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Further species diversity in Neotropical *Oropogon* (Lecanoromycetes: *Parmeliaceae*) in Central America

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Abstract: The new species *Oropogon evernicus* Essl. & S. Leavitt and *O. protocetraricus* S. Leavitt & Essl. are described from montane regions of Central America, further increasing the diversity of this genus in the New World. *Oropogon evernicus* is separated from *O. americanus* by the presence of medullary tissue directly beneath the pseudocyphellae, while *O. protocetraricus* is separated from *O. caespitosus* by the presence of protocetraric acid. The segregation of both species is confirmed by molecular sequence data (nuclear ITS, nuLSU, and β-tubulin). Both species appear to have split from their most recent common ancestor during the Miocene, supporting Miocene-dominated diversification of neotropical *Oropogon* species found in Central America.

Key words: divergence times, integrative taxonomy, Lecanoromycetes, lichens, Mexico, molecular phylogeny

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Introduction

Species within the genus *Oropogon* (Fée) Th. Fr. 1861 (*Parmeliaceae*) produce white-grey to brown-black fruticose thalli, which range from caespitose to pendent, and frequently branch isodichotomously (Esslinger 1989). *Oropogon* species occur on trees, shrubs, soil and stone from the mid to high elevations of Central and South America (Esslinger 1989; Sipman 1989, 1992, 1995, 2002), and East and South Asia (Esslinger 1989; Chen 1996; Harada & Wang 2008; Singh & Sinha 2010). Species diversity within *Oropogon* has received relatively little attention since the genus was first proposed. However, the number of species was dramatically and somewhat controversially increased with the recognition of 30 *Oropogon* species in the New World, where previously only three had been known (Esslinger 1989). At present, eight species are known from Asia, with only two species, *O. barbaticus* Essl. and *O. formosanus* Asahina, appearing to occur in both Asia and the New World (Esslinger 1989).

Oropogon is most diverse in northern South America where *c*. 90% of the currently known species occur, including several apparent endemics (Esslinger 1989; Sipman 1992, 2002). Consequently, montane regions of the Neotropics appear to be centres of diversification for *Oropogon* (Sipman 1992, 1995). Warming and cooling periods during the Quaternary have been suggested as a possible catalyst for speciation in *Oropogon* in the páramos of northern South America (Esslinger 1989). However, molecular dating analyses have suggested the Neogene as an important period for the diversification of extant *Oropogon* lineages, and the Pleistocene as important for the generation of intraspecific variation in extant species (Leavitt *et al*. 2012*a*).

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The cortex of *Oropogon* species is similar to that produced by *Bryoria*, and is composed of periclinally arranged hyphae forming a layer of prosoplectenchymatous tissue (Hawksworth 1969; Brodo & Hawksworth 1977; Esslinger 1989). Medullary tissue consists of loosely arranged, interwoven hyphae similar to that of other alectorioid genera (Brodo & Hawksworth 1977; Esslinger 1989). However, the form of the medulla, ranging from mostly hollow to filled, has proved useful for discriminating among *Oropogon* species (Esslinger 1989). In addition, the presence or absence, and type of pseudocyphellae (shape and perforation) play an important role in distinguishing species (Esslinger 1989).

Approximately half of the described *Oropogon* species regularly produce lateral, thalline apothecia (Esslinger 1989, 2002). These apothecia contain large, broadly clavate, thick-walled asci, consistent with the *Alectoria*-form of *Lecanora*-type of ascus (Esslinger 1989, 2002; Thell *et al.* 1995). *Oropogon* species typically produce a single large, broadly ellipsoid, brown muriform spore per ascus (Esslinger 1989). Spore size is quite variable within the genus; however, intraspecific variation renders this character of little use for segregating species from one another (Esslinger 1989). Additionally, pycnidia have been found in a few *Oropogon* species, but do not appear useful for distinguishing among *Oropogon* species (Esslinger 1989; Harada & Wang 2008). The production of asexual propagules appears rare in *Oropogon*, with only about two species regularly forming soredia (*O. aliphaticus*) or spinules (*O. loxensis*); consequently the presence or absence of varying asexually-derived reproductive structures is not widely used for taxonomic purposes in *Oropogon* (Esslinger 1989).

Oropogon is a chemically diverse genus, producing a greater number of substances than other alectorioid genera (Culberson & Culberson 1978; Esslinger 1980, 1989). This chemical diversity, coupled with high intraspecific variation in numerous morphological characters, has resulted in secondary chemistry playing an important role in the delimitation of species (Esslinger 1989). Most extrolites are restricted to medullary tissue, though some cortical substances are also known (Esslinger 1989). In addition, all *Oropogon* species examined are known to produce the cell wall polysaccharide lichenan (*Cetraria*-type), while only one species also synthesized isolichenan (*O. caespitosus*); these findings are consistent with other *Parmeliaceae* genera where the production of lichenan is typically conserved at the genus level, while the synthesis of isolichenan often appears variable within genera (Common 1991).

Based on collections made largely from the Oaxacan Highlands in southern Mexico, Leavitt *et al.* (2012*a*) demonstrated that species boundaries developed by Esslinger (1989) for *Oropogon*, based on morphological, anatomical and chemical data, are largely supported by molecular sequence data, with the exception of *O. caespitosus* Essl. *Oropogon caespitosus* isolates could be placed into three putative species-level lineages, two of which ('*O. caespitosus* A' and '*O. caespitosus* B'), were closely related to one another and to *O. mexicanus* Essl. However, samples from the third lineage, '*O. caespitosus* C', were distantly related to other *O. caespitosus* samples, forming a separate clade with *O. loxensis*, *O. atranorinus* and *O. bicolor* (Leavitt *et al.* 2012*a*). The separation of '*O. caespitosus* C' as a distinct species was corroborated by the subsequent discovery of differences in their production of extrolites, '*O. caespitosus* C' having protocetraric acid as the major extrolite, in contrast to fumaroprotocetraric acid found in *O. caespitosus* s. str. (Esslinger 1989; Leavitt *et al.* 2012*a*).

Esslinger (1989) had previously noted that *O. americanus* Essl. specimens from the northern part of its range differed in their type of pseudocyphellae from specimens in the southern part of its distribution. In contrast to the pseudocyphellae that are open to a hollow thallus centre in *O. americanus* s. str., *Oropogon* aff. *americanus* from Mexico and Guatemala produce pseudocyphellae that perforate the cortex but are closed by a thin medullary layer which is continuous beneath them (Fig. 1; Esslinger 1989). Recently, *Oropogon* aff. *americanus* specimens from the Oaxacan Highlands were included in a phylogenetic study of *Oropogon* diversity in Mexico (Leavitt *et al.*

2012*a*). However, due to a lack of fresh specimens of southern *O. americanus* s. str. (with pseudocyphellae that open to a hollow thallus centre), the relationship of the northern and southern elements has not yet been assessed within a molecular phylogenetic framework.

In the present study, we assessed the morphology and chemistry of *O. caespitosus* s. lat. collections from southern Mexico, together with representatives of *O. americanus* s. str. and *O.* aff. *americanus*, and inferred phylogenetic relationships among these groups using two nuclear ribosomal markers (ITS and partial nuLSU) and a fragment of the protein-coding β-tubulin gene. Here, we formally describe *Oropogon protocetraricus* as a new species, which is distinguished from *O. caespitosus* by its secondary chemistry as well as molecular data. Furthermore, molecular data confirm that *Oropogon* aff. *americanus* from Mexico and Guatemala, with pseudocyphellae only opening to the medullary layer, is a species distinct from *O. americanus* s. str., and it is here described as *O. evernicus.*

Materials and Methods

A total of 73 *Oropogon* specimens were included in the molecular dataset (Table 1), including all *Oropogon* specimens from Leavitt *et al.* (2012*a*). In the present study, we generated sequence data from additional *O. caespitosus* s. lat. specimens collected from the Oaxacan Highlands and collections made from Cerro de la Muerte, Talamanca Range, Costa Rica. The specimens collected from Cerro de la Muerte included a single collection of *O. americanus* s. str. Overall, our sampling included the following species: *O. americanus* s. lat., *O. atranorinus*, *O. bicolor*, *O. caespitosus* s. lat., *O. colibor*, *O. granulosus*, *O. lopezii*, *O. lorobic*, *O. loxensis*, *O. mexicanus*, and *O. sperlingii*. The position of *Oropogon* within *Parmeliaceae* remains unresolved, and we selected *Alectoria ochroleuca*, *A. sarmentosa*, *Brodoa intestiniformis*, *Bryoria fremontii*, *Flavocetraria cucullata*, *Letharia columbiana*, *Masonhalea richardsonii*, *Parmelia barrenoae*, *Platismatia glauca*, *Pseudephebe pubescens*, *Usnea florida*, and *U. longissima* as outgroups (Blanco *et al.* 2004, 2006; Crespo *et al.* 2007, 2010). For some outgroup taxa, sequences were combined from different individuals representing the same species using available sequences from GenBank, in order to maximize the number of loci represented for each taxon (see Table 1). Although some GenBank accessions represent misidentified taxa, we assumed that sequences from distinct individuals in chimeric operational taxonomic units (OTUs) were at least correctly identified to genus.

Laboratory methods

All *Oropogon* specimens were studied using standard techniques of light microscopy and extrolites were identified by thin-layer chromatography (TLC; Orange *et al.* 2001; Lumbsch 2002). DNA extraction, polymerase chain reactions (PCR), and sequencing reactions followed Leavitt *et al.* (2012*a*). In short, total genomic DNA was extracted from a small section of thallus material using the Prepease DNA Isolation Kit (USB, Cleveland, Ohio, USA), and the plant leaf extraction protocol. The ITS and nuLSU markers were amplified from new specimens via PCR using the primers ITS1F (Gardes & Bruns 1993) with either ITS4 (White *et al.* 1990) or ITS4a (Kroken & Taylor 2001), and LROR (Cubeta *et al.* 1991) with LR3 (Vilgalys & Hester 1990), respectively. PCR products were quantified on 1% agarose gel and stained with ethidium bromide. Amplification products were cleaned using ExoSAP-IT (USB, Cleveland, Ohio, USA), following the manufacturer's instructions. Complementary strands were sequenced from cleaned PCR products using BigDye v3.1 (Applied Biosystems, Foster City, CA, USA). Products were run on an ABI 3730 automated sequencer according to recommended protocols (Applied Biosystems) at the Pritzker Laboratory for Molecular Systematics and Evolution at The Field Museum (Chicago, IL, USA).

Sequence alignment

We assembled and edited sequences using the program Sequencher version 4.10.1 (Gene Codes Corporation, Ann Arbor, MI). Sequence identity was confirmed using the 'megaBLAST' search function in GenBank (Wheeler *et al.* 2006).

Although aligning the β-tubulin sequences was straightforward, both nuclear ribosomal markers (ITS and LSU) contained a number of difficult-to-align regions (see Leavitt *et al.* 2012*a*). Regions that are difficult to align may carry substantial phylogenetic signal, and excluding gaps and variable regions has been shown to be detrimental in some cases (Lee 2001; Liu *et al.* 2009; Dessimoz & Gil 2010; Lücking *et al.* 2011), including a previous study of *Oropogon* (Leavitt *et al.* 2012*a*). In order to include variable regions in the ITS and LSU markers, we used the program SATé version 2.2.5 (Liu *et al.* 2009). SATé has been shown to improve alignment accuracy, compared to other currently available programs, making possible the use of otherwise difficult to align regions (Liu *et al.* 2009, 2012). We aligned the ITS and LSU sequences in SATé using the following options: 'Aligner' = MAFFT, 'Merger' = MUSCLE, 'Tree Estimator' = RAxML, and 'RAxML Model' = GTRGAMMA. Each alignment was run for 24 h (>10 000 iterations) under the remaining default SATé settings.

Phylogenetic analyses

In order to assess the relationships of the new specimens included in this study, we estimated phylogenetic relationships from the concatenated three-gene dataset

Table 1. *Voucher information for material and NCBI GenBank accession numbers for all sequences included in the present study.*

Taxon	DNA ID #	Collection locality	Voucher	GenBank Accession Numbers		
				ITS	LSU	β-tubulin
Oropogon taxa						
O. americanus	6713	Costa Rica, Talamanca Range	*Nelsen* 4186G (F)	KC667030	KC667049	–
O. atranorinus	4036	Mexico, Estado de Oaxaca	*Esslinger* 18213A (F)	JQ813794	–	JQ813874
O. atranorinus	4398	Mexico, Estado de Oaxaca	*Esslinger* 18042 (F)	JQ813795	–	–
O. atranorinus	4399	Mexico, Estado de Oaxaca	*Esslinger* 17983 (F)	JQ813796	–	–
O. atranorinus	4401	Mexico, Estado de Oaxaca	*Esslinger* 18032 (F)	JQ813797	–	–
O. bicolor	4040	Mexico, Estado de Oaxaca	*Esslinger* 18255 (F)	JQ813798	–	–
O. bicolor	4042	Mexico, Estado de Oaxaca	*Esslinger* 17988 (F)	JQ813799	JQ813843	JQ813875
O. bicolor	4043	Mexico, Estado de Oaxaca	*Esslinger* 18532D (F)	JQ813800	JQ813844	JQ813876
O. bicolor	4402	Mexico, Estado de Oaxaca	*Esslinger* 18255 (F)	JQ813801	–	–
O. bicolor	4403	Mexico, Estado de Oaxaca	*Esslinger* 18384 (F)	JQ813802	–	–
O. '*caespitosus* A'	4046	Mexico, Estado de Oaxaca	*Esslinger* 18209 (F)	JQ813803	–	–
O. '*caespitosus* A'	4047	Mexico, Estado de Oaxaca	*Esslinger* 18364 (F)	JQ813804	JQ813845	JQ813877
O. '*caespitosus* A'	4405	Mexico, Estado de Oaxaca	*Esslinger* 18364 (F)	JQ813805	JQ813846	JQ813878
O. '*caespitosus* A'	6054	Mexico, Estado de Oaxaca	*Esslinger* 18590-d (F)	JQ813806	–	–
O. '*caespitosus* A'	6064	Mexico, Estado de Oaxaca	*Esslinger* 18590-n (F)	JQ813807	–	–
O. '*caespitosus* A'	6068	Mexico, Estado de Oaxaca	*Esslinger* 18590-r (F)	JQ813808	–	–
O. '*caespitosus* A'	6394	Mexico, Estado de Oaxaca	*Esslinger* 18366 (TLE)	KC667031	–	–
O. '*caespitosus* A'	6395	Mexico, Estado de Oaxaca	*Esslinger* 18368 (TLE)	KC667032	–	–
O. '*caespitosus* A'	6396	Mexico, Estado de Oaxaca	*Esslinger* 18355 (TLE)	KC667033	–	–
O. '*caespitosus* A'	6401	Mexico, Estado de Oaxaca	*Esslinger* 18230A(TLE)	KC667034	–	–
O. '*caespitosus* B'	4048	Mexico, Estado de Oaxaca	*Esslinger* 18387A (F)	JQ813809	JQ813847	–
O. '*caespitosus* B'	6071	Mexico, Estado de Oaxaca	*Esslinger* 18387A (F)	JQ813810	–	–
O. colibor	EF105414	Peru, Ancash	*Lumbsch et al.* 19326a (F)	EF105414	–	–
O. cf. *colibor*	6398	Mexico, Estado de Oaxaca	*Esslinger* 18332A (TLE)	KC667036	–	–
O. cf. *colibor*	6403	Mexico, Veracruz	*Esslinger* 17829 (TLE)	KC667035	–	–
O. cf. *colibor*	6704	Costa Rica, Talamanca Range	*Nelsen* 4186A (F)	KC667037	KC667050	–
O. colibor	6714	Costa Rica, Talamanca Range	*Nelsen* 4186B (F)	KC667041	–	–
O. colibor	6715	Costa Rica, Talamanca Range	*Nelsen* 4186C (F)	KC667042	KC667051	–
O. colibor	6716	Costa Rica, Talamanca Range	*Nelsen* 4186D (F)	KC667043	–	–
O. colibor	6711	Costa Rica, Talamanca Range	*Nelsen* 4186E (F)	KC667039	–	–
O. colibor	6712	Costa Rica, Talamanca Range	*Nelsen* 4186F (F)	KC667040	–	–
O. colibor	6706	Costa Rica, Talamanca Range	*Nelsen* 4197B (F)	KC667038	–	–
O. evernicus	4031	Mexico, Estado de Oaxaca	*Esslinger* 18286 (F)	–	–	JQ813871
O. evernicus	4032	Mexico, Estado de Oaxaca	*Esslinger* 18591 (F)	JQ813791	JQ813840	JQ813872
O. evernicus	4033	Mexico, Estado de Oaxaca	*Esslinger* 18357 (F)	JQ813792	JQ813841	–
O. evernicus	4034	Mexico, Estado de Oaxaca	*Esslinger* 18233 (F)	JQ813793	JQ813842	JQ813873
O. granulosus	4049	Mexico, Estado de Oaxaca	*Esslinger* 18243 (F)	JQ813815	JQ813849	JQ813880
O. granulosus	4050	Mexico, Estado de Oaxaca	*Esslinger* 18357A (F)	JQ813816	JQ813850	JQ813881
O. granulosus	4051	Mexico, Estado de Oaxaca	*Esslinger* 18374A (F)	JQ813817	JQ813851	JQ813882
O. granulosus	4052	Mexico, Estado de Oaxaca	*Esslinger* 18592 (F)	JQ813818	JQ813852	JQ813883
O. lopezii	4039	Mexico, Estado de Oaxaca	*Esslinger* 18376A (F)	JQ813819	–	–
O. lopezii	4056	Mexico, Estado de Oaxaca	*Esslinger* 18487 (F)	JQ813820	JQ813853	JQ813884
O. lopezii	4411	Mexico, Estado de Oaxaca	*Esslinger* 18487 (F)	JQ813821	JQ813854	–
O. lorobic	4057	Mexico, Estado de Oaxaca	*Esslinger* 18377 (F)	JQ813822	JQ813855	–
O. lorobic	4058	Mexico, Estado de Oaxaca	*Esslinger* 18383 (F)	JQ813823	–	–
O. lorobic	4413	Mexico, Estado de Oaxaca	*Esslinger* 18377 (F)	JQ813824	JQ813856	JQ813885

Table 1. *Continued*

Taxon	DNA ID #	Collection locality	Voucher	GenBank Accession Numbers		
				ITS	LSU	β-tubulin
O. lorobic	4414	Mexico, Estado de Oaxaca	*Esslinger* 18383 (F)	JQ813825	JQ813857	JQ813886
O. loxensis	4059	Mexico, Estado de Oaxaca	*Esslinger* 17894 (F)	JQ813826	JQ813858	JQ813887
O. loxensis	4060	Mexico, Estado de Oaxaca	*Esslinger* 18344 (F)	JQ813827	JQ813859	JQ813888
O. loxensis	4061	Mexico, Estado de Oaxaca	*Esslinger* 18382 (F)	JQ813828	JQ813860	JQ813889
O. loxensis	4062	Mexico, Estado de Oaxaca	*Esslinger* 18469 (F)	JQ813829	JQ813861	JQ813890
O. loxensis	4064	Mexico, Estado de Oaxaca	*Esslinger* 18592A (F)	JQ813830	JQ813862	JQ813891
O. loxensis	6705	Costa Rica, Talamanca Range	*Nelsen* 4197A (F)	KC667044	–	–
O. loxensis	6707	Costa Rica, Talamanca Range	*Nelsen* 4197C (F)	KC667045	–	–
O. loxensis	6709	Costa Rica, Talamanca Range	*Nelsen* 4204 (F)	KC667046	–	–
O. loxensis	6712a	Costa Rica, Talamanca Range	*Nelsen* 4186H (F)	KC667047	–	–
O. mexicanus	4065	Mexico, Estado de Oaxaca	*Esslinger* 17895 (F)	JQ813831	JQ813863	JQ813892
O. mexicanus	4066	Mexico, Estado de Oaxaca	*Esslinger* 18241 (F)	JQ813832	JQ813864	JQ813893
O. mexicanus	4067	Mexico, Estado de Oaxaca	*Esslinger* 18375 (F)	JQ813833	JQ813865	JQ813894
O. mexicanus	4068	Mexico, Estado de Oaxaca	*Esslinger* 18532 (F)	JQ813834	–	–
O. mexicanus	4419	Mexico, Estado de Oaxaca	*Esslinger* 18589 (F)	JQ813835	JQ813866	–
O. protocetraricus	4044	Mexico, Estado de Oaxaca	*Esslinger* 17898 (F)	JQ813811	JQ813848	JQ813879
O. protocetraricus	4420	Mexico, Estado de Oaxaca	*Esslinger* 18590 (F)	JQ813812	–	–
O. protocetraricus	6069	Mexico, Estado de Oaxaca	*Esslinger* 18590-s (F)	JQ813813	–	–
O. protocetraricus	6072	Mexico, Estado de Oaxaca	*Esslinger* 18387A (F)	JQ813814	–	–
O. 'sp. 1'	AY251435	Bolivia, locality unknown	*Elvebakk* 00-299 (TROM)	AY251435	–	–
O. 'sp. 1'	AY251451	Tibet, Sichuan Prominence	*Obermayer* 08657 (GZU)	AY251451	–	–
O. sperlingii	4069	Mexico, Estado de Oaxaca	*Esslinger* 17922 (F)	JQ813836	JQ813867	JQ813895
O. sperlingii	4070	Mexico, Estado de Oaxaca	*Esslinger* 18258A (F)	JQ813837	JQ813868	JQ813896
O. sperlingii	4071	Mexico, Estado de Oaxaca	*Esslinger* 18332 (F)	JQ813838	JQ813869	JQ813897
O. sperlingii	4072	Mexico, Estado de Oaxaca	*Esslinger* 18374B (F)	JQ813839	JQ813870	JQ813898
O. aff. *sperlingii*	6710	Costa Rica, Talamanca Range	*Nelsen* (F)	KC667048	KC667052	–
Outgroup taxa						
Alectoria ochroleuca	NA	ITS, BT = Austria	ITS, BT = *Feuerer & Thell* s. n. (HBG)	AF451735		AF457926
		LSU = Austria	LSU = *Wedin* Aug. 1998 (BM)		DQ899288	
A. sarmentosa	NA	Canada, British Columbia	*Articus* 7070 (UPS)	AJ748111	AJ748111	–
Brodoa intestiniformis	NA	ITS, BT = Austria, Tirolia	ITS, BT = *Feuerer & Thell* s. n. (HBG)	AY340873		AY340868
		LSU = Sweden: Härjedalen	LSU = *Wedin* 6329 (UPS)		DQ923653	
Bryoria fremontii	NA	Sweden: Västerbotten	*Wedin* 6349 (UPS)	DQ980004	DQ923656	–
Flavocetraria cucullata	NA	ITS, BT = Canada, North-west Territories	ITS, BT = FC10 (L)	FJ914765		FJ914813
		LSU = Austria, locality unknown	LSU = *Kärnefelt* 1996 (LD)		JN000263	
Letharia columbiana	NA	USA, locality unknown	*Moberg* 11301 (UPS)	DQ980013	DQ923664	–
Masonhalea richardsonii	NA	ITS, BT = Canada, Yukon Territories	ITS, BT = *Westberg* 1246 (LD)	AF254634		AF449730
		LSU = USA, Alaska	LSU = AFTOL-ID 1710		DQ973031	
Parmelia barrenoae	NA	Spain, La Barranca	*Crespo* MAF-Lich 6750 (MAF)	AY295103	–	AY295111
Platismatia glauca	NA	Sweden, Uppland	*Articus* 673 (UPS)	AF058035	AF058035	AF502271
Pseudephebe pubescens	NA	ITS = Antarctica	ITS = ANT050779 (HUR)	DQ534480		–
		LSU = Antarctica	LSU = ANT050954 (HUR)		EF489953	
Sulcaria sulcata	NA	ITS = China, locality unknown	ITS = 040113 (HUR)	DQ001294		–
		LSU = India, locality unknown	LSU = *Tibell* 22073 (UPS)		DQ923672	
Usnea florida	NA	Sweden: Östergötland	*Articus* 428 (UPS)	AJ457143	AJ457143	AF502262
U. longissima	NA	Canada, British Columbia	*Articus* 728 (UPS)	AJ748109	AJ748109	AJ748094

(ITS, nuLSU, and β-tubulin) (see justification in Leavitt *et al.* 2012*a*). We performed a maximum likelihood analysis of the concatenated three-gene dataset in RAxML v7.3.2 (Stamatakis 2006; Stamatakis *et al.* 2008). We used the GTRGAMMA model, which includes a parameter (Γ) for rate heterogeneity among sites, and chose not to include a parameter for estimating the proportion of invariable sites (Stamatakis 2006; Stamatakis *et al.* 2008). A search combining 200 separate maximum likelihood searches (to find the optimal tree) and 1000 'fast bootstrap' replicates (Stamatakis *et al.* 2008) to evaluate support for each node was conducted. Relationships were considered supported if they had ML bootstrap support (BS) values of ≥70%.

We also estimated relationships and divergence times from the concatenated data matrices using a Bayesian approach implemented in the program BEAST version 1.7.4 (Drummond & Rambaut 2007), following methods described in Leavitt *et al.* (2012*a*). In short, the concatenated data matrix was analyzed using a relaxed clock model (uncorrelated lognormal), with a birth-death model prior for the node heights and unlinked substitutions models across the loci. Models of DNA sequence evolution for each marker were selected with the program jModeltest v0.1 (Posada 2008), using the Akaike Information Criterion (AIC). To estimate the time to the most recent common ancestor (MRCA) for all clades, we used the LSU rate of $0{\cdot}70 \times 10^{-9}$ s/s/y estimated for *Parmeliaceae* (*Protoparmelia* excluded; Amo de Paz *et al.* 2011), and for the ITS, we used the rate estimated for the parmelioid genus *Melanohalea* ($3{\cdot}30 \times 10^{-9}$ s/s/y; Leavitt *et al.* 2012*b*). Branch rates were drawn from a lognormal distribution. Two independent analyses were run for 50 million generations and parameter values were sampled every 1000 generations. The output from each analysis was visualized using Tracer version 1.5 (Rambaut & Drummond 2003) to assess convergence and effective sampling size (ESS), and we also compared summarized tree topologies from separate runs. Based on these results, the first 12·5 million generations from each run were discarded as burn-in, and remaining samples were summarized as a maximum clade credibility tree with mean divergence times and highest posterior density intervals (HPD) of age estimates using the program TreeAnnotator version 1.7.4 (Rambaut & Drummond 2009).

Results and Discussion

The complete three-locus data matrix included 85 samples (including outgroups), consisting of 1740 aligned nucleotide position characters (ITS: 549; nuLSU: 537; β-tubulin: 654; TreeBase ID: 13959). All new sequences generated for this study have been deposited in GenBank under accession nos. KC667030–KC667052 (Table 1).

The partitioned ML analysis and Bayesian analysis yielded identical topologies and we present the Bayesian phylogeny in Fig. 1 (the ML topology is shown in Appendix, Fig. A1). Well-supported relationships among *Oropogon* lineages were similar to those presented in Leavitt *et al.* (2012*a*), with well-supported monophyletic clades generally corresponding to traditionally circumscribed species. All *O. caespitosus* s. lat. specimens containing protocetraric acid as the major extrolite were recovered in a well-supported monophyletic clade (labelled as '*O. protocetraricus*') (Fig. 1). The single specimen representing *O. americanus* s. str. was recovered with strong support as sister to *Oropogon* aff. *americanus* from the Oaxacan Highlands, Mexico, with pseudocyphellae only open to the medullary layer (labelled here as '*O. evernicus*') (Fig. 1).

The new species *Oropogon protocetraricus* was recovered in a well-supported clade along with *O. atranorinus*, *O. bicolor*, *O. lopezii*, and *O. loxesnsis* (Fig. 1). Species within this clade are all known to produce varying levels of protocetraric acid, although protocetraric acid is not consistently found in *O. bicolor*. In contrast, protocetraric acid is not present in the clade containing *O. caespitosus*, including lineages identified previously as '*O. caespitosus* A' and '*O. caespitosus* B', *O. lorobic*, and *O. mexicanus* (Fig. 1). Apart from differences in the production of extrolites, no morphological characters were observed corroborating the separation of *O. caespitosus* and *O. protocetraricus*. Within the *O. caespitosus*/*O. mexicanus* clade, *O. caespitosus* was not recovered as monophyletic (Fig. 2). The two species-level lineages representing *O. caespitosus* have been previously recognized as '*O. caespitosus* A' and '*O. caespitosus* B' (Leavitt *et al.* 2012*a*). However, diagnostic morphological/chemical characters have not been observed corroborating the distinction of these two lineages as separate species. Improved molecular sampling and additional morphological investigations will be required to confirm the independence of these lineages and relationships within the *O. caespitosus*/*O. mexicanus* clade.

Based on these data, the initial radiation of *Oropogon* is estimated to have occurred *c.*

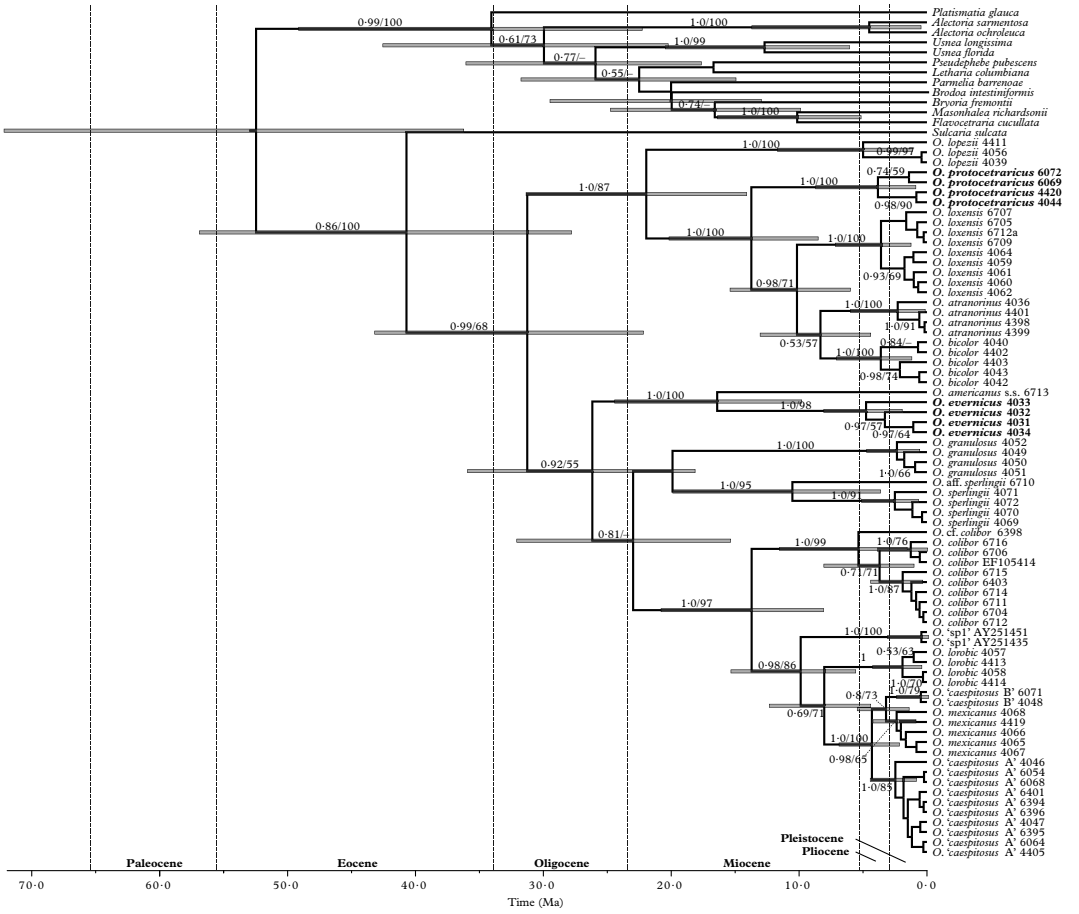

FIG. 1. *Oropogon* chronogram estimated from a partitioned dataset consisting of three loci (ITS, nuLSU and β-tubulin) under a relaxed molecular clock. The divergence times correspond to the mean posterior estimate of their age (in millions of years). The bars indicate the 95% highest posterior density interval for the estimated divergence times. Values on branches indicate posterior probabilities from a Bayesian analysis using the program BEAST and bootstrap values from a ML analysis using the program RAxML.

31·4 Ma (HPD = 22·3–43·3), as illustrated in Fig. 1. The split between *O. americanus* and '*O. evernicus*' is estimated to have occurred *c.* 16·5 Ma (HPD = 9·9–24·5), and the separation of '*O. protocetraricus*' and its sister clade *c.* 13·9 Ma (HPD = 8·6–20·3) (Fig. 1). Our estimates of the timing of diversification events in *Oropogon* support a Miocene-dominated diversification scenario in neotropical *Oropogon* species in Central America (Leavitt *et al.* 2012*a*).

Taxonomy. The results of this study (and Leavitt *et al.* 2012*a*) necessitate the formal description of these two species, which is made below.

Oropogon evernicus Essl. & S. Leavitt sp. nov.

MycoBank No.: MB 803457

Morphologically similar to *Oropogon americanus* Essl. but differs by having pseudocyphellae that open only to the medulla and not all the way to the hollow thallus centre.

Type: Mexico, Estado de Oaxaca: *c.* 65 km (by air) east of Oaxaca City, off Hwy 179 along secondary road to Mixistlan, 17° 09·473′N, 96° 04·138′W, *c.* 2640 m elev., remnant oak–madrone cloud forest on mountain

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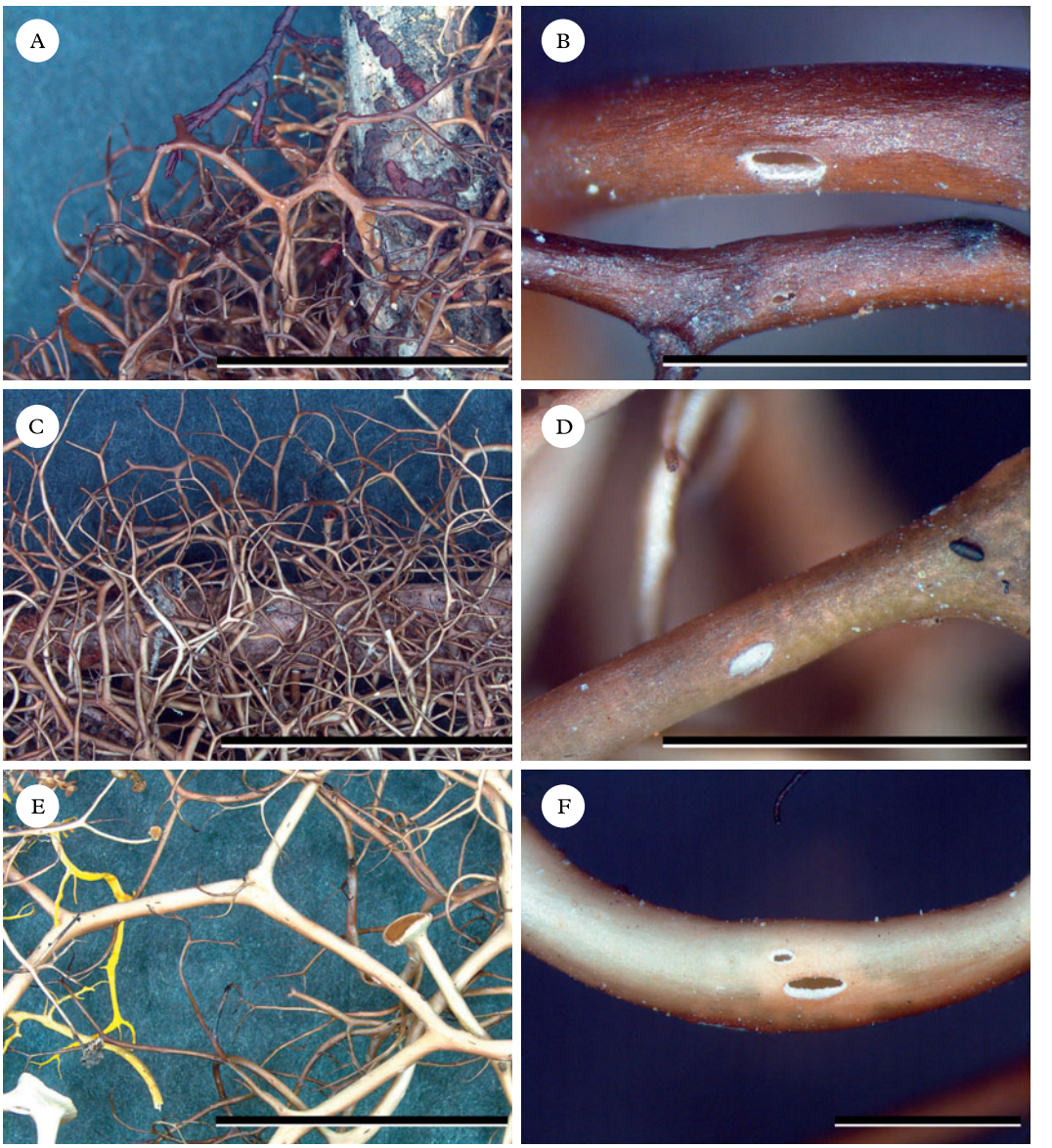

FIG. 2. *Oropogon* species, habit and close-ups of pseudocyphellae. A & B, *Oropogon americanus* (*Arvidsson et al.* 5898, TLE); C & D, *O. evernicus* (*Esslinger* 18591 – holotype, F); E & F, *O. protocetraricus* (*Esslinger* 18590 – holotype, F). Scales: A, C & E = 1 cm; B, D & F = 1 mm. In colour online.

ridge, with deforested strip on top of ridge, on bark, *Esslinger* 18591 (F—holotype; B, DUKE, MEXU, TLE—isotypes).

(Fig. 2)

Thallus caespitose, up to 10 or occasionally 12 cm long; branching mostly isodichotomous to rarely anisodichotomous, internodes mostly 2–8 (–12) mm long, main branches mostly up to 0·6 mm or rarely 0·8 mm in diam., pale tan to tan-brown or brown; *pseudocyphellae* frequent to often rather infrequent, rather narrow and sometimes nearly

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fissural, open only to the medulla layer. Thallus interior hollow, usually with a thin medulla, but occasionally narrower segments appearing loosely filled; *medulla* hyphal but soon becoming somewhat granular, off-white to pale yellow or dingy orange.

Apothecia frequent, up to 3 mm diam.; *disc* more or less flat to weakly concave; *margin* entire to weakly crenate; *hymenium* 148–157 μm thick, subhymenium 50–77 μm thick; spores muriform, 1 per ascus, 97–112 × 35–42 μm.

Pycnidia rare; *conidia* 4–5 × 1 μm, weakly and more or less unequally bifusiform.

Chemistry. Thallus reactions: cortex all spot tests negative; medulla PD−, K+ yellow-orange, C+ yellow-orange. Constituents: evernic acid (+++), usually with unknown yellow pigment.

Etymology. The epithet *evernicus* refers to the major extrolite, evernic acid.

Notes. In the monograph of New World *Oropogon* (Esslinger 1989), this taxon was included within the concept of species *O. americanus*, considered to be distributed from southern Mexico into South America and down the Andes to Peru. It was noted there, however, that the small number of specimens seen from Mexico and Guatemala differ from those further south in the range by having pseudocyphellae that do not open all the way to the hollow thallus centre, but only to the medulla. The additional material now available from Mexico has confirmed that these distinctions are consistent, and that the northern specimens should be recognized as a distinct species. This decision was further confirmed when we had the opportunity to sequence a specimen of *O. americanus* s. str. from the northern part of its range [Costa Rica, Cerro de la Muerte, *Nelsen* 4186G (F)].

Additional specimens examined. **Mexico:** no locality, *Muller* s. n. (BM, M). *Estado de Oaxaca*: along Hwy 175 between Tuxtepec and Oaxaca City, *c.* 25 km north of Ixtlán de Juárez, north of Cerro Machin, 17° 33·154′N, 96° 30·674′W, *c.* 2745 m elev., pine-oak forest, *Esslinger* 17912, 17923 (hb. Esslinger); mountain tops *c.* 6 km (by air) north/north-east of Ixtlán de Juárez, 17° 23·217′N, 96° 28·109′W, *c.* 2865 m elev., pine-oak-madrone forest, *Esslinger* 18208A, 18219, 18225B, 18233, 18235, 18241C, 18244 (hb. Esslinger); mountains *c.* 1·7 km (by air) north-east of Ixtlán de Juárez, 17° 21·424′N, 96° 28·236′W, *c.* 2660 m elev., pine-oak-madrone forest, *Esslinger* 18286 (hb. Esslinger); *c.* 7·7 km (by air) north of Ixtlán de Juárez, off Hwy 175 *c.* 1·5 km along secondary road to San Pedro Yaneri, 17° 24·416′N, 96° 30·037′W, *c.* 2890 m elev., pines and hardwoods in partly logged area with many downed branches, *Esslinger* 18350A, 18356, 18357 (hb. Esslinger); along Hwy 175 between Tuxtepec and Oaxaca City, *c.* 25 km north of Ixtlán de Juárez, north of Cerro Machin, 17° 33·154′N, 96° 30·674′W, *c.* 2745 m elev., *c.* 2840 m elev., pine-oak forest, *Esslinger* 18387 (hb. Esslinger); mountains N of the city of Oaxaca, La Cumbre, 3150 m, on madrone, *Beharrell* 855A (hb. Esslinger); 2900 m, on top of a felled oak, *Beharrell* 895 (ASU), 948 (hb. Esslinger), 1119 (US), 1182A (hb. Esslinger), 1246 (DUKE). *Estado de Chiapas*: Rancho El Pervenir, *c.* 3 km adelante de El Escalon., mpio. Huixtán, 16°42′39″N, 92° 30′26″W, *c.* 2400 m elev., epifita sobre tronco de Pinus *Wolf & Sipman* 1979 (B).—**Guatemala:** *San Marcos*: Volcan Tajumulco, between Las Canojas and top of ridge, 7 mi from San Sebastian, 3300–3900 m, 1940, *Steyermark* (US).

Oropogon protocetraricus S. Leavitt & Essl. sp. nov.

MycoBank No.: MB 803458

Morphologically similar to *Oropogon caespitosus* Essl. but differs by the presence of protocetraric acid rather than fumarprotocetraric acid.

Type: Mexico, Estado de Oaxaca, *c.* 65 km (by air) east of Oaxaca City, off Hwy 179 along secondary road to Mixistlan, 17° 09·473′N, 96° 04·138′W, *c.* 2640 m elev., remnant oak-madrone cloud forest on mountain ridge, with deforested strip on top of ridge, on bark, *Esslinger* 18590 (F—holotype; B, DUKE, MEXU, TLE—isotypes).

(Fig. 2)

Thallus caespitose, up to 9 or 10 cm long; branching mostly isodichotomous, internodes mostly 6–14 (–18) mm long, main branches up to 0·8 mm or rarely 1 mm in diam., pale tan to tan-brown or brown, only occasionally with blackened areas; *pseudocyphellae* usually frequent and conspicuous, open to the hollow thallus centre. Thallus interior hollow, with a thin medulla; *medulla* hyphal to somewhat granular, white to off-white.

Apothecia frequent, up to 3 or 4 mm diam.; *disc* more or less flat to weakly concave; *margin* entire to weakly crenate; *hymenium* 150–170 μm thick, subhymenium 51–80 μm thick; spores muriform, 1 per ascus, 114–128 × 38–48 μm.

Pycnidia not seen.

Chemistry. Thallus reactions: cortex all spot tests negative; medulla PD+ orange or red-orange, K− or often K+ yellow-orange, C−, KC+ fleeting pale rose or occasionally KC−. Constituents: protocetraric acid (+++), occasional trace unknowns.

Etymology. The epithet protocetraricus refers to the diagnostic extrolite, protocetraric acid, separating *O. caespitosus* from *O. protocetraricus*.

Notes. *Oropogon protocetraricus* is morphologically indistinguishable from *O. caespitosus*, and in fact was first recognized as a unique genetic entity during a molecular study of *O. caespitosus* and other common Mexican species of the genus (Leavitt *et al*. 2012*a*). Subsequent study by thin-layer chromatography showed that this new entity is chemically distinct, producing protocetraric acid as its major secondary compound, whereas the major compound produced by *O. caespitosus* s. str. is the related depsidone fumarprotocetraric acid, occasionally accompanied by trace amounts of protocetraric acid. The new species is known from four localities in Oaxaca State, southern Mexico, where it is sympatric with *O. caespitosus* and often grows with that species. In fact, the type specimen of *O. protocetraricus* was segregated from a large mixed collection of the two species.

Additional specimens examined. **Mexico:** *Estado de Oaxaca*: mountain tops *c*. 6 km (by air) north/north-east of Ixtlán de Juárez, 17° 23·217′N, 96° 28·109′W, *c*. 2865 m elev., pine-oak-madrone forest, *Esslinger* 18230A, 18241A, 18269 (hb. Esslinger); along Hwy 175 between Tuxtepec and Oaxaca City, *c*. 25 km north of Ixtlán de Juárez, north of Cerro Machin, 17° 33·154'N, 96° 30·674′W, *c*. 2745 m elev., pine-oak forest, *Esslinger* 17898 (hb. Esslinger); 17° 33·065′N, 96° 30·695′W, *c*. 2840 m elev., pine-oak forest, *Esslinger* 18387A/2 (hb. Esslinger).

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References

Amo de Paz, G., Cubas, P., Divakar, P. K., Lumbsch, H. T. & Crespo A. (2011) Origin and diversification of major clades in parmelioid lichens (*Parmeliaceae*, Ascomycota) during the Paleogene inferred by Bayesian analysis. *PLoS ONE* **6:** e28161.

Blanco, O., Crespo, A., Elix, J. A., Hawksworth, D. L. & Lumbsch, H. T. (2004) A molecular phylogeny and a new classification of parmelioid lichens containing *Xanthoparmelia*-type lichenan (Ascomycota: *Lecanorales*). *Taxon* **53:** 959–975.

Blanco, O., Crespo, A., Ree, R. H. & Lumbsch, H. T. (2006) Major clades of parmelioid lichens (*Parmeliaceae*, Ascomycota) and the evolution of their morphological and chemical diversity. *Molecular Phylogenetics and Evolution* **39:** 52–69.

Brodo, I. M. & Hawksworth, D. L. (1977) *Alectoria* and allied genera in North America. *Opera Botanica* **42:** 1–164.

Chen, J. (1996) The lichen genus *Oropogon* from China. *Acta Mycologica Sinica* **15:** 173–177.

Common, R. S. (1991) The distribution and taxonomic significance of lichenan and isolichenan in the *Parmeliaceae* (lichenized Ascomycotina), as determined by iodine reactions. I. Introduction and methods. II. The genus *Alectoria* and associated taxa. *Mycotaxon* **41:** 67–112.

Crespo, A., Lumbsch, H. T., Mattsson, J.-E., Blanco, O., Divakar, P. K., Articus, K., Wiklund, E., Bawingan, P. A. & Wedin, M. (2007) Testing morphology-based hypotheses of phylogenetic relationships in *Parmeliaceae* (Ascomycota) using three ribosomal markers and the nuclear RPB1 gene. *Molecular Phylogenetics and Evolution* **44:** 812–824.

Crespo, A., Kauff, F., Divakar, P. K., del-Prado, R., Pérez-Ortega, S., Amo de Paz, G., Ferencova, Z., Blanco, O., Roca-Valiente, B., Nunez-Zapata, J., *et al*. (2010) Phylogenetic generic classification of parmelioid lichens (*Parmeliaceae*, Ascomycota) based on molecular, morphological and chemical evidence. *Taxon* **59:** 1735–1753.

Cubeta, M. A., Echandi, E., Abernethy, T. & Vilgalys, R. (1991) Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. *Phytopathology* **81:** 1395–1400.

Culberson, C. F. & Culberson, W. L. (1978) Beta-orcinol derivatives in lichens: biogenetic evidence from *Oropogon loxensis*. *Experimental Mycology* **2:** 245–257.

Dessimoz, C. & Gil, M. (2010) Phylogenetic assessment of alignments reveals neglected tree signal in gaps. *Genome Biology* **11:** R37.

Drummond, A. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 214.

Esslinger, T. L. (1980) Typification of *Oropogon loxensis* and description of two related species. *Bryologist* **83:** 529–532.

Esslinger, T. L. (1989) Systematics of *Oropogon* (*Alectoriaceae*) in the New World. *Systematic Botany Monographs* **28:** 1–111.

Esslinger, T. L. (2002) *Oropogon*. In *Lichen Flora of the Greater Sonoran Desert Region. Vol. I* (T. H. Nash III, B. D. Ryan, C. Gries & F. Bungartz, eds): 302–304. Tempe, Arizona: Lichens Unlimited.

Gardes, M. & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology Notes* **2:** 113–118.

Harada, H. & Wang, L.-S. (2008) Taxonomic study on *Bryoria* (lichenized Ascomycota, *Parmeliaceae*) of east Asia (4). External morphology and anatomy of pycnidia. *Lichenology* **7:** 159–168.

Hawksworth, D. L. (1969) The scanning electron microscope. An aid to the study of cortical hyphal orientation in the lichen genera *Alectoria* and *Cornicularia*. *Journal de Microscopie* **8:** 753–760.

Kroken, S. & Taylor, J. W. (2001) A gene genealogical approach to recognize phylogenetic species boundaries in the lichenized fungus *Letharia*. *Mycologia* **93:** 38–53.

Leavitt, S. D., Esslinger, T. L. & Lumbsch, H. T. (2012*a*) Neogene-dominated diversification in neotropical montane lichens: dating divergence events in the lichen-forming fungal genus *Oropogon* (*Parmeliaceae*). *American Journal of Botany*. **99:** 1764–1777.

Leavitt, S. D., Esslinger, T. L., Divakar, P. K. & Lumbsch, H. T. (2012*b*) Miocene and Pliocene dominated diversification of the lichen-forming fungal genus *Melanohalea* (*Parmeliaceae*, Ascomycota) and Pleistocene population expansions. *BMC Evolutionary Biology* **12:** 176.

Lee, M. S. Y. (2001) Unalignable sequences and molecular evolution. *Trends in Ecology & Evolution* **16:** 681–685.

Liu, K., Raghavan, S., Nelesen, S., Linder, C. R. & Warnow, T. (2009) Rapid and accurate large-scale coestimation of sequence alignments and phylogenetic trees. *Science* **324:** 1561–1564.

Liu, K., Warnow, T. J., Holder, M. T., Nelesen, S. M., Yu, J., Stamatakis, A. P. & Linder, C. R. (2012) SATé-II: very fast and accurate simultaneous estimation of multiple sequence alignments and phylogenetic trees. *Systematic Biology* **61:** 90–106.

Lücking, R., Hodkinson, B., Stamatakis, A. & Cartwright, R. (2011) PICS-Ord: unlimited coding of ambiguous regions by pairwise identity and cost scores ordination. *BMC Bioinformatics* **12:** 10.

Lumbsch, H. T. (2002) Analysis of phenolic products in lichens for identification and taxonomy. In *Protocols in Lichenology: Culturing, Biochemistry, Ecophysiology and Use in Biomonitoring* (I. Kranner, R. P. Beckett & A. K. Varma, eds): 281–295. Berlin: Springer.

Orange, A., James, P. W. & White, F. J. (2001) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.

Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25:** 1253–1256.

Rambaut, A. & Drummond, A. J. (2003) *Tracer v1.5*. Available at: http://tree.bio.ed.ac.uk/software/tracer/

Rambaut, A. & Drummond, A. J. (2009) *TreeAnnotator v1.7.4*. Available at: http://beast.bio.ed.ac.uk/TreeAnnotator.

Singh, K. P. & Sinha, G. P. (2010) *Indian Lichens: an Annotated Checklist. Botanical Survey of India*. Kolkata: Ministry of Environment and Forests.

Sipman, H. J. M. (1989) Lichen zonation in the Parque Los Nevados transect. *Studies on Tropical Andean Ecosystems* **3:** 461–483.

Sipman, H. J. M. (1992) The origin of the lichen flora of the Columbian páramos. In *Páramo: an Andean Ecosystem Under Human Influence* (H. Balslev & H. L. Luteyn, eds): 95–109. London: Academic Press.

Sipman, H. J. M. (1995) Preliminary review of the lichen biodiversity of the Colombian montane forests. In *Biodiversity and Conservation of Neotropical Montane Forests* (S. P. Churchill, H. Balslev, E. Forero & J. L. Luteyn, eds): 313–320. Bronx: The New York Botanical Garden Press.

Sipman, H. J. M. (2002) The significance of the northern Andes for lichens. *The Botanical Review* **68:** 88–99.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57:** 758–771.

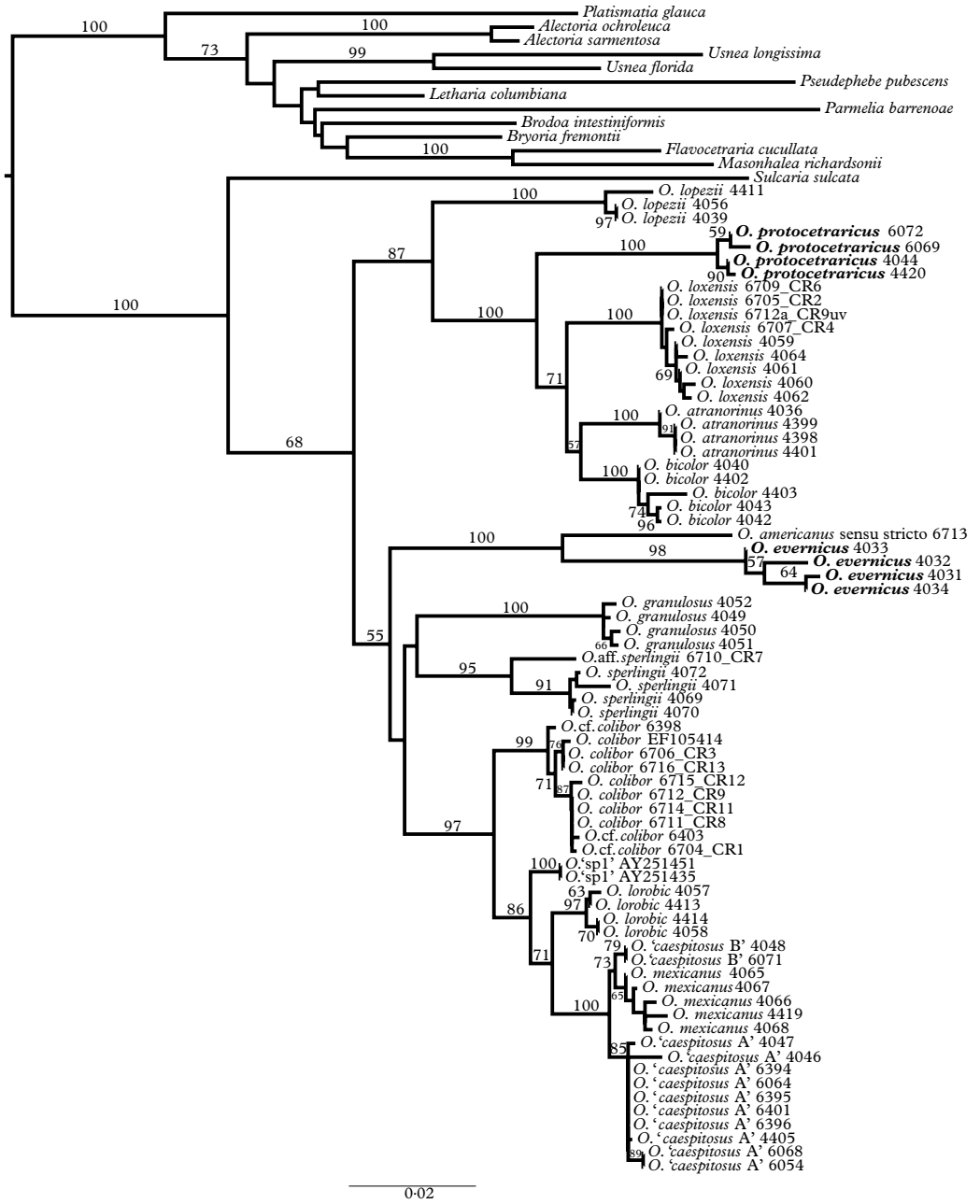
Thell, A., Mattsson, J.-E. & Kärnefelt, I. (1995) Lecanoralean ascus types in lichenized families *Alectoriaceae* and *Parmeliaceae*. *Cryptogamic Botany* **5:** 120–127.

Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172:** 4238–4246.

Wheeler, D. L., Barrett, T., Benson, D. A., Bryant, S. H., Canese, K., Chetvernin, V., Church, D. M., DiCuccio, M., Edgar, R., Federhen, S., *et al.* (2006) Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research* **35:** D5–D12.

White, T. J., Bruns, T. D., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (N. Innis, D. Gelfand, J. Sninsky & T. White, eds): 315–322. San Diego: Academic Press.

Appendix, Figure A1. Maximum likelihood phylogenetic analysis for sampled *Oropogon* estimated from a partitioned dataset consisting of three loci (ITS, nuLSU and β-tubulin) in RAxML. Values on branches indicate bootstrap values.



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