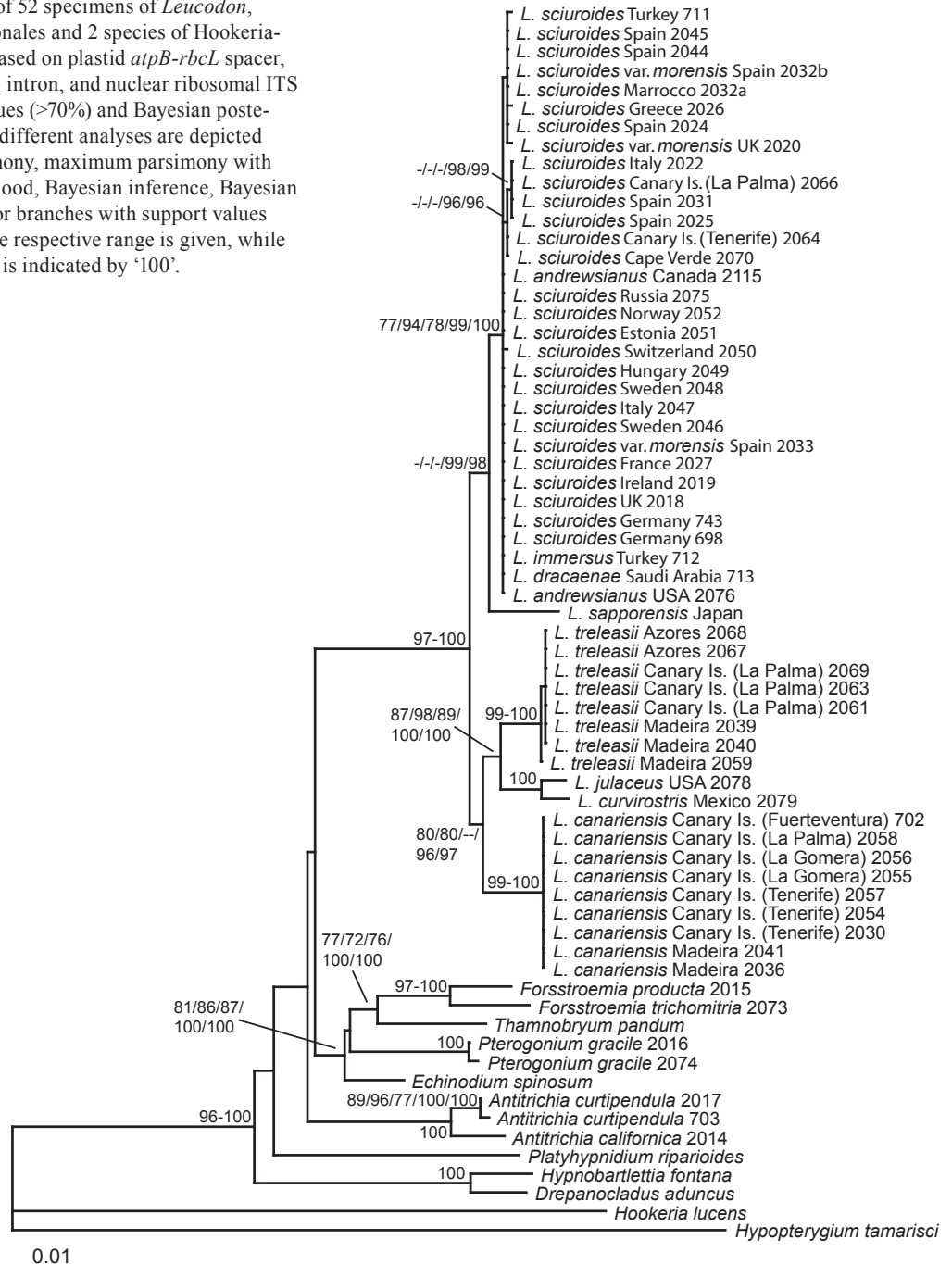
Within *L.* subg. *Leucodon*, the present data do not support the distinction of sect. *Macrosporiella* and sect. *Leucodon*, as *L. sapporensis* (sect. *Macrosporiella*) is not resolved as sister to

all taxa of sect. *Leucodon*. This topology, however, is statistically supported in the Bayesian calculations only, and inclusion of further species of sect. *Macrosporiella* is necessary to arrive at a final conclusion.

The two Macaronesian endemic species *Leucodon canariensis* and *L. treleasei* are clearly separated from each other and from the widespread *L. sciuroides* (Fig. 1). Consequently, three distinct *Leucodon* species occur in Macaronesia. They are morphologically distinguished by different shapes of the alar cell groups in the leaves as well as by size differences of gametophytic and sporophytic characters, for example the

mid-leaf lamina cells (Hedenäs, 1992; González-Mancebo & al., 2009). These differences are rather slight and difficult to apply in some specimens (own observations). According to the molecular data, however, the diagnostic morphological characters in principle are suitable for species identification and reflect the existence of separate species. In addition, habitat preferences and distribution frequencies on the Macaronesian archipelagos slightly differ between the three species (Hedenäs, 1992; Dierßen, 2001; Sérgio & al., 2006; Azorean Biodiversity Portal, 2008; González-Mancebo & al., 2009). *Leucodon treleasei* is the most widespread species, common

Fig. 1. Maximum likelihood tree of 52 specimens of *Leucodon*, with 12 further specimens of Hypnales and 2 species of Hookeriales as outgroup representatives, based on plastid *atpB-rbcL* spacer, *trnT_{ACC}-trnE_{GAA}* spacer, *trnG_{GGA}* intron, and nuclear ribosomal ITS sequences. Bootstrap support values (>70%) and Bayesian posterior probabilities (>95) from five different analyses are depicted at the branches: maximum parsimony, maximum parsimony with indels included, maximum likelihood, Bayesian inference, Bayesian inference with indels included. For branches with support values of >95 in all five analyses only the respective range is given, while maximum support in all analyses is indicated by ‘100’.



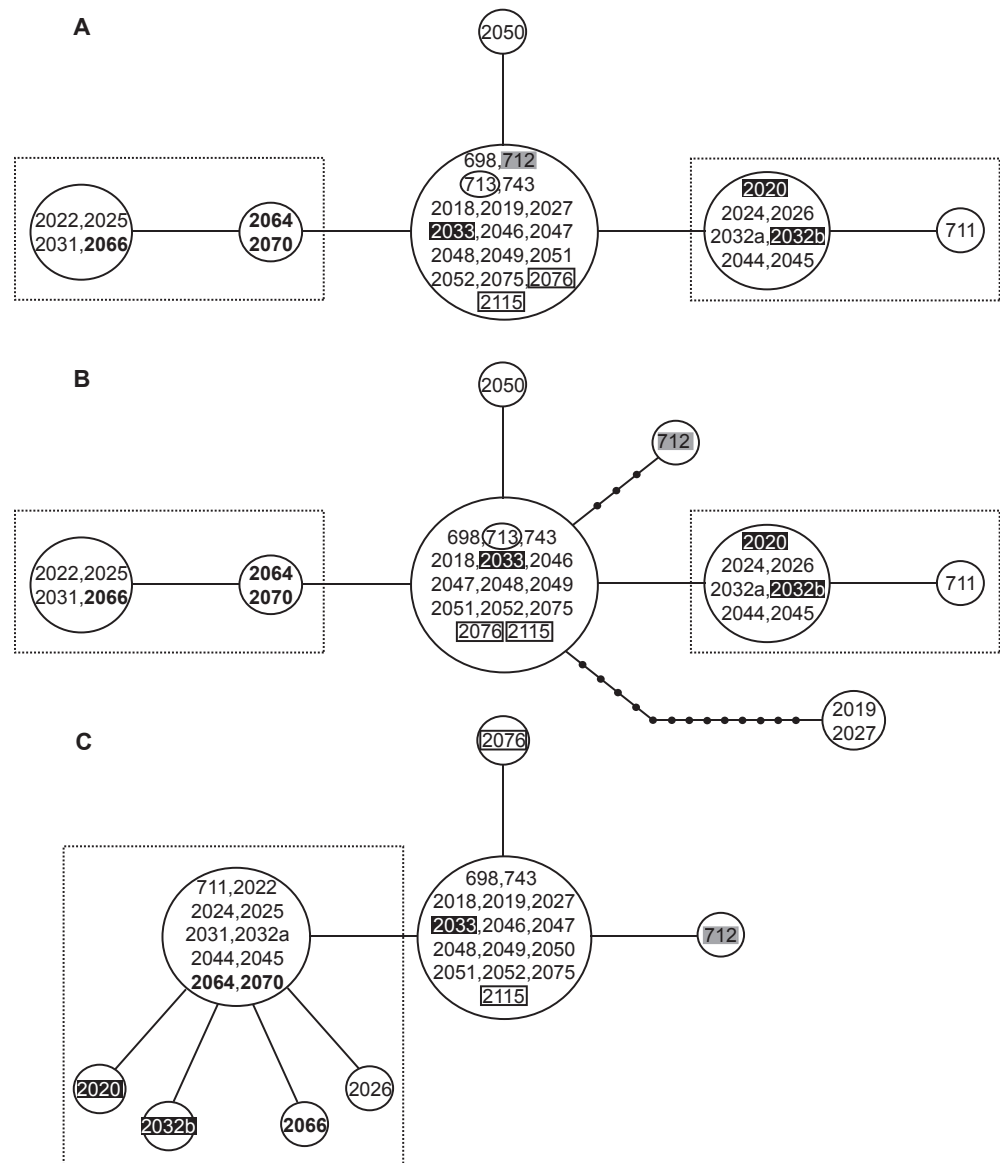
on the Azores and Madeira but rather rare on Canary Islands (González-Mancebo & al., 2009), except for La Palma, which has the highest mean annual precipitation in the Canary Islands (Afonso, 1988). On Madeira, *L. treleasei* grows as an epiphyte and on rocks or boulders, at an altitudinal range of 50–1500 m a.s.l. in several exposed and forested habitats in both the northern and southern zones of the island (González-Mancebo & al., 2009); most frequently in the natural laurel forest up to 1200 m a.s.l. *Leucodon sciuroides* is most tolerant to aridity and mainly found on Madeira and the Canary Islands, but rarely on the Azores. *Leucodon canariensis* is considered an (aero) hygrophyte-mesophyte restricted to the areas with the highest frequency of fogs (González-Mancebo & al., 2009), and is locally abundant on Canary Islands and rare on Madeira Island.

The separation of *Leucodon immersus* from *L. sciuroides* is less obvious at the molecular level, as the analyzed specimen is part of the large *L. sciuroides* clade (Fig. 1) and part

of the largest plastid haplotype based on substitutions only (Fig. 2A). *Leucodon immersus* differs from *L. sciuroides* by a single indel in the *trnT-trnE* spacer as well as by two substitutions plus one indel in the ITS, which is reflected by the separation of *L. immersus* in the haplotype networks shown in Fig. 2B–C. These differences are smaller than those observed between the *L. sciuroides* samples (nine substitutions and two indels in total). The most important morphological character to distinguish *L. immersus*, the short seta and immersed capsule, probably reflects an adaptation to epiphytic habitats. Shorter setae are generally characteristic for epiphytic pleurocarpous mosses (Hedenäs, 2001), and in *Leucodon* the transition from long to short setae can be found repeatedly and to different degrees, even within a species (e.g., 3–17 mm in *L. sapporensis*; Akiyama, 1986). Four species consistently display short setae (0.5–6.0 mm) and capsules that are immersed or laterally exserted from the perichaetial leaves. Of these, *L. immersus*

Fig. 2. Statistical parsimony haplotype networks of samples of *Leucodon sciuroides* and its related species *L. andrewsianus*, *L. dracaenae*, and *L. immersus*.

A, Haplotype network based on combined plastid *atpB-rbcL* spacer, *trnT_{ACC}-trnE_{GAA}* spacer, and *trnG_{GGA}* intron sequences; **B**, respective plastid haplotype network with indels coded as fifth character state; **C**, haplotype network based on nuclear ribosomal ITS sequences. Specimen numbers correspond to those in Fig. 1. Taxa or geographic origins are indicated as follows: *L. andrewsianus* (in boxes), *L. dracaenae* (in ellipse), *L. immersus* (grey background), *L. sciuroides* var. *morensis* (white on black background), *L. sciuroides* s.str. from Macaronesia (bold), *L. sciuroides* s.str. from Continental Europe (numbers without special formatting). Mediterranean/Macaronesian haplotypes are surrounded by boxes with dotted lines (see text for further information). Hypothetical missing haplotypes are indicated by black dots.



is gametophytically most similar to *L. sciuroides* and to the East Asian *L. secundus* (Harv.) Mitt., e.g., by the presence of a central strand in the stem (Akiyama, 1986; Ignatov & Czerdantseva, 1995). According to the present molecular data, *L. immersus* might thus represent a separate entity that evolved from within *L. sciuroides* and is distinguished by the reduction of seta length and by (slight) molecular differentiation. Until the significance of seta length as a taxonomic character is not evaluated further and more specimens of *L. immersus* and *L. secundus* are sequenced, we therefore refrain from synonymizing *L. immersus* with *L. sciuroides*.

The presence of *Leucodon sciuroides* in North America has long been discussed by different authors since Lesquereux & James (1884) listed it for the first time for this continent (cf. Reese & Anderson, 1997, for an historical overview). Crum & Anderson (1971) proposed a new name, *L. brachypus* var. *andrewsianus*, for the North American plants, arguing that they “can scarcely be distinguished from *L. brachypus* Brid. except for the constant production of small, slender, flexuose branchlets clustered in leaf axils, sometimes sparsely, sometimes profusely.” Later Reese & Anderson (1997) recognized this taxon at the rank of species as *L. andrewsianus*, based on new information discovered during a review of the genus for the *Flora of North America*. A comparison of the European specimens of *L. sciuroides* and American ones named *L. andrewsianus*, however, showed that there are no morphological features to distinguish the two taxa. The most important character for identifying *L. andrewsianus*, the presence of caducous branchlets, can also be observed in European material named *L. sciuroides* (Smith, 2004; Frey & al., 2006). Furthermore, the apices of young leaves terminating in a slender, elongate, mostly 34–60 µm long hyaline cell, as described for *L. andrewsianus* (Crum & Anderson, 1981), are also observed in *L. sciuroides*. The present molecular data support the idea that American and European specimens belong to the same species (Figs. 1–2), which is thus not restricted to the Palearctic but of Holarctic distribution. Intercontinental distribution patterns across the North Atlantic have recently been investigated for a number of moss taxa (see discussion in Huttunen & al., 2008). No or little intercontinental differentiation was detected in several species that commonly produce numerous, small spores, suggesting frequent long-distance dispersal (e.g., McDaniel & Shaw, 2005). In contrast, barriers to gene flow seem to exist in intercontinentally disjunct taxa, having led to clear phylogeographic signals (e.g., Huttunen & al., 2008). However, these disjunct distribution patterns may also be better explained by occasional (recent) long-distance dispersal than by ancient vicariance (Shaw & al., 2003; Huttunen & al., 2008; Vanderpoorten & al., 2008). The close molecular connection between eastern North America and the Old World indicated by the present data for *Leucodon sciuroides*/*L. andrewsianus* has also been shown for *Antitrichia curtispindula* (Hedenäs, 2008) and suggests ongoing gene flow, despite the fact that sporophytes of *L. andrewsianus* are unknown. One possible explanation would be that the morphologically very similar *L. andrewsianus* and *L. brachypus* are conspecific (cf. Crum & Anderson, 1971) and actually both belong to *L. sciuroides*. Spores that reach Europe from North America could then originate from plants formerly

named *L. brachypus*, from which sporophytes are known. Concerning the distinction between *L. brachypus* s.str. and *L. sciuroides*, the following morphological differences have been described in the literature and were also observed by us in a morphological study of selected specimens: seta 2–4 mm long in *L. brachypus* vs. 3–10 mm in *L. sciuroides*, capsule immersed to shortly exerted in *L. brachypus* but clearly exerted in *L. sciuroides*, spore size 44–52 µm in *L. brachypus* vs. 30–55 µm in *L. sciuroides*, and hyaline cell of young leaves 21–29 µm long in *L. brachypus* vs. 34–60 µm in *L. sciuroides*. Ignatov & Czerdantseva (1995) concluded that the difference in seta length, resulting in emergent versus exerted capsules, is the only reliable character to distinguish both species. Unfortunately we were not able to sequence material of *L. brachypus* in order to infer the significance of the reported morphological differences and consequently, the taxonomic status of *L. brachypus* s.str. and its relationship with *L. sciuroides*.

The sequenced specimen of *Leucodon dracaenae* differs from *L. sciuroides* by one substitution only and consequently is not separated in the phylogenetic reconstructions (Fig. 1) and plastid DNA haplotype networks (Fig. 2A–B). The morphological characters of *L. dracaenae*, as described by Kürschner (2000), are very similar to those of *L. sciuroides*: “Plants very robust, corticolous; primary stems stoloniform, secondary stems numerous, robust, ascending, sometimes pendulous, to 7 cm long; leaves densely imbricate when dry, plicate; lamina cells smooth.” The morphological and molecular data suggest that *L. dracaenae* actually belongs to *L. sciuroides*. Nevertheless, the synonymization of the species should be made based on (molecular) study of typical African material of *L. dracaenae*.

Intraspecific morphological variation in *L. sciuroides* has led to the distinction of var. *morensis*, which comprises the more robust plants with cylindrical instead of oval capsules (Fuertes & al., 1997; Smith, 2004; Frey & al., 2006). Such plants are most common in the Mediterranean where they almost replace *L. sciuroides* s.str. in certain areas, for example in mainland Portugal (Sérgio & Carvalho, 2003). The morphological differences between var. *morensis* and *L. sciuroides* s.str., however, are rather slight and not significant in the light of the present molecular results. The three sequenced specimens of var. *morensis* are molecularly almost indistinguishable from the remaining samples of the *L. sciuroides*/*L. andrewsianus*/*L. dracaenae* clade, although two of them represent separate ITS haplotypes (Fig. 2C). We therefore conclude that var. *morensis* does not deserve taxonomical distinction.

The single other study on genetic diversity within *Leucodon sciuroides* in Europe was based on isozyme data (Cronberg, 2000) and compared populations from Scandinavia and the eastern Mediterranean (Greece), which formed separate clusters in a phenetic analysis. In addition, the observed genetic depletion of the Scandinavian populations compared to most Mediterranean ones was considered to be in accordance with the expected lower genetic diversity in formerly glaciated areas in general. The present DNA sequence data support the basic separation of Mediterranean (plus Macaronesian) versus non-Mediterranean haplotypes (Fig. 2). This separation is especially obvious for the two specimens from Italy: the one (no. 2047) that belongs to the

main non-Mediterranean plastid and ITS haplotypes originates from the northern, Alpine part of Italy, whereas the other (no. 2022) originates from Mediterranean Central Italy. The only exceptions are two samples of var. *morensis* (no. 2020 from U.K. and 2033 from Spain), which may have reached the Mediterranean or Western Europe, respectively, by (recent) dispersal. Further genetic diversification is observed mainly in Macaronesia and the Mediterranean. The two plastid haplotypes on the left in Fig. 2A include Macaronesian and western/central Mediterranean haplotypes, whereas the larger haplotype on the right seems to have a circum-Mediterranean (and Macaronesian) distribution. The ITS analysis supports the presence of one main circum-Mediterranean (and Macaronesian) haplotype, from which several haplotypes with putatively smaller distribution might have been derived. Despite these differences, the higher haplotype diversity in the Mediterranean in contrast to the other parts of Europe supports Cronberg's (2000) conclusion of genetic depletion in formerly glaciated areas. However, considering the differences in haplotype diversity in pleurocarpous mosses, as far as can be inferred from the yet relatively few published studies, this does not seem to be a general pattern. For example, haplotype diversity in Central to Northern Europe can be considerably higher in species with a more northern (temperate to arctic) distribution, such as *Scorpidium cossonii* and *S. scorpioides* (cf. Hedenäs, 2009).

Molecular inferences revealed rather complex biogeographic relationships of bryophyte species from the Macaronesian islands, with connections to different regions of both the New and the Old World, such as the Neotropics, Paleotropics, or Europe (e.g., Sim-Sim & al., 2005; Stech & al., 2006, 2007, 2010; Vanderpoorten & Long, 2006; Feldberg & al., 2007; Aigoïn & al., 2009). Especially for liverworts a pronounced Neotropical affinity has been revealed (e.g., Schumann & al., 2005; Vanderpoorten & Long, 2006; Feldberg & al., 2007; Stech & al., 2010). The well-supported sister group relationship of *Leucodon treleasei* with the American species *L. curvirostris* and *L. julaceus* in the present study strongly supports a geographic affinity of *L. treleasei* with the New World, probably North America, as well. It is therefore likely that *L. treleasei* does not represent a palaeoendemic species from the Tertiary that survived in Macaronesia, but should be considered a neoendemic that possibly originated from a New World ancestor. The neoendemic status of *L. treleasei* is in accordance with that of other Macaronesian endemic bryophyte species, such as *Rhynchostegiella* spp. (Aigoïn & al., 2009) and the recently reinstated *Homalothecium mandonii* (Mitt.) Geh. (Huttunen & al., 2008).

For *Leucodon canariensis* the molecular data are less indicative, as its sister-group relationship with the *L. curvirostris/L. julaceus/L. treleasei* clade is less well-supported (Fig. 1). *Leucodon canariensis* may thus have the same origin and relationships as *L. treleasei*. Its closest relatives, however, remain to be detected by further taxon sampling covering the whole distribution area of *Leucodon*. The deviating ecology of *L. canariensis*, viz., its relative rarity and restriction to laurel forest habitats (see discussion above), may be supportive of a relict origin of the species (cf. Vargass, 2007).

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Appendix. List of taxa studied.

Taxon; DNA no.; geographic origin; herbarium voucher; GenBank accession numbers (*atpB-rbcL*, *trnT-trnE*, *trnG*, ITS).

Antitrichia californica Sull.; 2014; Spain; 18383 (MUB); HQ268441, HQ268312, HQ268377, HQ268248. *Antitrichia curtispindula* (Timm ex Hedw.) Brid.; 703; Spain (Canary Is., La Gomera); 15138 (TFC); HQ268440, HQ268311, HQ268376, HQ268247. *A. curtispindula*; 2017; Spain; 18581 (MUB); HQ268439, HQ268310, HQ268375, HQ268246. *Drepanocladus aduncus* (Hedw.) Warnst.; –; New Zealand; *Frahm* 13.8.1998 (L); HQ268438, HQ268308, HQ268373, AF152388/AF152391. *Echinodium spinosum* (Mitt.) Jur.; –; Portugal (Madeira); 198434 (LISU); HQ268442, HQ268314, HQ268379, EU477604. *Forsstroemia producta* (Hornsch.) Paris; 2015; Kenya; 18189 (MUB); HQ268445, HQ268317, HQ268382, HQ268251. *Forsstroemia trichomitria* (Hedw.) Lindb.; 2073; U.S.A.; 0031853 (DUKE); HQ268446, HQ268318, HQ268383, HQ268252. *Hookeria lucens* (Hedw.) Sm.; –; Germany; *Stech* B880404.8 (L); EU186635, HQ268305, HQ268370, AF230980/AF230995. *Hypnobarlettia fontana* Ochrya; –; New Zealand; *Frahm* 13.8.1998 (L); HQ268437, HQ268307, HQ268372, AF152386/AF152389. *Hypopterygium tamarisci* (Sw.) Brid. ex Müll.Hal.; –; Germany (Botanical Garden Berlin); *Pfeiffer & Sipman* 99-69 (L); AY864294, HQ268306, HQ268371, DQ007172. *Leucodon andrewsianus* (H.A. Crum & L.E. Anderson) W.D. Reese & L.E. Anderson; 2115; Canada; 0002028 (DUKE); HQ268448, HQ268320, HQ268385, HQ268254. *L. andrewsianus*; 2076; U.S.A.; 0029011 (DUKE); HQ268449, HQ268321, HQ268386, HQ268255. *Leucodon canariensis* (Brid.) Schwägr.; 702; Spain (Canary Is., Fuerteventura); 15145 (TFC); HQ268490, HQ268361, HQ268427, HQ268296. *L. canariensis*; 2030; Spain (Canary Is., La Gomera); 11875 (MUB); HQ268491, HQ268362, HQ268428, HQ268297. *L. canariensis*; 2036; Portugal (Madeira); B4569 (S); HQ268492, HQ268363, HQ268429, HQ268298. *L. canariensis*; 2041; Portugal (Madeira); B9645 (S); HQ268493, HQ268364, HQ268430, HQ268299. *L. canariensis*; 2054; Spain (Canary Is., Tenerife); 19000 (TFC), 27430 (MUB); HQ268494, HQ268365, HQ268431, HQ268300. *L. canariensis*; 2055; Spain (Canary Is., La Gomera); 19001 (TFC), 27426 (MUB); HQ268495, HQ268366, HQ268432, HQ268301. *L. canariensis*; 2056; Spain (Canary Is., La Gomera); 19002 (TFC), 27427 (MUB); HQ268496, HQ268367, HQ268433, HQ268302. *L. canariensis*; 2057; Spain (Canary Is., Tenerife); 19003 (TFC), 27429 (MUB); HQ268497, HQ268368, HQ268434, HQ268303. *L. canariensis*; 2058; Spain (Canary Is., La Palma); 19004 (TFC), 27428 (MUB); HQ268498, HQ268369, HQ268435, HQ268304. *Leucodon curvirostris* Hampe 2079; Mexico; 0031855 (DUKE); HQ268489, –; HQ268426, HQ268295. *Leucodon dracaenae* Solms ex Venturi; 713; Saudi Arabia; 2995 (herb. Kürschner); HQ268450, HQ268322, HQ268387, HQ268256. *Leucodon immersus* Lindb.; 712; Turkey; 10205 (herb. Kürschner); HQ268451, HQ268323, HQ268388, HQ268257. *Leucodon julaceus* (Hedw.) Sull.; 2078; U.S.A.; 007045 (DUKE); HQ268488, HQ268360, HQ268425, HQ268294. *Leucodon saponensis* Besch.; –; Japan; C6-043329 (HYO); HQ268447, HQ268319, HQ268384, HQ268253. *Leucodon sciuroides* (Hedw.) Schwägr.; 698; Germany; 10131 (herb. Kürschner); HQ268452, HQ268324, HQ268389, HQ268258. *L. sciuroides*; 711; Turkey; 10314 (herb. Kürschner); HQ268466, HQ268338, HQ268403, HQ268272. *L. sciuroides*; 743; Germany; *Stech* B051230.2 (L); HQ268453, HQ268325, HQ268390, HQ268259. *L. sciuroides*; 2018; United Kingdom; 00206522 (E); HQ268454, HQ268326, HQ268391, HQ268260. *L. sciuroides*; 2019; Ireland; 00206516 (E); HQ268455, HQ268327, HQ268392, HQ268861. *L. sciuroides*; 2022; Italy; 00206493 (E); HQ268468, HQ268340, HQ268405, HQ268274. *L. sciuroides*; 2024; Spain; 18002 (MUB); HQ268472, HQ268344, HQ268409, HQ268278. *L. sciuroides*; 2025; Spain; 17677 (MUB); HQ268469, HQ268341, HQ268406, HQ268275. *L. sciuroides*; 2026; Greece; I2031 (MUB); HQ268473, HQ268345, HQ268410, HQ268279. *L. sciuroides*; 2027; France; 13598 (MUB); HQ268456, HQ268328, HQ268393, HQ268262. *L. sciuroides*; 2031; Spain; 11250 (MUB); HQ268470, HQ268342, HQ268407, HQ268276. *L. sciuroides*; 2032a; Morocco; 10750 (MUB); HQ268474, HQ268346, HQ268411, HQ268280. *L. sciuroides*; 2044; Spain; 27447 (MUB); HQ268476, HQ268348, HQ268413, HQ268282. *L. sciuroides*; 2045; Spain; 18450 (MUB); HQ268477, HQ268349, HQ268414, HQ268283. *L. sciuroides*; 2046; Sweden; 18435 (MUB); HQ268458, HQ268330, HQ268395, HQ268264. *L. sciuroides*; 2047; Italy; 18436 (MUB); HQ268459, HQ268331, HQ268396, HQ268265. *L. sciuroides*; 2048; Sweden; 27450 (MUB); HQ268460, HQ268332, HQ268397, HQ268266. *L. sciuroides*; 2049; Hungary; 27446 (MUB); HQ268461, HQ268333, HQ268398, HQ268267. *L. sciuroides*; 2050; Switzerland; B98892 (S); HQ268462, HQ268334, HQ268399, HQ268268. *L. sciuroides*; 2051; Estonia; B98627 (S); HQ268463, HQ268335, HQ268400, HQ268269. *L. sciuroides*; 2052; Norway; B94486 (S); HQ268464, HQ268336, HQ268401, HQ268270. *L. sciuroides*; 2064; Spain (Canary Is., Tenerife); 19009 (TFC), 27442 (MUB); HQ268465, HQ268337, HQ268402, HQ268271. *L. sciuroides*; 2066; Spain (Canary Is., La Palma); 19010 (TFC), 27439 (MUB); HQ268471, HQ268343, HQ268408, HQ268277. *L. sciuroides*; 2070; Cape Verde; 19011 (TFC), 27443 (MUB); HQ268478, HQ268350, HQ268415, HQ268284. *L. sciuroides*; 2075; Russia; 0031852 (DUKE); HQ268479, HQ268351, HQ268416, HQ268285. *Leucodon sciuroides* var. *morensis* (Schwägr.) De Not.; 2020; United Kingdom; 00206513 (E); HQ268467, HQ268339, HQ268404, HQ268273. *L. sciuroides* var. *morensis*; 2032b; Spain; 27188 (VIT); HQ268475, HQ268347, HQ268412, HQ268281. *L. sciuroides* var. *morensis*; 2033; Spain; 33465 (VIT); HQ268457, HQ268329, HQ268394, HQ268263. *Leucodon treleasei* (Cardot) Paris; 2039; Portugal (Madeira); B4521 (S); HQ268480, HQ268352, HQ268417, HQ268286. *L. treleasei*; 2040; Portugal (Madeira); B22458 (S); HQ268481, HQ268353, HQ268418, HQ268287. *L. treleasei*; 2059; Portugal (Madeira); 19005 (TFC), 27433 (MUB); HQ268482, HQ268354, HQ268419, HQ268288. *L. treleasei*; 2061; Spain (Canary Is., La Palma); 19006 (TFC), 27435 (MUB); HQ268483, HQ268355, HQ268420, HQ268289. *L. treleasei*; 2063; Spain (Canary Is., La Palma); 19007 (TFC), 27437 (MUB); HQ268484, HQ268356, HQ268421, HQ268290. *L. treleasei*; 2067; Portugal (Azores, Terceira); 27431 (MUB); HQ268486, HQ268358, HQ268423, HQ268292. *L. treleasei*; 2068; Portugal (Azores, Terceira); 27432 (MUB); HQ268487, HQ268359, HQ268424, HQ268293. *L. treleasei*; 2069; Spain (Canary Is., La Palma); 19008 (TFC), 27440 (MUB); HQ268485, HQ268357, HQ268422, HQ268291. *Platyhypnidium riparioides* (Hedw.) Dixon; –; Portugal (Madeira); *Stech* 04-237 (L); EU186644, HQ268309, HQ268374, HQ268245. *Pterogonium gracile* (Hedw.) Sm.; 2016; Spain; 15943 (MUB); HQ268443, HQ268315, HQ268380, HQ268249. *P. gracile*; 2074; South Africa; 0016797 (DUKE); HQ268444, HQ268316, HQ268381, HQ268250. *Thamnobryum pandum* (Hook. f. & Wilson) I.G. Stone & G.A.M. Scott; –; New Zealand; *Frey & Frey* 94-117 (herb. Frey); EU186642, HQ268313, HQ268378, AY010311.