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Single origin and subsequent diversification of central Andean endemic *Umbilicaria* species

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Abstract: We studied an Andean endemic group of species of the lichen-forming fungal genus *Umbilicaria* from the subalpine and low-alpine zone, with their biogeographic center in Bolivia and Peru. A number of species and varieties have been described from this element, but apparent instability in several morphological traits has made it difficult to precisely delimit taxa. Based on DNA sequences of nuclear ITS, LSU and mitochondrial SSU from extensive collections from Argentina, Bolivia, Chile, Colombia, Ecuador and Peru, we present here a molecular phylogenetic analysis of this Andean endemic element within genus *Umbilicaria*. All analyses (MP, ML and Bayesian) support a single origin for the element and a division into two major groups characterized by different apothecium types: the *Umbilicaria dichroa* group and *U. calvescens* group. Taxa *U. krempelhuberi*, *U. peruviana* and *U. subcalvescens* are nested within *U. calvescens* and are treated as conspecific with the latter species. The endemic element shares a most recent common ancestor with the *Umbilicaria vellea* group, which has a worldwide distribution and contains several asexually reproducing (sorediate) species. Independent reversals to sexual reproduction might explain the evolution of two types of apothecia in this monophyletic endemic lineage. A number of cosmopolitan, mostly high-alpine, species of *Umbilicaria* also present in the central Andes are related only remotely to the endemic element and do not exhibit speciation into endemics. Because the Andean element dominates the *Umbilicaria* habitats of the low- and subalpine zones we propose that the founder colonized the Andes at a time when the mountains had not yet reached their current elevation while the high-alpine species arrived more recently.

Key words: apothecium types, endemism, evolutionary radiation, lichen-forming Lecanoromycetes, Neotropics, reproductive strategies

INTRODUCTION

The lichen-forming fungal genus *Umbilicaria* (Umbilicariales, Lecanoromycetes) has a worldwide distribution and constitutes a major element of the saxicolous lichen flora in boreal and alpine regions (Frey 1933, 1936a; Llano 1950; Krog and Swinscow 1986; Wei and Jiang 1993). In an early review of the biogeography of the Umbilicariaceae the Swiss expert Eduard Frey (1936a) particularly lamented the scarcity of collections from the high Andes (subalpine to alpine) and suggested that this region would provide the key to a further understanding of the systematics of the family. This situation did not substantially improve with Llano's (1950) monograph of the Umbilicariaceae in the western hemisphere. Llano himself had not collected in South America and had to rely on the scant material found in herbaria. Umbilicariaceae in several Andean nations recently have received separate treatments or have been listed in national surveys or checklists (Sipman and Topham 1992; Marcano and Morales Mendez 1993; Hestmark, 1997, 2009; Galloway and Quilhot 1998; Calvelo and Liberatore 2002; Feuerer and Thell 2008). These studies reveal that most *Umbilicaria* species found in the alpine zone of the Andes are well known from other high elevation or arctic areas and that the diversity of the Andean *Umbilicaria* flora is fairly low compared to that found in the northern hemisphere. However one major taxonomic challenge has remained: the central Andean endemic element.

Since the mid-19th century, when the earliest collections of *Umbilicaria* lichens from South America were described, it has been known that low alpine and subalpine zones of the Andes are the habitats of an apparently endemic group of *Umbilicaria* species, with their biogeographic center in Bolivia and Peru (Nylander 1855, 1859, 1861, 1869; Hestmark 2010). More recent investigations reported that this element extends southward into parts of Argentina and Chile

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TABLE I. Species and intraspecific taxa of *Umbilicaria* of the endemic Andean element

Taxa	References	Diagnostic character states
Species with leiodisc apothecia		
Umbilicaria dichroa	Nyl. 1855	lower surface black and verrucose
Umbilicaria haplocarpa	Nyl. 1859	lower surface beige to black with abundant rhizinomorphs
var. *convexa*	Räsanen 1944	apothecia convex
var. *subhirsuta*	Frey 1949	isidiate and sorediate
f. *tenuis*	Frey 1949	intermediate between the type and var. *subhirsuta*
var. *friesii*	Llano 1950	upper surface olive
var. *kühnemannii*	Llano 1950	thallus indentions under apothecia
Species with gyrodisc apothecia		
Umbilicaria calvescens	Nyl. 1861	no or few rhizinomorphs
var. *subvellea*	Nyl. 1869	lower surface pale with abundant rhizinomorphs
var. *hypomelaena*	Nyl. 1869	lower surface black with abundant rhizinomorphs
Umbilicaria krempelhuberi	Müll.Arg. 1883	no or few rhizinomorphs
Umbilicaria leprosa	Zahlbr. 1906	with soredia
Umbilicaria peruviana	Llano 1950	lower side black, no or few rhizinomorphs
Umbilicaria subcalvescens	Sipman and Topham 1992	lower surface black with rhizinomorphs and thalloconidia

and northward into southern Ecuador (Frey 1936a, 1949; Llano 1950; Hestmark 1997, 2009). From extensive field observations and collections made by the first author in Argentina, Bolivia, Chile, Ecuador and Peru in the past 15 y it can be ascertained that the central Andean endemic *Umbilicaria* element dominates the lowermost parts (low alpine to subalpine) of the altitudinal range of the genus (ca. 2500–4400 m) in Bolivia, Peru and northern Chile and Argentina. The variation in several morphological characters of the endemic Andean *Umbilicaria* taxa however has led to much confusion, and a number of species and varieties have been named, often based on scant or badly preserved material (Frey 1949, Llano 1950) (TABLE I). Another problem was that several researchers, before establishing new species or intraspecific taxa, did not examine the herbarium material on which William Nylander based his original descriptions of many of the endemic taxa (Hestmark 2010). Further contributing to the taxonomical confusion is the fact that characters that have proved fruitful for the delimitation of species in many other lichen genera, such as ascospore size, color and septation as well as secondary compound chemistry, are identical over the entire central Andean element; ascospores are all unicellular, hyaline, and in size, 6–12 × 10–18 μm (Nylander 1869, Llano 1950, Frey 1949); the secondary compound chemistry is uniformly restricted to gyrophoric and lecanoric acid (Posner et al. 1992, Narui et al. 1996). In addition most members of the endemic element may look broadly the same in the field, that is monophyllous, gray-white thalli usually less than 6 cm diam, and often with abundant organs of sexual reproduction—black apothecia producing hyaline ascospores—on the upper surface. Furthermore they commonly co-occur in the same locality, sometimes in mixtures on the same rock surface. This morphological similarity could be an indication of recent common ancestry. Yet on closer inspection two groups might be distinguished tentatively within the endemic element with reference to the morphology of their apothecia: one group with plane (leiodisc) apothecia and one group with gyrose (gyrodisc) apothecia (TABLE I). Some taxonomists (e.g. Scholander 1934, Llano 1950) considered apothecium type a diagnostic character sufficient to divide genus *Umbilicaria* into several genera, but this taxonomic treatment was never widely accepted. Even if the Andean *Umbilicaria* element is divided into two groups based on apothecium morphology, the problem remains that in both groups several species, varieties and forms have been described. Furthermore in both groups forms, varieties or even species have been described to accommodate the identification of individual thalli that reproduce by symbiotic asexual propagules known as soredia (containing both the fungal and algal partners), instead of the more usual sexual reproduction of the fungal partner, via apothecia, followed by a re-association with a compatible alga.

The apparent instability of several thallus characters has led to the suggestion that these endemic Andean *Umbilicaria* species ''are still in the process of development'' (Llano 1950). Hestmark (2009) suggested that the dominance of the element in the subalpine and low-alpine zones could indicate that members are derived from a colonizer taxon arriving in the Andes when the mountain chain was lower than today and preceded the colonization of high alpine *Umbilicaria* species. The early colonizer then had time for evolutionary differentiation and a radiation into several niches before further rise of the mountains took place and the arrival of the high elevation *Umbilicaria* species, common in arctic and alpine areas of the world, which today dominate the high Andes. The high central Andes have been ''high elevation'' only since the last major uplift that took place in the late Pliocene or early Pleistocene, 2–4 MYA (Gregory-Wodzicki 2000). This might explain why the high alpine *Umbilicaria* flora of the central Andean region (4400–5600 m) consists of species well known from other high alpine regions; they are a recent addition and have had less time for local differentiation (e.g. *U. africana*, *U. aprina*, *U. cinereorufescens*, *U. decussata*, *U. nylanderiana* and *U. vellea*) (Hestmark 1997, 2009). A number of molecular phylogenetic studies have indicated that members of Umbilicariaceae were among the oldest groups to evolve within Lecanoromycetideae or Lecanoromycetes in general (Reeb et al. 2004, Lutzoni et al. 2004, Miadlikowska et al. 2006). The distinct phenotypic traits and their early divergence led to their recognition as a separate suborder (Umbilicarinae), order (Umbilicariales) and might require classification as a subclass (Umbilicariomycetidae) within Fungi (Poelt 1974, Miadlikowska et al. 2006, Spatafora et al. 2006, Hibbett et al. 2007). Thus the entire *Umbilicaria* flora in a young high-elevation habitat such as the central Andes is a comparatively recent influx, most probably from comparable arctic and alpine areas of the northern hemisphere, where the greatest species diversity is found, including the other genus in Umbilicariaceae, *Lasallia*, which has not been reported from South America. This absence of *Lasallia* suggests it encountered major obstacles, such as absence of suitable stepping stone habitats in the dense lowland jungle of the Panamanian land bridge, to the movement of low elevation taxa between North and South America. *Umbilicaria* species are found almost exclusively on sun-exposed igneous or metamorphic rocks.

Traditional studies, based on phenotypic variation, have failed to resolve taxonomic ambiguities within the central Andean element. PCR-based methods have been used to evaluate the status of taxa within lichen species complexes (Grube and Kroken 2000, Miadlikowska et al. 2003), including genus *Umbilicaria* (Ivanova et al. 1999, Ott et al. 2004). In the present study we provide a molecular analysis of the central Andean element of *Umbilicaria*. We address these questions: To what degree are the species or specimens with the same apothecium type phylogenetically related, and are the Andean endemics of the genus *Umbilicaria* derived from a single origin? To what degree are the endemics related to the other *Umbilicaria* species found in the central Andean area? We also examined the phylogenetic placement of these endemic species within the broader context of family Umbilicariaceae to shed light on their evolutionary history with regard to mode of reproduction and the colonization of subalpine and low-alpine Andes.

MATERIALS AND METHODS

Taxon sampling.—We compiled extensive collections of fresh lichen material 1994–2009 from Argentina, Bolivia, Chile, Colombia, Ecuador and Peru. From this material a total of 49 specimens of *Umbilicaria* were included in our molecular study (SUPPLEMENTARY TABLE I). Of these 35 belonged to the presumed endemic element (low alpine to subalpine) while 14 were from the other *Umbilicaria* species present in the high alpine zone of the central Andes (*U. aprina*, *U. africana*, *U. cinereorufescens*, *U. decussata*, *U. nylanderiana* and *U. vellea*). Specimens in the central Andean endemic element were selected to represent the range of morphological variation in thallus traits and reproductive traits (TABLE I). In addition a number of northern hemisphere *Umbilicaria* taxa (*U. crustulosa*, *U. grisea*, *U. hirsuta* and *U. spodochroa*) with morphological similarities to the endemic Andean taxa were selected to trace the possible origin of the endemic Andean element. Together with *U. vellea* and *U. cinereorufescens*, which are present in the central Andes, these northern hemisphere taxa form a distinct morphological group that we refer to as the *U. vellea* group. For comparative purposes we also used all nuclear ITS, LSU and mitochondrial SSU sequences from members of Umbilicariaceae (genera *Umbilicaria* and *Lasallia*) in the AFTOL (Assembling the Fungal Tree of Life, WASABI) database (http://www.aftol.org, cf. Lutzoni et al. 2004, Miadlikowska et al. 2006, Kauff et al. 2007). In addition we selected three taxa, *Boreoplaca ultrafrigida*, *Hypocenomyce scalaris* and *Ophiopharma ventosa*, as outgroup to the family, based on the phylogenetic relationships established by Wedin et al. (2005) and Miadlikowska et al. (2006).

DNA isolation, sequencing and sequence alignment.—Genomic DNA was extracted from recently collected specimens with a protocol modified from Zolan and Pukkila (1986) with 2% sodium dodecyl sulphate (SDS) as extraction buffer. Isolated DNA was resuspended in sterile water and stored at −20 C. When pigments or polysaccharides were thought to be inhibiting PCR, the DNA isolates were

cleaned with the E.Z.N.A.® Fungal DNA Miniprep Kit (Omega Biotech). PCR amplification followed a modified Vilgalys and Hester (1990) procedure with 1.5–3.0 mM of $MgCl_2$, 0.4 mg μL^{-1} bovine serum albumin (Hillis et al. 1996), Red Hot® DNA Polymerase and chemistries from ABgene® (ABgene Inc., Rochester, New York). Cloning, when required, was performed with a TOPO TA Cloning® Kit (Invitrogen™, Life Technologies, Carlsbad, California). Amplified PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN, Valencia, California) or Exo-SAP (exonuclease I and shrimp alkaline phosphatase, USB Corp., Cleveland, Ohio) before automated sequencing with Big Dye chemistry with 3700 or 3730xl DNA analyzers (PE Applied Biosystems, Foster City, California).

We amplified and sequenced these three loci: the nuclear ITS with primers ITS1F or NS24R and ITS4 (White et al. 1990, Gardes and Bruns 1993, Miadlikowska et al. 2003), ≈ 1.4 kb nuclear LSU with primers LR0R–LR7 (or LR5) (Vilgalys and Hester 1990) and ≈ 0.8 kb mitochondrial SSU with primers mitSSU1–mitSSU3R (Zoller et al. 1999). PCR and sequencing conditions followed Hofstetter et al. (2002). Sequences were assembled and edited with the software package Sequencher™ 4.1 (Gene Codes Corp., Ann Arbor, Michigan). GenBank accession and identification numbers are provided and a total of 178 new sequences were generated (SUPPLEMENTARY TABLE I). The sequences were aligned manually with MacClade 4.07 (Maddison and Maddison 2005). The alignments were carefully inspected for the presence of ambiguously aligned regions caused by the insertion of gaps. The unequivocal coding of these ambiguous regions and the elaboration of symmetric step matrices for each of these coded characters were generated with the programs INAASE 2.3b (Lutzoni et al. 2000) or ARC (Miadlikowska et al. 2003). The nexus file was deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S10614) and is available for download at http://www.lutzonilab.net/publications.

Phylogenetic analyses.—Each single-locus alignment was analyzed separately to detect significant conflicts among these datasets with ML bootstrap (1000 replicates and GTRCAT model) as implemented in RAxML-VI-HPC (Stamatakis et al. 2005). Models of evolution for Bayesian phylogenetic analyses were estimated with the Akaike information criterion (AIC) as implemented in MrModeltest 2.3 (Nylander 2004). A conflict among single-locus datasets was considered significant if a well supported monophyletic group (i.e. bootstrap value ≥ 75%) was found to be well supported as nonmonophyletic with a different locus. Sequences causing these conflicts were removed from the final phylogenetic analyses. When no further interlocus conflict was detected single-locus datasets were concatenated into a single dataset of 73 operational taxonomic units (OTUs). This concatenated dataset included 4254 sites of which 1950 were excluded from all analyses because their alignments were ambiguous. For MP analysis 1921 characters also were excluded because they were constant.

The combined dataset was analyzed with maximum parsimony (MP) as the optimization criterion with PAUP* 4.0b10 (Swofford 2003) and with ML with RAxML-VI-HPC. The Bayesian analysis was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2005). For the weighted MP analysis unambiguously aligned sites of the ITS-1, 5.8S, ITS-2, nucLSU and mitSSU were subjected to five step matrices (one for each data partition), which were generated with the program STMatrix 2.2 (written by S. Zoller and available at http://www.lutzonilab.net/downloads). Ambiguously aligned regions were excluded or replaced by coded characters with INAASE or ARC. MP searches were conducted with the five step matrices generate by STMatrix as well as the INAASE and ARC characters simultaneously. For ITS six of the excluded ambiguously aligned regions were coded with INAASE and six regions, which resulted in more than 32 character states when using INAASE, were coded with ARC. For nucLSU and mitSSU four and eight ambiguously aligned regions respectively were coded with INAASE. In total 158 coded characters were added to the 4254 sites of the concatenated dataset. Of this grand total of 4412 characters 637 (450 of which were parsimony informative) were subjected to MP analyses. Of these 507 had a weight of 1, 24 had a weight of 0.25, 59 had a weight of 0.10 and 47 had a weight of 0.50 (see Reeb et al. 2004 for the weighting scheme used here that is associated with ARC characters). MP search was done with 1000 random addition sequences (RAS) and TBR swapping. A total of 243 equally most parsimonious trees of 2710.59 steps were recovered for 408 of 1000 RAS. Based on this frequency, nonparametric MP bootstrap support (MP-BS) values were generated with the same settings as for the analysis on the original combined dataset, except that eight RAS were implemented, for each of the 1000 bootstrap replicates saving the 100 best trees. To assess the contribution of ARC characters to MP analysis we performed an additional MP bootstrap analysis on the same concatenated data matrix but without ARC characters. No topological conflicts were detected with and without ARC characters when the same criterion used to detect among partition conflicts was implemented as described above. Maximum likelihood analysis with RAxML was performed with 1000 replicates and GTRGAMMA model with gamma distribution, approximated with four categories.

In addition to MP-BS, phylogenetic confidence for relationships inferred from the combined dataset was estimated with Bayesian posterior probabilities (PP) and maximum likelihood bootstrap proportions (ML-BS). The combined dataset was separated into five partitions (mitSSU, nucLSU, ITS-1, 5.8S, ITS-2), and the Bayesian analyses were run with four independent chains 20 000 000 generations, sampling every 500th tree. As estimated by MrModeltest, a six-parameter model for nucleotide substitution (GTR + I + G, Rodríguez et al. 1990) with a gamma distribution approximated with four categories and a proportion of invariable sites was used for all data partitions except the 5.8S, for which a two-parameter model with a proportion of invariable sites (K80 + I, Kimura 1980) was implemented. Two independent Bayesian runs were conducted. To ensure that the runs reached stationarity and converged on the same ln-likelihood score, chains were examined by eye and with AWTY (http://ceb.csit.fsu.edu/awty). After discarding the burn-in, the last 20 000 trees of

each run were used to calculate a 50% majority rule consensus tree. Bootstrap proportions were calculated with 1000 bootstrap pseudoreplicates with RAxML implementing the GTRCAT model with gamma distribution, approximated with four categories. Bootstrap proportions (ML-BS and MP-BS) equal to or greater than 70%, and posterior probability values (PP, resulting from MrBayes analyses) equal to or greater than 95% were considered significant. Internal branches with significant support values revealed from at least two out of the three methods implemented were interpreted as well/strongly supported.

RESULTS

Monophyly of the Andean endemic element and its phylogenetic placement.—We provided the strict consensus of 243 equally most parsimonious trees (tree length = 2710.5900 steps) (FIG. 1) and the maximum likelihood tree (ln likelihood = −8201.260234) (SUPPLEMENTARY FIG. 1). All results from phylogenetic analyses of the concatenated dataset were concordant for the well supported (BS ≥ 70%, PP ≥ 95%) portions of their respective topologies. All specimens of the central Andean complex were recovered as a monophyletic group (MP-BS = 100, ML-BS = 98, PP = 100), sister of the *U. vellea* group (MP-BS = 100, ML-BS = 100, PP = 100) (FIG. 1). The other central Andean taxa of *Umbilicaria*—*U. africana*, *U. aprina*, *U. dendrophora*, *U. nylanderiana*—are seen to be more distantly related to the endemic element, with the exception of *U. cinereorufescens* and *U. vellea* that are part of the *U. vellea* group.

Two major endemic groups diverging from a single origin.—The overall topology of the trees suggests a division of the central Andean *Umbilicaria* element into two major groups, corresponding to apothecium morphology: a group with gyrose (gyrodisc) apothecia and a group with plane (leiodisc) apothecia (FIG. 1). We designated the former as the *calvescens* group and the latter as the *dichroa* group, with reference to the first member described within each clade. The *calvescens* group is well supported as monophyletic (MP-BS = 100, ML-BS = 100, PP = 100) (FIG. 1), as is the monophyly of the *dichroa* group (MP-BS = 90, ML-BS = 95, PP = 100). Within the *calvescens* group the longer branches in both the MP tree (FIG. 1) and the ML tree (SUPPLEMENTAL FIG. 1) might indicate an increase in the rate of nucleotide substitution, which could have resulted in higher genetic variation and structure compared to its sister *dichroa* group (FIG. 1).

In all analyses the majority of specimens from the central Andean endemic *Umbilicaria* with leiodisc apothecia (*U. dichroa* group) sorted into two separate, highly supported, monophyletic subgroups: H1 (MP-BS = 89, ML-BS = 87, PP = 100) and H2 (MP-BS = 99, ML-BS = 100, PP = 100) (FIG. 1). The morphology of the specimens in both HI and H2 corresponds to what has been referred to as *Umbilicaria haplocarpa*. There is no easily observable morphological distinction between these two subgroups, and H1 and H2 do not correspond to any of the varieties described previously for *U. haplocarpa*. Both subgroups comprise specimens with more or less abundant apothecia, more or less abundant rhizinomorphs and more or less pronounced reticulation around the holdfast. The only difference seems to be that H1 never exhibits a non-black lower surface. These two subgroups appear to reflect geographical origin; the first subgroup (H1) comprises specimens from Peru, Bolivia (Tunari) and Ecuador, the other (H2) comprises specimens from Chile and Bolivia. Thus the latter subgroup has a slightly more southern distribution but both subgroups comprise specimens from the western as well as eastern Andean ranges. Neither of the two subgroups can be distinguished by elevation preferences. Outside the two well supported H1 and H2 subgroups three more individuals are part of the *dichroa*-group. There is however no support for their placement within this group. Two of these individuals, without apothecia, from the same locality at Puno, Peru, are identical morphologically to the type specimen of *Umbilicaria dichroa*. The third individual is distinctly sorediate and isidiate and without apothecia. The latter specimen corresponds morphologically to the taxon *U. haplocarpa* var. *subhirsuta* (Frey 1949: 449). Because it does not exhibit a black, granular lower side as *U. dichroa* (TABLE I) it here is labeled *U. haplocarpa* var. *subhirsuta*, pending further collections and phylogenetic analyses of more variable loci.

The increase in nucleotide substitution, reported here for the *U. calvescens* group, resulted in more phylogenetic structure than observed for the *dichroa* group. The first divergence within the *calvescens* group involves three slow evolving sorediate individuals, corresponding to the taxon *Umbilicaria leprosa* (Zahlbr.) Frey (*U. leprosa* 2, 3 and 4) (MP-BS = 88, ML-BS = 88, PP = 99) (FIG. 1). One of these specimens (*U. leprosa* 2) is from the type locality of *U. leprosa*, the volcano Chimborazo, Ecuador (Zahlbruckner 1906). The clade further includes a specimen from the volcano Cayambe, Ecuador, and one specimen from western Bolivia (volcano Sajama). The first divergence detected within the lineage sister of the *U. leprosa* subgroup, although not supported, involves a sorediate specimen with some apothecia, tentatively named *U. leprosa* 1, which is sister of a nonsorediate group of 16 specimens with mostly abundant gyrose apothecia.

Within the group of specimens labeled *U. calvescens* 1–15 there is little or no support for most suggested

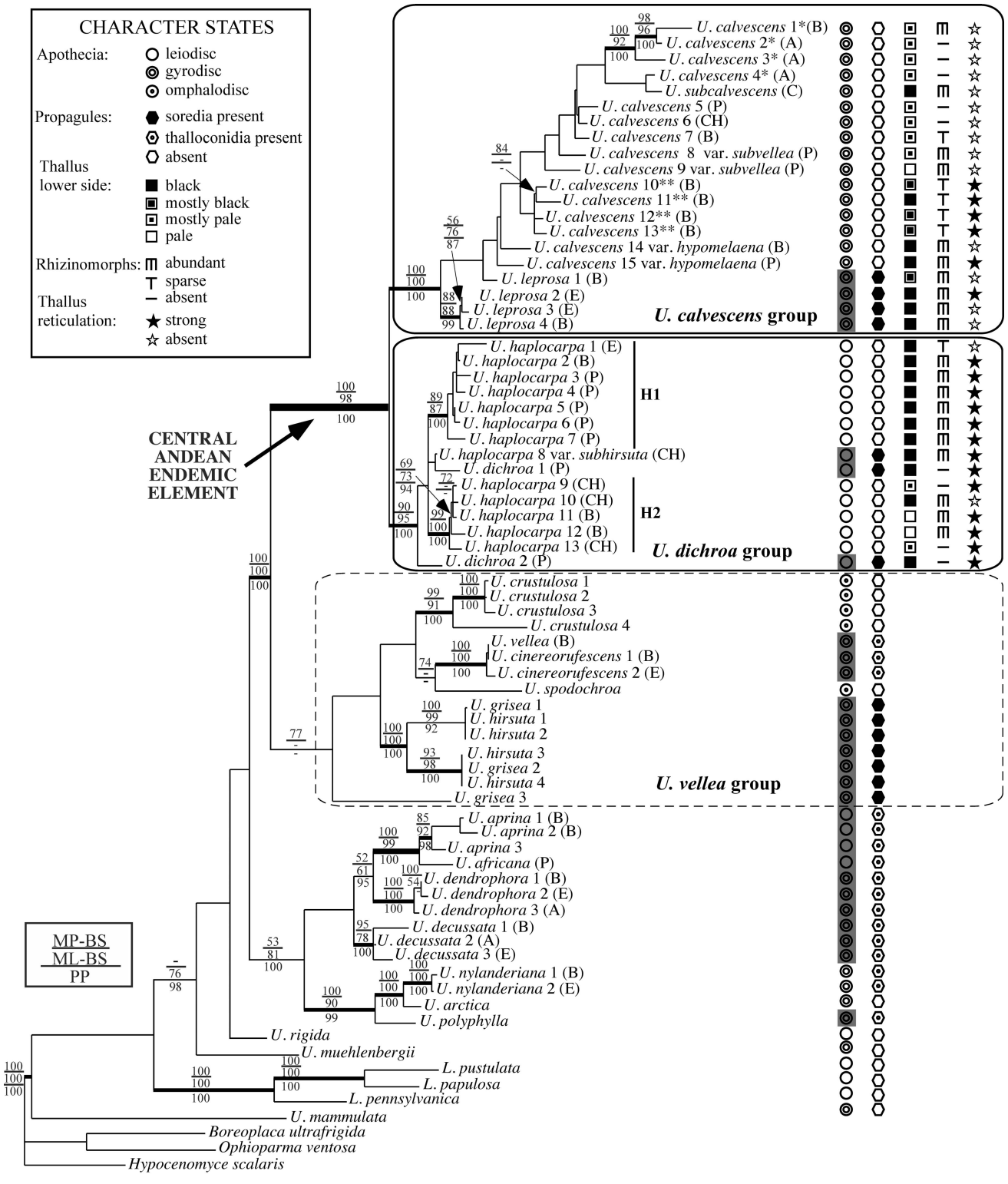

FIG. 1. Phylogenetic relationships of central Andean endemic Umbilicariaceae based on combined nuclear ITS, LSU and mitochondrial SSU. Strict consensus of 243 equally most parsimonious trees. Branch lengths were estimated with the PHYLOGRAM option of PAUP* with the same settings as for the phylogenetic search. Internal branches are shown as thick lines when both bootstrap values were ≥ 70% and posterior probability was ≥ 95%. Boxes with continuous lines circumscribe the two main monophyletic groups within the central Andean endemic Umbilicariaceae-*calvescens* and *dichroa* groups. The

relationships (FIG. 1). At the bottom of this paraphyletic group are two specimens (*U. calvescens* 14 and 15), corresponding to the taxon *U. calvescens* var. *hypomelaena*, with a black lower side, abundant rhizinomorphs and sparse gyrose apothecia. The next subgroup (*U. calvescens* 10–13), with some support (MP-BS = 84), consists of specimens from the middle alpine zone that have pronounced reticulation on the lower side, which is pitch black and with scant or no rhizinomorphs. Three of these four specimens are from the same area in Bolivia, the peak Huayna Potosi, and the fourth is from Tunari, Bolivia. Among the specimens selected these come closest in morphology to the synonymous taxon *U. peruviana*. Two individuals with light lower sides densely covered with rhizinomorphs, corresponding to the variety *U. calvescens* var. *subvellea* Nyl. (*U. calvescens* 8 and 9), are paraphyletic (not supported). With the exception of *U. subcalvescens*, all remaining specimens (*U. calvescens* 1–7) correspond to the typical form, with mostly no or only a few rhizinomorphs. All phylogenetic analyses (including single-gene analyses of mitSSU and nucLSU) revealed a nested position of *U. subcalvescens* within the *U. calvescens* group. Because it is represented in our phylogeny by only one specimen and the backbone of the *calvescens* group is mostly without statistical support no formal taxonomic changes are proposed. Two internodes within *U. calvescens* typicum (1–7) exhibit strong support but do not correspond to clear morphological distinctions. Three specimens (here labeled *U. calvescens* 2, 3, 4) come from the type locality of the synonymous taxon *U. krempelhuberi* in Argentina but are not supported as monophyletic. In the *calvescens* group there appears to be an evolutionary trend from a mainly sorediate ancestor with rhizinomorphs to a sexually reproducing lichen-forming fungus without rhizinomorphs.

DISCUSSION

Results from all phylogenetic analyses conducted in this study support a single origin of the entire central Andean endemic element of *Umbilicaria*. This common ancestry is surprising when considering the variation in apothecium morphology. Gyrose and leiodisc apothecia often have been considered two extremes along an evolutionary line, where leiodisc was considered the ancestral state and gyrose a derived state (Scholander 1934, Llano 1950). However this was challenged by Frey (1936b, 1949) who pointed to intermediate apothecial morphologies in some species and by Henssen (1970) who showed the ontogeny of the different apothecium types to be fairly similar. Our results suggest that switches between these two types of apothecia occurred repeatedly within family Umbilicariaceae and therefore might result from only few genetic changes. This hypothesis also is supported by phylogenetic relationships of the non-endemic species within Umbilicariaceae; several other clades contain species with both apothecium types (FIG. 1). Thus, while the molecular analyses confirm the utility of apothecium morphology for taxonomic decisions and determinations at the species level, the use of this trait to establish several new genera and reorder the entire family Umbilicariaceae as proposed by Scholander (1934) and Llano (1950) is not valid if we want taxonomy to reflect phylogeny. So far no adaptive function can be attributed to the various apothecium morphologies of *Umbilicaria*.

The molecular phylogenetic analysis further places the central Andean endemic *Umbilicaria* element as sister of the *U. vellea* group, which includes members with both gyrose and omphalodisc apothecia, as well as several mainly sorediate taxa. *U. vellea* and its smaller close relative, *U. cinereorufescens*, both are present in the central Andes, although not frequently due to habitat specialization (Hestmark 1997, 2009). The two species have a worldwide distribution, occurring in almost all alpine areas with acidic rocks. This suggests that the endemic element at some time branched from the ancestral population from which evolved *U. vellea* group (*U. vellea*, *U. cinereorufescens*, *U. crustulosa*, *U. grisea*, *U. hirsuta*). Future molecular studies might reveal to what degree the central Andean endemics are related to putative specimens of *U. haplocarpa* reported from a few high mountain localities in Natal, South Africa (Frey 1949, Almborn 1987, Wei and Biazrov 1991).

Another unexpected result came from the phylogenetic placement of all included sorediate Andean specimens within the endemic element. Although sorediate varieties have been described for both *U.*

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monophyletic sister *U. vellea* group is delimited with a discontinuous line. Character states reported here for members of the central Andean endemic clade and sister group apply only to individual specimens included in this tree. However for comparative purposes and completeness we have included literature data on apothecium type for the species for the specimens lacking apothecia (shown in gray shaded boxes). Capital letters in parentheses refer to country of origin for each specimen, A = Argentina, B = Bolivia, C = Colombia, CH = Chile, and P = Peru. One asterisk follows the name of specimens traditionally referred to (but unsupported) as '*U. krempelhuberi*'. Two asterisks are used for specimens that traditionally were referred to (but unsupported) as '*U. peruviana*'.

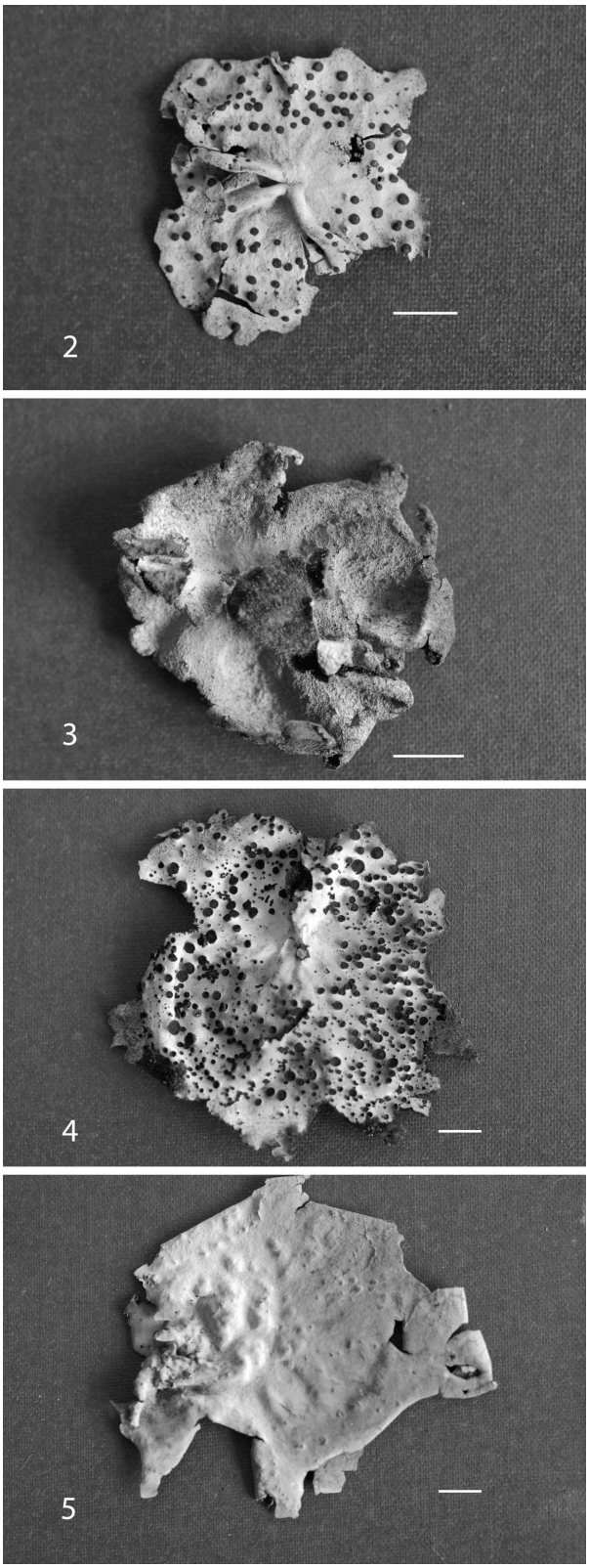
calvescens and *U. haplocarpa*, the majority of sorediate specimens from the Andes generally have been referred to the taxon *Umbilicaria leprosa* (Zahlbruckner 1906), which so far has not been considered to be associated with either of the two main groups revealed by our molecular phylogenetic study. Many of these sorediate individuals appear to have discarded sexual reproduction completely or almost completely. When no apothecia are present it is practically impossible to assign a sorediate specimen to the *calvescens* or *dichroa* group. Because soredia originated multiple times during the evolution of lichens (Bowler and Rundel 1975) there is an ongoing discussion about whether two specimens, which differ only by the presence or absence of soredia, should be considered to represent two separate species. For example Ott et al. (2004) argued that the sorediate taxon *Umbilicaria kappenii* was indistinguishable from the taxon *U. antarctica* in a molecular study based on nuclear ITS and LSU rDNA and mitochondrial LSU rDNA and accordingly should be reduced to synonymy with the latter. The anatomy of species in the Andean element, with a continuous, dense algal layer, well separated from the lower parts of the thallus by a thick layer of dense hyphae, might aid the evolution of soredia. Algae from the photobiont layer may easily detach from the thallus, carrying with them hyphae of the upper cortex. The sorediate taxa from the northern hemisphere, *U. grisea* and *U. hirsuta*, are part of the *U. vellea* group, although morphologically *U. hirsuta* is difficult to distinguish from the endemic *U. leprosa*. This suggests that all previous reports of *U. hirsuta* from the Andes must be re-evaluated.

The tentative phylogenetic placement of sorediate individuals and their presence in both clades of the endemic element, as well as in the sister *U. vellea* group (FIG. 1), indicates that the central Andean endemic species might have evolved from a sorediate ancestor. If so we might have a case of long-distance dispersal via asexual propagules containing both symbionts, followed by local speciation and evolutionary reversal to sexuality and/or loss of asexual reproduction via soredia in many of the branches of the central Andean element. The two putative independent reversals to sexuality in the central Andean endemic group could be an explanation for the evolution of two apothecium types within this element. Similar reversals from predominantly asexually reproducing to sexually reproducing taxa have been described in other groups of lichen-forming fungi (Cornejo et al. 2009, Tehler et al. 2009). This scenario suggests that the genes for sexual reproduction were not lost through evolution but only temporarily repressed. The loss of sexual reproduction is considered an evolutionary disadvantage for several reasons in non-obligate symbiotic organisms (Bell 1982, Zeyl and Otto 2007). However for an obligate mutualistic system asexual propagules containing both symbionts often have been considered a more effective means of dispersal than fungal propagules such as ascospores or thalloconidia alone, which necessitate the proximity of a compatible photobiont to enable the establishment of the next generation of lichens (Hestmark 1991). Although some evidence supports the hypothesis that species of *Umbilicaria* are not very selective in their requirements for a compatible algal partner (Romeike et al. 2002), it nevertheless would have been advantageous for the first *Umbilicaria* to colonize an emerging mountain chain, arriving with a propagule containing both mycobiont and photobiont. To what degree the Andean *Umbilicaria* species share the same algal species or strains and their algae are related to algae found in lichens of the *U. vellea* group versus other lichens remains to be investigated.

Rapid species diversification linked to adaptive radiation is a common occurrence when new, largely unoccupied, isolated habitats are colonized by a few pioneer species (Schluter 2000) and has been documented for many genera of flowering plants on the comparatively recent central Andean ''alpine island'' (Hughes and Eastwood 2006). Many of these genera, such as *Draba*, *Lupinus*, *Quercus*, *Salix*, *Sambucus*, *Valeriana* and *Viburnum*, are thought to have arrived in the Andes from North America after the uplift (van der Hammen and Cleef 1986, Burnham and Graham 1999). In general flowering plants seem to have much more plasticity to evolve new life histories, dispersal options, etc. (Hughes and Eastwood 2006). In contrast Ahti (1992) and Sipman (1992, 2002) discussed the evolutionary rates of lichens in the Páramo vegetation zone and concluded that lichen endemism there is rare, confirming the opinion that lichen-forming fungi speciate slowly. Slow evolutionary rates in part might be ascribed to the long generation of alpine lichens. A study on growth and reproduction in several alpine *Umbilicaria* species by Hestmark et al. (2004) demonstrated generation times much longer than that of most vascular plants: 50–80 y from establishment to first reproduction. For *Umbilicaria* lichens the plasticity and possible morphological and structural changes also seem restricted. In the *calvescens* group there seems to be an evolutionary trend toward the elimination of rhizinomorphs, culminating in the *U. calvescens* typicum (and its synonym *U. krempelhuberi*). Species in the *vellea* group usually have abundant rhizinomorphs, sometimes considered beneficial for water uptake in the habitats of species within this group. *U. calvescens* varieties with abundant rhizino-

morphs (var. *subvellea* and var. *hypomelaena*) both tend to grow in similar habitats, with trickling water, in the Andes. In contrast the typical *U. calvescens*, with no or sparse rhizinomorphs, frequently are found on small and large boulders or on sun-exposed surfaces that exhibit no persistent water trickle. Thus it could be that the loss of rhizinomorphs is associated with an extension of the ancestral niche into drier and more open habitats. The absence of other *Umbilicaria* and *Lasallia* species in subalpine and low-alpine regions have left these drier niches open for expansion of the endemic element. The amplitude of variation in rhizinomorph presence seen in the *calvescens* complex is similar to that observed in the sorediate members of the *vellea* group from the richly rhizinomorphic *U. hirsute*, which means hairy, to the non-hirsute *U. grisea*.

Genetic differences observed among individuals in the Andean endemic *Umbilicaria*, notably in the *calvescens* group, explains some of the taxonomic confusion and the problem of field identification that has been associated with this group of lichens since its discovery in the mid-19th century. The phylogenetic tree presented here does not suggest obvious solutions to these problems, and in general the hypothetical nature of any such tree should always be emphasized (Hestmark 2000). The many unsupported relationships in the *calvescens* and *dichroa* groups indicate that new loci (preferentially fast evolving) need to be sequenced and a population genetic approach should be implemented, in addition to phylogenetics if new species are to be delimited and described within the central Andean endemic group. In any case, with morphological traits exhibiting gradual transitions, it will be difficult to use a taxonomy based on such genetic markers for the practical purposes of field and herbarium identification if species are found in addition to the two main species groups defined here for the central Andean element. Therefore to aid field identification we suggest that with the exception of the distinctly sorediate taxon *U. leprosa* all other specimens in the *calvescens* group should be named *U. calvescens*, if desirable with some indication of variety. The typical specimens of *U. calvescens* (cf. Hestmark 2010) correspond to the later described taxon *U. krempelhuberi* Müll-Arg., which accordingly should be reduced to synonymy with *U. calvescens*. The taxon *U. peruviana* also is considered best a synonym of *U. calvescens*. Although the taxon *U. haplocarpa* might appear to be divided into two fairly distinct genetic subpopulations (H1 and H2), practical considerations of identification suggest that this taxon should be kept as one unit, species *U. haplocarpa*. The taxon *U. dichroa* can be maintained for specimens with few



FIGS. 2–5. 2. *Umbilicaria calvescens*. 3. *U. leprosa*. 4. *U. dichroa*. 5. *U. haplocarpa*. Bars = 1 cm.

or no apothecia, a black lower side with wart-like protrusions until more data is gathered. Field work by the first author indicates that *U. dichroa* has a restricted range around Lake Titicaca. The central high Andean element thus is seen to be in active, diverging evolution, potentially resulting in several new and separate species. (Currently recognized species are depicted in FIGS. 2–5.)

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