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

Juliana Chacón[1, 2], Santiago Madriñán[1, 2], Mark W. Chase[2] & Jeremy J. Bruhl[3]

[1] *Laboratorio de Botánica y Sistemática, Universidad de los Andes, Apartado Aéreo 4976 Bogotá, D. C., Colombia. samadrin@uniandes.edu.co (author for correspondence)*
[2] *Molecular Systematics Section, Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, U.K.*
[3] *Botany, Centre for Ecology, Evolution and Systematics, The University of New England, Armidale NSW 2351, Australia.*

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 (Goetghebeur, 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 Rhynchosporeae (i.e., including Schoeneae sensu Goetghebeur, 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 Schoeneae on the basis of morphological and molecular characters (Goetghebeur, 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 (Kükenthal, 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 Goetghebeur (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 phlogenetic 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 of 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 (*TrN*G) 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 invariable 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

Table 1. Statistics obtained from maximum parsimony analyses of separate data matrices of *Oreobolus*.

	ITS	*trnL-F*
Length of matrix	792	1028
No. of variable sites	59 (7.5%)	55 (5.4%)
No. of parsimony-informative sites	40 (5%)	31(3%)
No. of included taxa	15	15
No. of trees (Fitch)	1	30
No. of steps (Fitch)	72	63
Consistency Index	0.82	0.89
Retention Index	0.90	0.91
Average no. of steps/variable sites	1.22	1.15

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 4.89×10^{-9} substitutions per site per year assuming a 5.1 mya age of the *O. furcatus*/*O. acutifolius* node.

DISCUSSION

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

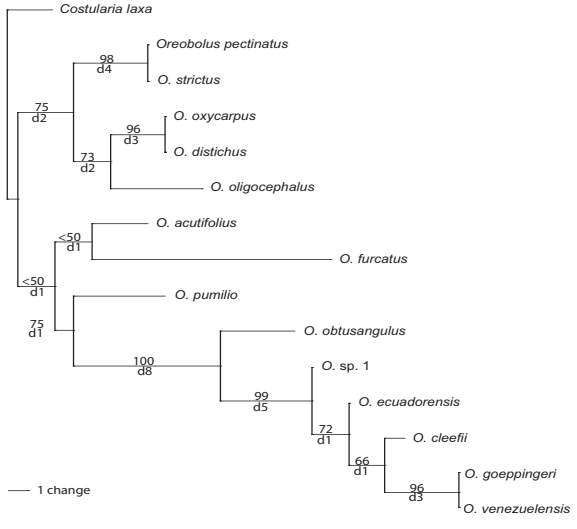

Fig. 1. The single most parsimonious tree obtained from the Branch and Bound analysis of the ITS dataset. This tree is identical in topology to the Maximum Likelihood tree. Support for internal nodes is reported as bootstrap percentages (above) and Bremer support (below).

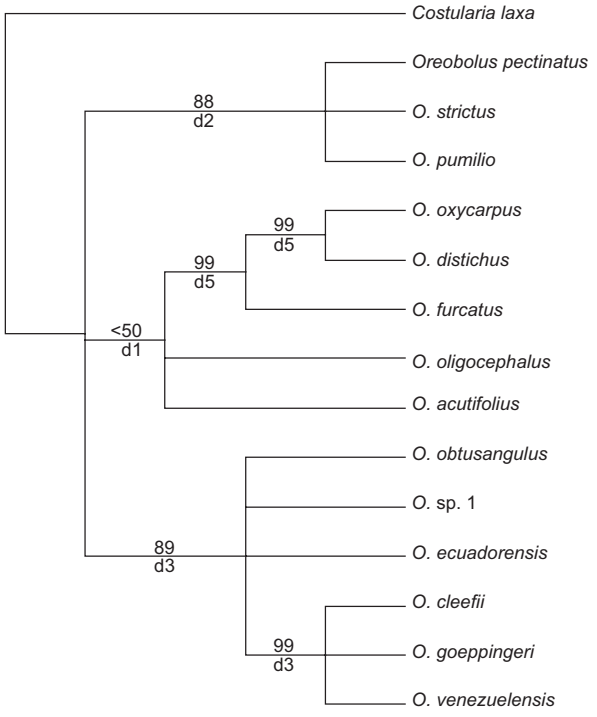

Fig. 2. Strict consensus of 30 equally parsimonious trees obtained from the Branch and Bound analysis of the *trnL-F* dataset. Support for internal nodes is reported as bootstrap percentages (above) and Bremer support (below).

(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.

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 4.89×10^{-9} substitutions per site per year. This falls within the range of

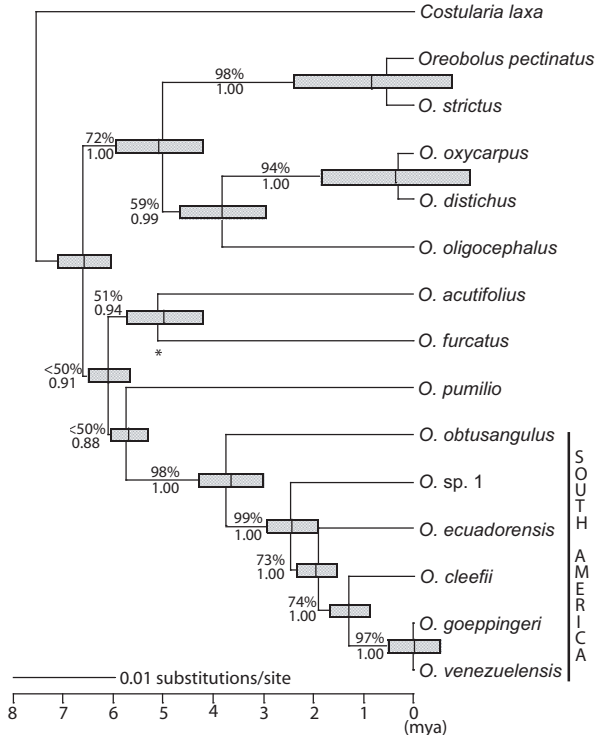

Fig. 3. Chronogram obtained from the ITS dataset with Maximum Likelihood branch lengths transformed with non parametric rate smoothing. The asterisk indicates the calibration point used, based on the origin of *O. furcatus* estimated at 5.1 mya. Bootstrap percentages (above) are given for each node. Boxes indicate the standard deviation and the mean of the estimated dates.

published substitution rates for several perennial herbaceous 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 8.34×10^{-9} substitutions per site per year in *Soldanella* (Zhang, L.-B. & al., 2001).

The presence of *Oreobolus* in the Hawaiian archipelago can only be explained as a result of long distance dispersal from Australasia or Malesia (Fig. 3). The position 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 hypothesis is that there was a long distance dispersal event starting from Australasia, as described for *O. furcatus*. The latter explanation is commonly used for explaining distributions 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 continent 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 diversification 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 witnessed a process of species adaptation, with groups such as *Gunnera* (Wanntorp & al., 2001, 2002) and *Pyrrhobryum* (McDaniel & Shaw, 2003), that repeatedly immigrated from austral Pacific regions. Against this background, new species of *Oreobolus* emerged in a progressive 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 respectively (Fig. 3). During this time, páramo vegetation dominated 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) demonstrated that a continuous alternation between forest and páramo vegetation started 1 mya, reflecting a cycle of glacial and interglacial periods that may have permitted the diversification of the flora. According to our estimates, *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. obtusangulus*, 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 vicariance and migration towards the northern part of South America is supported by our results. In Seberg's biogeographic 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.

ACKNOWLEDGEMENTS

This study was part of the undergraduate honor's thesis of the first author at the Universidad de los Andes, Bogotá. The molecular work was carried out at the Jodrell Laboratory, Royal Botanic Gardens, Kew. We thank David Simpson (K) for assistance with plant specimens, Julio Betancur (COL) for plant samples provided, Jonathan Davis for help with the molecular clock calibration and phylogenetic dating procedure, Stuart J. Davies (AA), Favio González (COL), James Richadson (RBGE), Karen Wilson (NSW) and two anonymous reviewers for comments on early drafts of the manuscript. Preliminary results from this work were

presented at the VII Latin American Botanical Congress in Cartagena, Colombia 2002, in a symposium on the Evolution of Neotropical flora sponsored by the IA The second author acknowledges the support of the Kew-Mellon Latin American Fellowship 2001-2002 under which part of the work was performed.

LITERATURE CITED

Baldwin, B. G., Sanderson, M. J., Porter, J. M., Wojciechowski, M. F., Campbell, C. S. & Donoghue, M. J. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Ann. Missouri Bot. Gard*. 82: 247–277.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.

Bremer, K. 2002. Gondwanan evolution of the grass alliance of families (Poales). *Evolution* 56: 1374–1387.

Brown, R. 1810. *Prodromus Florae Novae Hollandiae et Insulae Van-Diemen.* Taylor, London.

Bruhl, J. J. 1995. Sedge genera of the world: relationships and new classification of the Cyperaceae. *Aust. Syst. Bot.* 8: 125–305.

Bruhl, J. J., Watson, L. & Dallwitz, M. J. 1992. Genera of Cyperaceae: interactive identification and information retrieval. *Taxon* 41: 225–234.

Carson, H. L. & Clague, D. A. 1995. Geology and biogeography of the Hawaiian Islands. Pp. 14–29 in: Wagner, W. L. & Funk, V. A. (eds.), *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago.* Smithsonian Institution Press, Washington D. C.

Coates, A. G. & Obando, J. A. 1996. The geologic evolution of the Central American isthmus. Pp. 21–56 in: Jackson, J., Budd, N. & Coates, A. (eds.), *Evolution and Environments in Tropical America.* Univ. Chicago Press, Chicago.

de Queiroz, A. 2005. The resurrection of oceanic dispersal in historical biogeography. *Trends Ecol. Evol.* 20: 68–73.

Doyle, J. J. & Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19:11–15.

Dymond, J. 1975. K-Ar ages of Tahiti and Moorea, Society Islands, and implications for hot-spot model. *Geology* 3: 236–240.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

Fitch, W. M. 1971. Toward defining the course of evolution: minimum change for a specified tree topology. *Syst. Zool.* 20: 406–416.

Ghamkhar, K., Marchant, A., Wilson, K. L. & Bruhl, J. J. In press. Phylogeny of *Abildgaardieae* (Cyperaceae) inferred from ITS and *trnL-F* data. In: Columbus, J. T., Friar, E. A., Hamilton, C. W., Porter, J. M., Prince, L. M. & Simpson, M. G. (eds.), *Monocots: Comparative Biology and Evolution*, 2 vols. Rancho Santa Ana Botanic Garden, Claremont.

Goetghebeur, P. 1986. *Genera* Cyperacearum. *Een Bijdrage tot de Kennis van de Morfologie, Systematiek en Fylogenese van de Cyperaceae-genera.* Doctoral Thesis, Rijksuniversiteit, Gent.

Goetghebeur, P. 1998. Cyperaceae. Pp. 141–190 in: Kubitzki, K. (ed.), *The Families and Genera of Vascular Plants: Flowering Plants, Monocotyledons.* Springer, Berlin.

Heads, M. 2003. Ericaceae in Malesia: vicariance biogeography, terrane tectonics and ecology. *Telopea* 10: 311–449.

Heads, M. 2005. Dating nodes on molecular phylogenies: a critique of molecular biogeography. *Cladistics* 21: 62–78.

Kelchner, S. A. 2000. The evolution of non-coding chloroplast DNA and its application in plant systematics. *Ann. Missouri Bot. Gard.* 87: 482–498.

Kroonenberg, S. B., Bakker, J. G. M. & van der Wiel, A. M. 1990. Late Cenozoic uplift and paleogeography of the Colombian Andes: constraints on the development of high-andean biota. *Geol. & Mijnb.* 69: 279–290.

Kubitzki, K. 1966. Untersuchungen über den Blütenbau von *Oreobolus*. R. Br. *Bot. Jahrb. Syst.* 85: 80–87.

Kükenthal, G. 1939. Vorarbeiten zu einer Monographie der Rhynchosporoideae IV. *Repert. Spec. Nov. Regni Veg.* 46: 13–32, 65–76.

Li, W. H. & Graur, D. 1991. Rates and pattern of nucleotide substitution. Pp. 67–98 in: Li, W. H. & Graur, D. (eds.), *Fundamentals of Molecular Evolution.* Sinauer Associates, Sunderland, Massachusetts.

Maddison, D. R. & Maddison, W. P. 2002. *MacClade 4: Analysis of Phylogeny and Character Evolution, Version 4.05 for OS X.* Sinauer Associates, Sunderland, Massachusetts.

McDaniel, S. F. & Shaw, A. J. 2003. Phylogeographic structure and cryptic speciation in the Trans-Antarctic moss *Pyrrhobryum minioides*. *Evolution* 57: 205–215.

Metcalfe, C. R. 1971. *Anatomy of the Monocotyledons. V. Cyperaceae.* Clarendon Press, Oxford.

Mora-Osejo, L. E. 1960. Beiträge zur Entwicklungsgeschichte und vergleichende Morphologie der Cyperaceen. *Beitr. Biol. Pflanzen* 35: 253–341.

Mora-Osejo, L. E. 1987. *Estudios Morfológicos, Autoecológicos y Sistemáticos en Angiospermas*. Academia Colombiana de Ciencias Exactas, Físicas y Naturales.

Morris, D. I. 2001. A new species of *Oreobolus, O. tholicarpus* (Cyperaceae), endemic to Tasmania. *Muelleria* 15: 28–29.

Muasya, A. M., Bruhl, J. J., Simpson, D. A., Culham, A. & Chase, M. W. 2000. Poales – Cyperaceae supragenericphylogeny of Cyperaceae: a combined analysis. Pp. 593–601 in: Wilson, K. L. & Morrison, D. A. (eds.), *Monocots: Systematics and Evolution.* CSIRO Publishing, Melbourne.

Muasya, A. M., Simpson, D. A. & Chase, M. W. 2002. Phylogenetic relationships in *Cyperus* L. s.l. (Cyperaceae) inferred from plastid DNA sequence data. *Bot. J. Linn. Soc.* 138: 145–153.

Muasya, A. M., Simpson, D. A., Chase, M. W. & Culham, A. 1998. An assessment of the supragenericphylogeny in Cyperaceae using rbcL DNA sequences. *Pl. Syst. Evol.* 211: 257–271.

Nelson, P. & Ladiges, P. Y. 2001. Gondwana, vicariance biogeography and the New York School revisited. *Aust. J. Bot.* 49: 389–409.

Price, J. P. & Clague, D. A. 2002. How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. *Proc. Roy. Soc. London, Ser. B, Biol. Sci.* 269: 2429–2435.

Posada, D. & Crandall. K. A. 1998. Modeltest: testing the

model of DNA substitution. *Bioinformatics* 14: 817–818.

Rambaut, A. & Charleston, M. 2002. *TreeEdit: Phylogenetic Tree Editor, v1.0 alpha 10.* (http://evolve.zoo.ox.ac.uk/software/TreeEdit/TreeEdit.html)

Raven, P. & Axelrod, D. 1974. Angiosperm biogeography and past continental movements. *Ann. Missouri. Bot. Gard.* 61: 539–673.

Richardson, J. E., Pennington, R. T., Pennington, T. D. & Hollingsworth, P. M. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. *Science* 293: 2242–2245.

Sanderson, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Molec. Biol. Evol.* 14: 1218–1231.

Sanmartín, I. & Ronquist, F. 2004. Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Syst. Biol.* 53: 216–243.

Seberg, O. 1985. The identity of *Chillania pusilla* Roivainen (Cyperaceae). *Syst. Bot*. 10: 239–240.

Seberg, O. 1986. *Schoenoides*, a new genus of Cyperaceae from Tasmania. *Willdenowia* 16: 181–186.

Seberg, O. 1988. Taxonomy, phylogeny and biogeography of the genus *Oreobolus* R. Br. (Cyperaceae), with comments on the biogeography of the South Pacific continents. *Bot. J. Linn. Soc.* 96: 119–195.

Seberg, O. 1991. Biogeographic congruence in the South Pacific. *Aust. Syst. Bot*. 4: 127–136.

Simpson, D. A., Furness, C. A., Hodkinson, T. R., Muasya, A. M. & Chase, M. W. 2003. Phylogenetic relationships in Cyperaceae subfamily Mapanioideae inferred from pollen and plastid DNA sequence data. *Amer. J. Bot.* 90: 1071–1086.

Sorenson, M. D. 1999. *TreeRot, version 2b.* (http://people.bu.edu/msoren/TreeRot.html)

Sun, Y., Skinner, D. Z., Liang, G. H. & Hulbert, L. H. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theor. Appl. Genet.* 89: 26–32.

Swofford, D. L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Version 4.0b10 for Macintosh*^{TM}. Sinauer Associates, Sunderland, Massachusetts.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Molec. Pl. Biol.* 17: 1105–1109.

van der Hammen, T. 1974. The Pleistocene changes of vegetation and climate in tropical South America. *J. Biogeogr.* 1: 3–26.

van der Hammen, T. & Cleef, A. M. 1986. Development of the high Andean páramo flora and vegetation. Pp. 153–201 in: Vuilleumier, F. & Monasterio, M. (eds.), *High Altitude Tropical Biogeography*. Oxford Univ. Press, Oxford.

Verboom, G. A. 2006. A phylogeny of the schoenoid sedges (Cyperaceae: Schoeneae) based on plastid DNA sequences, with special reference to the genera found in Africa. *Molec. Phylog. Evol.* 38: 79–89.

Wanntorp, L., Wanntorp, H.-E. & Källersjö, M. 2001. Phylogeny of *Gunnera*. *Pl. Syst. Evol.* 226: 85–107.

Wanntorp, L., Wanntorp, H.-E. & Källersjö, M. 2002. Phylogenetic relationships of *Gunnera* based on nuclear ribosomal ITS region, *rbc*L and *rps*16 intron sequences. *Syst. Bot.* 27: 512–521.

Whitten, W. M., Willians, N. H. & Chase, M. W. 2000. Subtribal and generic relationship of *Maxillarieae* (Orchidaceae) with emphasis on *Stanhopeinae*: combined molecular evidence. *Amer. J. Bot.* 87: 1842–1856.

Winkworth, R. C., Wagstaff, S. J., Glenny, D. & Lockhart, P. J. 2002. Plant dispersal N.E.W.S. from New Zealand. *Trends Ecol. Evol.* 17: 514–520.

Zachos, J. C., Brez, J. & Wise, S. 1992. Early Oligocene ice-sheet expansion on Antarctica: stable isotope and sedimentological evidence from Kerguelen Plateau, southern Indian Ocean. *Geology* 20: 569–573.

Zhang, L.-B., Comes, H. P. & Kadereit, J. W. 2001. Phylogeny and quaternary history of the European montane/alpine endemic *Soldanella* (Primulaceae) based on ITS and AFLP variation. *Amer. J. Bot.* 88: 2331–2345.

Zhang, X., Marchant, A., Wilson, K. L. & Bruhl, J. J. 2004. Phylogenetic relationships of *Carpha* and its relatives (Schoeneae, Cyperaceae) inferred from chloroplast *trnL* intron and *trnL-trn*F intergenic spacer sequences. *Molec. Phylog. Evol.* 31: 647–657.

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

Costularia laxa Cherm., Madagascar, *Gautier & Chatelain 2706* (K), DQ450465 (1), DQ456955 (2); *Oreobolus acutifolius* S.T. Blake, Tasmania, *Ockenden 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. ecuadorensis* T. Koyama, Colombia, *Sturn & Abouchar 103* (COL), DQ450469 (1), DQ456959 (2); *O. furcatus* M. Hann, Hawaii, *Carlquist 21111* (K), DQ450470 (1), DQ456960 (2); *O. goeppingeri* Suess., Colombia, *García 093* (COL), DQ450471 (1), DQ456961 (2); *O. obtusangulus* Gaudich, Argentina, *Moore 2817* (K), DQ450472 (1), DQ456962 (2); *O. oligocephalus* W.M. Curtis, Tasmania, *Bruhl 1889A* (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. pumilio* R. Br., Tasmania, *Bruhl 1879C* (NE), DQ450476 (1), DQ456966 (2); *O.* sp. 1[1], Ecuador, *Lægaard 70382* (COL), DQ450477 (1), DQ456967 (2); *O. strictus* Berggr., New Zealand, *McMillan 143395* (K), *, DQ450478 (1), DQ456968 (2), *O. venezuelensis* Steyerm., Colombia, *Jiménez 02* (COL), DQ450479 (1), DQ456969 (2).

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