Molecular phylogenetics of *Oreobolus* (Cyperaceae) and the origin and diversification of the American species

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Nuclear ribosomal DNA Internal Transcribed Spacer (ITS) and plastid trnL intron and trnL-F intergenic spacer regions were sequenced for 14 species of *Oreobolus* (Cyperaceae) from throughout most of its distribution range (South America, Australasia, and Hawai‘i), with the exception of the Malesian species; *Costularia laxa* was used as outgroup. Phylogenetic trees were produced for ITS and trnL-F datasets using maximum parsimony and maximum likelihood. We estimated species divergence times by enforcing a molecular clock on the Maximum Likelihood ITS tree, using the appearance of *O. furcatus* in Hawai‘i, no earlier than 5.1 mya, as a calibration point. Our results support the monophyly of the South American species with the southern *O. obtusangulus* as sister to the rest. This South American clade is sister to the Australian *O. pumilio*, and the Australasian and Hawaiian species sampled form a basal grade. *Oreobolus oligocephalus* is embedded within *Oreobolus*, rejecting its separation in the monotypic genus *Schoenoides*. The transformed branch lengths of the ITS tree indicate a recent (5.5–6 mya) origin of the South American clade, followed by a northward migration and diversification of species along the Andes.

**KEYWORDS:** long distance dispersal, molecular phylogenetics, *Oreobolus*, páramo, phylogenetic dating, South American biogeography.

**INTRODUCTION**

The genus *Oreobolus* R. Br. (Cyperaceae) is a group of low cushion plants, growing in bogs or mesic habitats in the mountains of Central and South America, Australasia, Malesia, Tahiti, and the Hawaiian Islands. *Oreobolus* has been characterized by the combined presence of one-flowered spikelets (Brown, 1810), a pseudopetiole in the leaf lamina (Seberg, 1988), and the usually flat perianth scales (Seberg, 1988). These characters, however, are not unique to *Oreobolus*. Pseudopetiolate leaf lamina (Bruhl & al., 1992; Bruhl, 1995) and flat perianth scales (Goetzhebeur, 1986, 1998; Bruhl & al., 1992; Bruhl, 1995) are also present in other members of Cyperaceae, although the occurrences of the latter attribute, are probably not strictly homologous to *Oreobolus*. Moreover, Seberg (1985) showed the presence of one-flowered spikelets in various genera in Rhynchosporae (i.e., including Schoenae sensu Goetzhebeur, 1986, and Bruhl, 1995). The inclusion of *Schoenoides* (with multi-flowered spikelets) in *Oreobolus*, as noted below, may represent a reversal of this character within the genus (cf. Zhang, X. & al., 2004).

*Oreobolus* has been placed in the tribe Schoenae on the basis of morphological and molecular characters (Goetzhebeur, 1986, 1998; Bruhl, 1995; Muasya & al., 1998). With species of *Costularia* C.B. Clarke it shares the presence of conical, “external”, silica bodies associated with the sinuous anticlinal walls of the epidermal cells of the leaves (Metcalfe, 1971: 401; Seberg, 1988; Bruhl & al., 1992; Bruhl, 1995), and a spiro- or orthodistichous phyllotaxy (Kikenthal, 1939; Seberg, 1986). Additionally, the presence of an innovation bud just below the inflorescence in *Oreobolus* and *Costularia* subgen. *Chamaedendron* Kük., responsible for the elevated, spherical habit of these plants (Mora-Osejo, 1960; Kubitzki, 1966), has been used as evidence of their close relationship (Seberg, 1986, 1988). As pointed out by Seberg (1988) on morphological grounds and recently confirmed by molecular analyses (Zhang, X. & al., 2004; Verboom, 2006), *Costularia* is non-monophyletic, calling for a reassessment of the genus.

*Oreobolus* was revised by Seberg (1988), who recognized 14 species and three subspecies. A new species from Tasmania *O. tholicarpus* D.J. Morris was recently added (Morris, 2001). *Oreobolus oligocephalus* W. M. Curtis was excluded from the genus and placed in its own monotypic genus *Schoenoides* Seberg, based on multi-flowered spikelets (viewed as plesiomorphic by Seberg, 1986), sub-capitate inflorescences, and split prophylls.
(Seberg, 1986). The pseudopetiole and scale-like tepals in two alternating whorls were considered synapomorphies of Oreobolus and Schoenoides (Seberg, 1988). In a recent taxonomic treatment of Cyperaceae, however, Goetghhebeur (1998) abandoned the usage of Schoenoides. Molecular data also argues for placement of Schoenoides oligocephalus within Oreobolus (Zhang, X. & al., 2004).

Oreobolus has fairly strict ecological preferences and occurs in mesic grasslands in southern temperate regions and at high elevations in the tropics (Mora-Osejo, 1987; Seberg, 1988). Its current range reflects a Gondwanian origin (see Bremer, 2002), and includes two widely disjunct regions. The first region comprises páramos, punas, and jalcas of the Andean cordillera (Colombia, Venezuela, Ecuador, Peru, Chile, Argentina) and southern Central America (Panama, Costa Rica) (Mora-Osejo, 1987). The second region includes alpine and subalpine sedgelands and herbfields of New Zealand, mainland Australia, Tasmania, New Guinea, Borneo, Sumatra, and Hawai‘i (Seberg, 1988). This distribution is paralleled by other groups of plants and animals, leading to the formulation of biogeographic hypotheses explaining the origins of the southern biota by means of vicariance events resulting from the breakup of the supercontinent Gondwana, approximately 100–130 million years ago (references in Nelson & Ladiges, 2001). Alternative hypotheses invoking long-distance dispersal events from different geographical sources are becoming more and more common as molecular clock data become available (Winkworth & al., 2002; Sanmartín & Ronquist, 2004; de Queiroz, 2005). Nevertheless, the various methods currently in use rely strongly on the source of the data used to calibrate the molecular clock, and thus must be interpreted with caution (Heads, 2005).

**Previous systematic studies on Oreobolus.** — Mora-Osejo (1987) studied the systematics and biogeography of the American species of Oreobolus (O. cleefii, O. ecuadorensis, O. goeppingeri, O. obtusangulus, O. venezuelensis), using O. pumilio from Australia as outgroup in his phylogenetic analysis. He described the general organization types of buds and inflorescences of Oreobolus, given their importance in species differentiation. Seberg (1988) performed a morphological phylogenetic and biogeographic analysis of Oreobolus including all species of the genus. Substantial incongruences exist between the two studies with respect to the relationships of the South American species. Oreobolus cleefii, included as a distinct species in the analysis of Mora-Osejo, was treated by Seberg as a subspecies of O. obtusangulus (O. obtusangulus subsp. unispicus Seberg), and Oreobolus ecuadorensis, sister to all other South American species in Seberg’s study was resolved within the South American clade in Mora-Osejo’s analysis.

In addition, two different biogeographical scenarios resulted from the two studies. According to Mora-Osejo (1987), diversification of the American species of Oreobolus began in southern South America. The first lineage to appear was O. obtusangulus; as the Andean Cordillera began to emerge, conditions similar to those of the southern part of the continent appeared, allowing dispersal and divergence of other species lineages further northward in South America during the Pleistocene. This hypothesis differs considerably from that of Seberg who proposed sister area relationships between Australia and Central America, O. distichus (from mainland Australia and Tasmania) being sister to O. goeppingeri (currently found in northern South America and Central America), and subsequent diversification southwards in the Andes (Seberg, 1988, 1991).

The present study uses molecular markers to infer a phylogenetic hypothesis of relationships among extant species of Oreobolus and the biogeographical history of the genus in South America. Additionally, we perform a molecular dating exercise to estimate the times when this may have taken place. We sequenced the nuclear ribosomal DNA Internal Transcribed Spacer (ITS), and plastid trnL intron and the trnL-F intergenic spacer. These DNA regions have been previously found useful in phylogenetic studies of Cyperaceae (Muasya & al., 2002; Simpson & al., 2003; Ghamkhar & al., in press).

**MATERIALS AND METHODS**

**Taxon sampling.** — We sampled 14 species of Oreobolus and one of Costularia (C. laxa). Specimens were obtained from the collection of silica gel-dried páramo plants of the Laboratorio de Botánica y Sistemática, Universidad de los Andes, field-collected specimens from Australia, and herbarium material from COL and K (Appendix).

**DNA extraction and sequencing.** — Total DNA was extracted from c. 0.5 g of leaf tissue dried in silica gel or c. 0.8 g of herbarium material, using a modified version of the 2X CTAB method of Doyle & Doyle (1987). DNA was precipitated using ethanol for the samples preserved in silica gel, and isopropanol for the herbarium samples because the latter is more effective in precipitating degraded DNA found in such samples. The DNA was purified by means of caesium chloride/ethidium bromide gradients (1.55 g/ml). In the case of herbarium samples, it was necessary to carry out a further purification with QIAquick silica columns (Qiagen, Inc.), following the protocol for PCR products provided by the manufacturer; this procedure both cleaned the DNAs as well as concentrating them.
Amplification (PCR) was carried out using the protocols of Muasya & al. (2000), except for lowering of the annealing temperature and an increase in the extension time for some of the herbarium samples. Amplification products were purified using QIAquick silica columns (Qiagen, Inc.) according to the manufacturer’s protocols and sequenced on an ABI 377 automated sequencer (Applied Biosystems, Inc.), using standard dye-terminator chemistry following the manufacturer’s protocols. The sequence reaction products were purified by ethanol precipitation.

For ITS, the ~700 base pair (bp) region between the 18S and 26S rDNA subunits was amplified, including the 5.8S subunit. To amplify the samples preserved in silica gel, the forward 17SE and reverse 26SE primers (Sun & al., 1994) were used, for herbarium samples two additional internal primers, ITS2 and ITS3 (Baldwin & al., 1995). The trnL-F region was amplified using the primer pairs c/f (Taberlet & al., 1991) for the silica-gel samples and two internal primer pairs c/d and e/f (Taberlet & al., 1991) for herbarium samples.

**Editing, alignment and phylogenetic analyses.** — Sequences were edited with Sequencher version 4.1 (Gene Codes Corporation). The sequences were aligned by eye using MacClade version 4.0b (Maddison & Maddison, 2002) following the guidelines of Kelchner (2000). The phylogenetic analyses were carried out using PAUP* version 4.0b10 for Macintosh (Swofford, 2002). Branch and Bound Maximum Parsimony searches were performed for each dataset separately, using unordered and equally weighted characters (Fitch parsimony; Fitch, 1971). Gaps were coded as missing data and treated as uninformative characters in the analyses. Costularia laxa was used as outgroup. Bootstrap support (BP; Felsenstein, 1985) for each clade was estimated with 1000 replicates of the Branch and Bound analysis. Bremer support (d; Bremer, 1988) for each clade was also determined by means of random addition of taxa and 20 repetitions using TreeRot.v2b f (Sorenson, 1999). Maximum Likelihood analysis was carried out for the ITS matrix; it was omitted for the trnL-F matrix due to incomplete sampling of this region (Appendix). In the ITS matrix we used the best-fit model (TrNG) selected by the Hierarchical Likelihood Ratio Tests (hLRTs) in Modeltest Version 3.06 (Posada & Crandall, 1998). The assumed nucleotide frequencies were estimated from the data: A = 0.1941, C = 0.2576, G = 0.3337, T = 0.2146. The proportion of invariant sites was 0, the Gamma distribution shape parameter was 0.0171, and the number of substitution types was 6. The following substitution rate matrices were estimated by Modeltest: A/C = 1, A/G = 3.3065, A/T = 1, C/G = 1, C/T = 16.0472, G/T = 1. Topological congruence between the three trees of the two datasets was determined by visual examination (Whitten & al., 2000).

**Molecular clock.** — Branch lengths obtained from the Maximum Likelihood analysis of the ITS matrix were used to estimate the evolutionary rate heterogeneity among lineages. We used the likelihood ratio test, which compares the logarithm of the likelihood (LnL) of two different hypotheses, one of them forcing a molecular clock and the other not. The null hypothesis, stating that our data behaved clocklike was rejected, and non-parametric rate smoothing (NPRS; Sanderson, 1997) was applied to produce an ultrametric tree with TreeEdit v.1.0 - alpha 4-61 (Rambaut & Charleston, 2002). Branch lengths on the ultrametric tree where used to produce a chronogram to determine the relative ages of all clades, using the presence of *O. furcatus* in the Hawaiian archipelago as a calibration point. The age of the branch leading to this species was set to 5.1 mya, corresponding to the earliest possible date of origin of *O. furcatus*, based on the age of Kauai (Carson & Clague, 1995), the oldest island where this species currently occurs. It is possible that *Oreobolus* may have been found on older now submerged islands at an earlier date. However, none of these submerged islands had high elevation habitats, thus limiting the successful colonization of *Oreobolus* (Carson & Clague, 1995). Confidence intervals of the resulting dates were determined by performing 1000 bootstrap replicates of the Branch and Bound analysis, estimating the divergence time in each replicate relative to the base tree. The proportion of nucleotide substitutions in the ITS sequences between *O. furcatus* and its sister species (from the phylogenetic analysis) was used to obtain an estimate of substitution rate, r, for this region, calculated by comparing the number of substitutions per site in the ultrametric tree to the time scale of the chronogram estimated with the calibration point above (Li & Graur, 1991). The resulting rate was compared to other published rates of nucleotide substitution for the ITS region.

**RESULTS**

**DNA sequencing and alignment.** — Complete amplification of ITS was successful in 14 of the 15 species sampled, either as a single sequence or as two adjacent ones; for *O. strictus*, only the second half of the whole sequence was obtained (Appendix). For trnL-F, complete amplification was successful in 10 species, either as a single sequence or as two adjacent ones, whereas for the remaining five, only partial sequences where obtained (Appendix). The ITS aligned matrix consisted of 792 bp of which 7.5% were variable and 5% were potentially parsimony-informative (Table 1). In the case of trnL-F, the length of the aligned matrix was 1028 bp long, but the percentages of variable positions and
potentially parsimony-informative characters were lower than for ITS (5.4\% and 3\%, respectively; Table 1). The combined aligned matrix is available in TreeBase (www.treebase.org).

**Phylogenetic analyses.** — The Maximum Parsimony search of the ITS matrix yielded a single most parsimonious tree of 72 steps with a consistency index (CI) of 0.82 and a retention index (RI) of 0.90 (Table 1, Fig. 1). The Maximum Likelihood analysis produced a tree with a Likelihood score of 1541.5546, and identical topology to the single most parsimonious tree. In this tree, the South American species comprise a well-supported clade (BP 100, d8); they are sister to *O. pumilio* from mainland Australia and Tasmania. The position of *O. obtusangulus* from southern South America as sister to the rest of the South American species is strongly supported. *Oreobolus oligocephalus* is embedded in the genus. The positions of *O. acutifolius* and *O. furcatus* are weakly supported (BP <50, d1).

Analysis of the trnL-F dataset yielded 30 trees of 63 steps with CI of 0.89 and RI of 0.91 (Table 1, Fig. 2). The strict consensus tree shows three clades in a basal polytomy, differing from the ITS tree in the position of *O. oligocephalus*, *O. pumilio* and *O. furcatus* (Fig. 2). Two clades are well-supported: the South American clade (BP 89, d3) and the clade formed by *O. pectinatus*, *O. strictus* and *O. pumilio* (BP 88, d2). As in the ITS tree *O. oligocephalus* is embedded among the rest of *Oreobolus* species.

**Molecular clock calibration.** — The likelihood ratio test rejected the hypothesis of no evolutionary rate heterogeneity among lineages (1559.2923 versus 1541.5546, \(P < 0.05\)), and thus we used NPRS to produce an ultrametric tree. The calibration point of 5.1 mya applied to the *O. furcatus/O. acutifolius* node, yielded a maximum age for the genus of 6.2 to 7.1 mya. The estimated age of divergence of the South American clade (Fig. 3) is approximately 5.5 to 6.1 mya, but this node has no support. The diversification of the genus in South America began approximately 3 to 4.4 mya (BP 98) and, probably 2 to 3 mya (BP 99), *Oreobolus* began colonizing the páramo habitat. Our analysis indicates that *O. oligocephalus* diverged approximately 3 to 4.8 mya (BP 59). The substitution rate for the ITS region is \(4.89 \times 10^{-9}\) substitutions per site per year assuming a 5.1 mya age of the *O. furcatus/O. acutifolius* node.

**Phylogenetic relationships of *Oreobolus*.** — The presence of a South American clade is common to the trees generated by each of the data partitions (Figs. 1–3). In the ITS tree, the Australasian species form a grade to the other members of the genus. These relationships are not present in Seberg’s (1988) morphological analysis, which included all species of the genus. The clade containing the New Zealand species, *O. pectinatus* and *O. strictus*, is the only component common with Seberg’s tree. Unfortunately, we could not obtain material of 4 Australasian/Malesian spp. (*O. kuekenthalii*, *O. ambiguus*, *O. tholicarpus*, *O. impar*), therefore we could not analyze the relationships among the species of the Australasia/Malesia region in detail.

Other components of the molecular tree are incongruous with Seberg’s hypothesis. The presence of *O. distichus* as the sister species of *O. oxycarpus* is one of the most relevant incongruities. The sister relationship between these two Australian species seems more plausible than that between *O. distichus* and the South American *O. goeppingeri* (Seberg, 1988). The position of *O. oligocephalus*, deeply embedded among other *Oreobolus* species, is another difference to Seberg’s hypothesis.
Origin and diversification of the American species of Oreobolus. — *Oreobolus furcatus* is the only species of the genus limited to extant islands of the Hawaiian archipelago (Hawai‘i, Maui, Molokai, Oahu and Kauai), with specimens also reported from Tahiti by Seberg (1988). This volcanic archipelago has been forming over a hot spot in the Earth’s mantle since 80 mya. The oldest extant islands are Nihoa (7.2 mya) and Kauai (5.1 mya; Carson & Clague, 1995). The molecular clock calibration of our tree is based on the date of emergence of Kauai (*O. furcatus* does not occur on Nihoa). Price & Clague (2002) concluded that extant montane species of the Hawaiian archipelago evolved after the formation of Kauai or are the product of more recent dispersal. The presence of *O. furcatus* in Tahiti, with an approximate age of 2 mya (Dymond, 1975), and the possibility that *O. furcatus* is a relatively recent arrival to the Hawaiian archipelago and not necessarily arrived soon after the formation of Kauai 5.1 mya., does not alter our maximum age estimation for *O. furcatus*.

The substitution rate for the ITS region in the genus estimated with our calibration point is $4.89 \times 10^{-9}$ substitutions per site per year. This falls within the range of (1988, 1991), in which *O. oligocephalus* is sister to the remaining species of *Oreobolus* justifying transfer of this species to *Schoenoides*. We propose that the multi-flowered spikelets distinguishing *Schoenoides* from *Oreobolus* are a reversal in *Oreobolus*. The position of *O. oligocephalus*, embedded within *Oreobolus* was also resolved in the molecular study of Zhang, X. & al. (2004), which included only three species of *Oreobolus*.

*Oreobolus pumilio* is sister to the South American clade, being most closely related to *O. obtusangulus*. This last species, from Argentina and Chile, differs clearly from *O. cleefii*, which is deeply embedded in the South American clade as sister to *O. goeppingeri* and *O. venezuelensis* (Figs. 1–3). Thus, species status of *O. cleefii* as proposed by Mora-Osejo (1986) is confirmed and its treatment as a subspecies of *O. obtusangulus* (*O. obtusangulus* subsp. *unispicus* Seberg) is not supported. The topologies of the South American clade (Fig. 1–3) are not congruent with those of Mora-Osejo (1987) and Seberg (1988) based on morphology. In Mora-Osejo’s analysis (1987), *O. venezuelensis* is sister to the other South American species and in Seberg’s (1988) it is *O. ecuadorensis*. The ITS tree in this study, however, reveal a progressive expansion of *Oreobolus* towards northern South America, from Argentina via Chile, Ecuador and Colombia, to Venezuela and Costa Rica.

![Fig. 2. Strict consensus of 30 equally parsimonious trees obtained from the Branch and Bound analysis of the *trnL-F* dataset. Support for internal nodes is reported as bootstrap percentages (above) and Bremer support (below).](image-url)

![Fig. 3. Chronogram obtained from the ITS dataset with Maximum Likelihood branch lengths transformed with non parametric rate smoothing. The asterisk indicates the calibration point used, based on the origin of *O. furcatus* estimated at 5.1 mya. Bootstrap percentages (above) are given for each node. Boxes indicate the standard deviation and the mean of the estimated dates.](image-url)
published substitution rates for several perennial herba-
ceous angiosperms with generation times of 1–2 years, of
1.72–5.69 \times 10^{-9} substitutions per site per year
(Richardson & al., 2001) to 8.34 \times 10^{-9} substitutions per
site per year in Soldanella (Zhang, L.-B. & al., 2001).

The presence of Oreobolus in the Hawaiian archi-
pelago can only be explained as a result of long distance
dispersal from Australasia or Malesia (Fig. 3). The posi-
tion of O. pumilio as sister to the South American clade
indicates a sister relationship between South America
and Australia. Malesian species were not included in our
study but we and others (e.g., K.L. Wilson pers. comm.,
2005) predict that Malesian and Australasian species of
Oreobolus group together (see also Heads, 2003). The
molecular clock calibration for the arrival of Oreobolus
in South America is relatively recent (approximately 5.5
to 6 mya) as compared with the date of separation of
Australia and South America (at least 45 mya), when the
connection with the Antarctic bridge was broken (Raven
& Axelrod, 1974). The hypothesis of a migration of species
to South America by vicariance after the Gondwanan breakup can probably be discarded, since it
would have only been possible through the Antarctic
connection. Stepping-stone dispersal through Antarctica,
before the formation of the ice sheet at ca. 34 mya
(Zachos & al., 1992), may not have been possible
according to our age estimates. The alternative hypothe-
sis is that there was a long distance dispersal event start-
ing from Australasia, as described for O. furcatus. The
latter explanation is commonly used for explaining dis-
tributions of widely disjunct plant species (e.g., de Queiroz,
2005).

After the arrival of O. pumilio in South America, species
diversification towards the northern part of the contin-
tent started with the emergence of O. obtusangulus
in Argentina and Chile (Fig. 3). The divergence of this
species from its Australasian ancestors probably occurred
5.5 to 6 mya, at the beginning of the Pliocene. At
that time the South American continent presented similar
climatic and edaphic conditions to those of the higher
Australian mountains. The formation of the three
Colombian cordilleras and the Cordillera de Mérida in
Venezuela occurred 4 mya (Kroonenberg & al., 1990). In
the Andean cordillera the first páramo elements were
beginning to appear at elevations not greater than 3000
meters (van der Hammen, 1974; van der Hammen & Cleef, 1986). The times of establishment and diversifica-
tion of Oreobolus in South America proposed under our
dating scheme are consistent with the uplift of the Andes
and origins of high elevation vegetation in the northern
Andes.

During the Pliocene, 2 to 4 mya, páramo vegetation
began to diversify with new species emerging as the
Andean cordillera began to rise. These mountains wit-
nessed a process of species adaptation, with groups such
as Gunnera (Wanntorp & al., 2001, 2002) and Pyrrh-
obyrum (McDaniel & Shaw, 2003), that repeatedly
immigrated from austral Pacific regions. Against this
background, new species of Oreobolus emerged in a pro-
gressive geographical pattern from south to north. This
would be the case for O. ecuadorensis and O. cleefii,
which probably arose in the region known today as
Ecuador between 2 to 3 mya and 1.5 to 2.5 mya respec-
tively (Fig. 3). During this time, páramo vegetation dom-
inated the high Andes and ocean cooling processes began
what would be one of the glaciations at the beginning of
the Pleistocene (van der Hammen & Cleef, 1986). These
conditions could have enabled O. cleefii to continue
migrating through páramos until reaching Colombia.
Pollen diagrams (van der Hammen & Cleef, 1986) dem-
onstrated that a continuous alternation between forest
and páramo vegetation started 1 mya, reflecting a cycle
of glacial and interglacial periods that may have permit-
ted the diversification of the flora. According to our esti-
mates, O. venezuelensis and O. goeppingeri probably
emerged at this time (approximately 1 mya to 20,000
years; Fig. 3), further migrating across the isthmus of
Panamá to Costa Rica through a land bridge that had
risen 3.5–3.1 mya (Coates & Obando, 1996).

The geographical processes described here are similar
to those proposed by Mora-Osejo (1987). This author
suggested that the diversification of the South American
species of Oreobolus began in Argentina with O. obtu-
sangulus, followed by O. venezuelensis, which continued
expanding towards the northern part of South America.
Although the succession of species in Mora-Osejo’s tree
(1987) is different from ours, the hypothesis of vicari-
ance and migration towards the northern part of South
America is supported by our results. In Seberg’s bioge-
ographic hypothesis (1988), the position of O. distichus
in the South American clade implies a recent long distance
dispersal event from Australia to Central America and
subsequent spreading southwards. Our results indicate
that the morphological similarities leading to the latter
scenario are due to convergence, not common ancestry.

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Appendix Taxa used in this study, geographical origin, voucher and Genbank accession numbers for ITS (1) and trnL-F (2).

Costularia laxa Chem., Madagascar, Gautier & Chatelain 2706 (K), DQ450465 (1), DQ456955 (2); Oreobolus acutifolius S.T. Blake, Tasmania, Ockendon s.n. (K), DQ450466 (1), DQ456956 (2); O. cleefii Mora-Osejo, Colombia, García 122 (COL), DQ450467 (1), DQ456957 (2); O. distichus F. Muell., Australia (NSW), Bruhl 1870 (NE), DQ450468 (1), DQ456958 (2); O. eucalycophyllis T. Koyama, Colombia, Stern & Abuchar 103 (COL), DQ450469 (1), DQ456959 (2); O. furcatus M. Hann, Hawaii, Carlquist 21111 (K), DQ450470 (1), DQ456960 (2); O. goeppeingrii Sus., Colombia, García 093 (COL), DQ450471 (1), DQ456961 (2); O. obtusangulus Gaudich, Argentina, Moore 2817 (K), DQ450472 (1), DQ456962 (2); O. ologecophalus W.M. Curtis, Tasmania, Bruhl 18894 (NE), DQ450473 (1), DQ456963 (2); O. oxycarpus S.T. Blake, Tasmania, Blake 18320 (K), DQ450474 (1), DQ456964 (2); O. pectinatus Hook. f., New Zealand, Melville & Connor 6528 (K), DQ450475 (1), DQ456965 (2); O. punctilobum R. Br., Tasmania, Bruhl 1879C (NE), DQ450476 (1), DQ456966 (2); O. sp. 11, Ecuador, Lagoa 70382 (COL), DQ450477 (1), DQ456967 (2); O. strictus Berggr., New Zealand, McMillan 13395 (K), DQ450478 (1), DQ456968 (2); O. venezuelensis Steyer, Colombia, Jiménez 02 (COL), DQ450479 (1), DQ456969 (2).

1This specimen, originally determined as O. cf. eucalycophyllis, has ITS and trnL-F sequences that differ considerably from the other O. eucalycophyllis specimen, and may represent a new species to be described elsewhere.