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Is *Drosera meristocaulis* a pygmy sundew? Evidence of a long-distance dispersal between Western Australia and northern South America

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• *Background and aims* South America and Oceania possess numerous floristic similarities, often confirmed by morphological and molecular data. The carnivorous *Drosera meristocaulis* (Droseraceae), endemic to the Neblina highlands of northern South America, was known to share morphological characters with the pygmy sundews of *Drosera* sect. *Bryastrum*, which are endemic to Australia and New Zealand. The inclusion of *D. meristocaulis* in a molecular phylogenetic analysis may clarify its systematic position and offer an opportunity to investigate character evolution in Droseraceae and phylogeographic patterns between South America and Oceania.
• *Methods Drosera meristocaulis* was included in a molecular phylogenetic analysis of Droseraceae, using nuclear internal transcribed spacer (ITS) and plastid *rbcL* and *rps16* sequence data. Pollen of *D. meristocaulis* was studied using light microscopy and scanning electron microscopy techniques, and the karyotype was inferred from root tip meristem.
• *Key Results* The phylogenetic inferences (maximum parsimony, maximum likelihood and Bayesian approaches) substantiate with high statistical support the inclusion of sect. *Meristocaulis* and its single species, *D. meristocaulis*, within the Australian *Drosera* clade, sister to a group comprising species of sect. *Bryastrum*. A chromosome number of $2n =$ approx. 32–36 supports the phylogenetic position within the Australian clade. The undivided styles, conspicuous large setuous stipules, a cryptocotylar (hypogaeous) germination pattern and pollen tetrads with aperture of intermediate type 7–8 are key morphological traits shared between *D. meristocaulis* and pygmy sundews of sect. *Bryastrum* from Australia and New Zealand.
• *Conclusions* The multidisciplinary approach adopted in this study (using morphological, palynological, cytotaxonomic and molecular phylogenetic data) enabled us to elucidate the relationships of the thus far unplaced taxon *D. meristocaulis*. Long-distance dispersal between southwestern Oceania and northern South America is the most likely scenario to explain the phylogeographic pattern revealed.

Key words: Droseraceae, *Drosera* sect. *Bryastrum*, America–Oceania disjunction, carnivorous plants, ITS, *rbcL*, *rps16*, phylogeny, pollen morphology, germination pattern, chromosome numbers.

INTRODUCTION

The carnivorous plants known as sundews of the genus *Drosera* (Droseraceae) comprise nearly 200 species spread worldwide, mostly in the Southern Hemisphere and especially in southwestern Australia (Diels, 1906; Schlauer, 2007; McPherson, 2010). Species of the most distinctive groups of *Drosera*, known as the pygmy sundews – because of their usually diminutive size – are all endemic to the southwestern tip of Western Australia, except for *D. pygmaea* which is also found in southeastern Australia and New Zealand (Lowrie, 1989).

The pygmy sundews make up sect. *Bryastrum* (following the sectional classification of Seine and Barthlott, 1994), consisting of approx. 50 species (Lowrie, 1989, 1998; Lowrie and Carlquist, 1992; Lowrie and Conran, 2007; Mann, 2007), and are characterized not only by their relatively diminutive size, but also by large translucent papery stipules which are arranged as a dense stipule bud in the centre of the rosette, three to five undivided styles, long fibrous roots and their unique capability to reproduce vegetatively by small leaf-derived propagules known as gemmae. The gemmae are modified leaves, which are chlorophyllous and rich in starch (Goebel, 1908; Karlsson and Pate, 1992). Recent molecular phylogenetic data (Rivadavia *et al.*, 2003) showed the pygmy sundews to be a well supported monophyletic group, which is part of a large clade containing mostly Australian species, and sister to a clade including mostly taxa native to the New World and southern Africa.

Botanical expeditions in the 1950s to the isolated highlands known as the Neblina massif on the Brazilian–Venezuelan border in the Amazonas lowlands of northern South America resulted in the description of numerous endemic species, including *Drosera meristocaulis* (Maguire and Wurdack, 1957) (Fig. 1). Because this species has only three undivided styles, a unique character among New World *Drosera* taxa, a monotypic sect. *Meristocaulis* was created for this taxon (Maguire and Wurdack, 1957; Seine and Barthlott, 1994),

FIG. 1. *Drosera meristocaulis* (A, C) from the Neblina massif in the Amazon and the Western Australian pygmy sundew *Drosera gibsonii* (B, D) show a remarkable similarity in overall habit and in flower morphology.

which was raised to subgeneric level by Schlauer (1996). Other conspicuous characters of this taxon include long stems up to 40 cm in length and nearly sessile flowers nested among the leaves and stipules (Fig. 1). Nonetheless, *D. meristocaulis* also presents characteristics reminiscent of pygmy sundews, such as diminutive leaves, large translucent papery stipules and long fibrous roots. The extreme isolation of the remote Neblina massif kept *D. meristocaulis* from being studied in more depth, thus heightening scientists' curiosity about the relationship of this species to other members of *Drosera*. Maguire and Wurdack (1957) were well aware of the similarities of *D. meristocaulis* to the pygmy *Drosera* of sect. *Bryastrum* from Australia (Fig. 1). Due to the undivided styles, however, they supposed a close relationship to the single South American member of sect. *Thelocalyx*, *D. sessilifolia*. Duno de Stefano (1995) studied the pollen morphology of *D. meristocaulis* for the first time and proposed a close relationship of sect. *Meristocaulis* with sect. *Drosera*.

The Neblina massif is a huge sandstone formation reaching nearly 3000 m above sea level and is covered in part by low vegetation ('Neblinaria scrub'; Brewer-Carías, 1988; Huber, 1995) composed of species not found in the hot surrounding lowlands. Several expeditions to Neblina and other mountains of the Guayana Highlands (known as *tepuis*) documented an

impressive number of endemic taxa and contributed to the idea of a diverse and unique flora with a high degree of endemism (Steyermark, 1979). In an attempt to explain this unique flora, the idea of 'Lost Worlds' was created, postulating that the origin of local biota was relictual as a result of a long history of evolution in isolation on the mountain summits (Rull, 2004). On the other hand, the 'Vertical Displacement' hypothesis assumes the lack of total geographical isolation among *tepui* summits, with extensive valleys and gentle slopes possibly being important paths connecting highlands with lowlands, thus providing hypothetical migrational pathways (Huber, 1988; Rull, 2004).

Long-distance dispersal (LDD) was accepted and rejected many times as a good theory to explain floristic similarities among continents since Darwin's experiments (1859). Besides the fact that LDD was accepted as a natural process that occurred on recent volcanic islands (Carlquist, 1966, 2010), the plate tectonics theory provided vicariance explanations for many cases of disjunctions (de Queiroz, 2005). Molecular clock techniques have revealed that many plant lineages have a recent origin, with radiation events occurring after continental splits (Givnish and Renner, 2004; Muñoz *et al.*, 2004; Sytsma *et al.*, 2004; Dick *et al.*, 2007). Now many dispersion routes are corroborated by multiple taxa in the Southern Hemisphere (de Queiroz, 2005), and LDD can explain the disjunction patterns of many groups.

In the present study, a multidisciplinary investigation was carried out in order to clarify the phylogenetic position of *D. meristocaulis* in Droseraceae and to test the hypothesis of a putative common ancestry with species from sect. *Bryastrum*. The pattern of seed germination, pollen morphology, chromosome counts and a molecular approach based on nuclear and plastid DNA sequences were investigated.

MATERIALS AND METHODS

Seed germination

Seeds of *Drosera meristocaulis* and *D. capillaris* were obtained from a commercial carnivorous plant seed source (A. Lowrie, Duncraig, Australia) and were sown on pure peat and on milled long fibre sphagnum in a greenhouse, and kept moist at 20–25 °C.

Chromosome counts

Root tips of greenhouse-grown seedlings were used for karyotype analysis. In addition, *in vitro* raised plants of *D. meristocaulis* were obtained from a commercial nursery (bestcarnivorousplants.com). For mitotic chromosome counts, root tips of *in vitro* and *ex vitro* plants were collected and pretreated with 0·002 M 8-hydroxyquinoline for 3 h to achieve mitotic arrest, and then fixed in ethanol:acetic acid (3:1) and stored at 4 °C. Fixed root tips were hydrolysed in 2 M hydrochloric acid at 60 °C for 10 min, and then enzymatically macerated with 5 % cellulase (Roth, Germany) at 37 °C for 20 min. Root tips were rinsed with distilled water, squashed on glass slides and the prepared root tip meristems were orcein stained (Orcein: Roth, Germany). Chromosome counts were made using a light microscope (Leitz, Germany), and slides were documented photographically using a digital camera (Nikon D5000, Germany).

Pollen analysis

Dried anthers were taken from herbarium specimens of *D. meristocaulis* deposited in SPF (voucher *F. Rivadavia et al. 1881*). The anthers were soaked in 10 % KOH overnight and then prepared by acetolysis following Erdtman (1960). After a final washing step, the acetolysed pollen grains were stored in acetone for light microscopy (LM) and scanning electron microscopy (SEM) analysis. Photomicrographs of pollen grains in LM were obtained with a video camera (Olympus) connected to a PC. SEM analyses were made using acetolysed pollen grains, which were washed in pure water at several steps to remove residual acetone, and then put on lightstub carbon plates. The samples were gold coated in a vacuum at 36 mA for 2 min using an SCD 050 sputter coater (BAL-TEC, Liechtenstein) and analysed with a 438VP scanning electron microscope (LEO, Germany).

Plant material and DNA extraction

Voucher specimens of *D. meristocaulis* were deposited at the University of São Paulo Herbarium SPF (*F. Rivadavia et al. 1881*). DNA from dried leaves was extracted using the cetyltrimethylammonium bromide (CTAB) buffer protocol (Doyle and Doyle, 1987). Genomic DNA of species of sect. *Bryastrum* and of *Drosera glanduligera*, *Drosera regia* and the outgroup taxon *Dionaea muscipula* (Droseraceae) (see Table 1) was extracted from fresh leaf tissue of greenhouse-grown plants from the private collection of A. Fleischmann, using a NucleoSpin® Plant Kit (Macherey-Nagel, Düren, Germany), following the manufacturer's protocol (Macherey-Nagel, 2007). Voucher specimens are listed in Table 1.

PCR conditions/DNA amplification and sequencing

Amplification of the plastid molecular marker *rbcL* was performed using the primers and protocol of Hasebe *et al.* (1994). The *rps16* intron was amplified and sequenced using the primers rpsF and rps2R and the protocol of Oxelman *et al.* (1997). The nuclear internal transcribed spacer (ITS) region was amplified using the PCR primers Leu1 (Walker and Sytsma, 2007) and ITS4 (White *et al.*, 1990), following the PCR protocol published in White *et al.* (1990). ITS amplification of *D. muscipula* and *D. regia* followed the protocol of Miranda *et al.* (2010).

PCR-amplified sequences were purified using a GFX™ PCR DNA and Gel Purification Kit (GE Healthcare, USA). Both strands of the spacer region were sequenced by the dideoxy chain terminator method in a thermal cycler (GeneAmp® PCR System 9700, Applied Biosystems, Foster City, CA, USA). The sequencing reactions were performed in a total volume of 10 μL containing 30–50 ng of DNA, 5 μM of each primer, 2 μL of the ABI Prism BigDye Terminator v3·1 cycle sequencing ready reaction kit (Applied Biosystems) and 1 μL of 5× Sequencing Buffer (Applied Biosystems). The thermal cycling parameters were as follows: one cycle of 4 min at 94 °C, 40 cycles at 94 °C

TABLE 1. *List of the* Drosera *species and outgroup taxa used for the combined phylogenetic analysis, including voucher data and GenBank accession numbers of the sequence data generated for this study*

Species	Source	Distribution	GenBank number *rbc*L	GenBank number ITS	GenBank number *rps16*
D. meristocaulis	Neblina, *F.Rivadavia et al. 1881* (SPF)	Neblina massif, Brazil–Venezuela border	JN388035	JN388038	JN388044
D. glanduligera	cult. Fleischmann (M; photo voucher)	SW Australia	AB072511*	JN388039	JN388045
D. barbigera	cult. Fleischmann (M; photo voucher)	SW Australia	JQ712489	JQ712490	JQ712488
D. nitidula	cult. Fleischmann (M; photo voucher)	SW Australia	JN388036	JN388040	JN388046
D. scorpioides	cult. Fleischmann (M; photo voucher)	SW Australia	AB072509*	JN388041	JN388047
D. occidentalis	cult. Fleischmann (M; photo voucher)	SW Australia	AB072506*	JN388042	JN388048
D. paradoxa	cult. Fleischmann (M; photo voucher)	Northern Australia	*D. petiolaris*: L01913	JN388043	JN388049
D. ordensis	cult. Fleischmann (M; photo voucher)	Northern Australia	JN388037	JN388075	JN388050
D. regia	*V.F.O. de Miranda 218* (HUMC)	South Africa	AB072566*	JN388077	JN388051
Dionaea muscipula	*V.F.O. de Miranda 208* (HUMC)	SE USA	AB072558*	JN388078	JN388052

Photographic vouchers are given as Supplementary Data, available online.
* Sequences published in Rivadavia *et al.* (2003).

for 40 s, 52 °C for 40 s and 72 °C for 1 min. Electrophoresis and fluorescence detection were carried out on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Phylogenetic reconstruction

The sequences were aligned using ClustalW 1·4 (Thompson *et al.*, 1994) followed by manual examination using BioEdit (Hall, 1999). Some of the ITS and *rbcL* sequences used here were obtained from previous studies (Rivadavia *et al.*, 2003; V. Miranda *et al.*, unpubl. res.) and are available from NCBI GenBank (accession numbers for all nucleotide sequences are listed in Table 1). Indels were treated as missing data. As a strategy of tree rooting, several taxa were initially employed as outgroups, most of them representatives from various families of Caryophyllales known to be closely related to Droseraceae (i.e. Ancistrocladaceae, Dioncophyllaceae and Nepenthaceae). Nevertheless most of these sequences resulted in pairwise similarity <75 %, compared with the sequences of the *Drosera* ingroup, a scenario that could increase noise in the analyses. Therefore, we chose to employ only the monotypic *Dionaea* as an outgroup in all the analyses, because of the higher values of pairwise similarities gained. The phylogenetic analyses were performed for each individual matrix (ITS, *rps16* and *rbcL*) and as combined matrices (ITS + *rps16* + *rbcL*). An additional analysis was carried out with the combined ITS + *rps16* data set, because of an incongruent position of *D. meristocaulis* compared with the topology of the *rbcL* data set. An analysis with a more complete *rbcL* data set of *Drosera* spp. was also performed (Table 2; all *rbcL* sequence data for *Drosera* from Rivadavia *et al.*, 2003 from GenBank). Further outgroup taxa were added to this *rbcL* analysis, based on sequences available in GenBank: *Armeria bottendorfensis*, *Limonium sinense* (Plumbaginaceae), *Drosophyllum lusitanicum* (Drosophyllaceae), and *Polygonum capitatum* and *Rheum delavayi* (Polygonaceae) (Tables 1 and 2).

Maximum parsimony

Phylogenetic analysis based on maximum parsimony (MP) of the sequence data was performed using PAUP* version 4b10 (Swofford, 2002). The phylogenetic trees were obtained by heuristic search through random addition with 5000 replications. The branch swapping followed the tree bisection–reconnection (tbr) algorithm. The robustness of the inferred trees was evaluated using decay indices (Bremer, 1988) and bootstrap resampling (Felsenstein, 1985) through 2000 replicates (pseudomatrices) with 40 heuristic search replicates and random taxon addition. Decay indices were calculated using TNT version 1·1 (Goloboff *et al.*, 2008) and only absolute values ≤ 50 were considered.

Maximum likelihood and Bayesian analyses

The likelihood ratio test as implemented in ModelTest version 3·7 (Posada and Crandall, 1998), with the help of MrMTgui version 1·0 (P. Nuin, GNU General Public License), was employed to determinate the best-fit model of DNA substitution for each data set (individual and combined data sets) under the Akaike information criterion (AIC;

TABLE 2. *List of the* Drosera *species and outgroup taxa additionally used for the enlarged* rbcL *data set from GenBank*

Species	GenBank number
Aldrovanda vesiculosa L.	AB072550
Armeria bottendorfensis A.Schulz	Z97640
Drosera adelae F.Muell.	AY096107
D. alba E.Phillips	AB072515
D. aliciae Raym.-Hamet	AB072516
D. anglica Huds.	AB072517
D. arcturi Hook.	AB072512
D. ascendens A.St.-Hil.	AB072542
D. brevifolia Pursh	AB072519
D. burkeana Planch.	AB072520
D. burmannii Vahl	L01908
D. caduca Lowrie	AB072510
D. capensis L.	L01909
D. capillaris Poir.	AB072521
D. chrysolepis Taub.	AB072522
D. cistiflora L.	AB072523
D.collinsiae N.E.Br. in Burtt Davy	AB072524
D. cuneifolia L.f.	AB072525
D. omissa Diels (as *D. ericksoniae* N.Marchant)	AB072507
D. felix Steyerm. & L.B.Smith	AB072527
D. filiformis Raf.	L01911
D. gigantea Lindl.	L19528
D. graminifolia A.St.-Hil.	AB072528
D. graomogolensis T.R.S.Silva	AB072529
D. hamiltonii C.R.P.Andrews	AB072921
D. hilaris Cham. & Schlechtd.	AB072530
D. hirtella A.St.-Hil.	AB072531
D. indica L.	L19529
D. macrantha Endl. subsp. *planchonii* N.G.Marchant	AB072549
D. longiscapa Debbert (as *D. madagascariensis* DC)	AB072533
D. montana A.St.-Hil.	AB072534
D. natalensis Diels	AB072537
D. pauciflora Banks ex DC.	AB072552
D. peltata Thunb.	L01912
D. pygmaea DC.	AB072505
D. rotundifolia L.	AB072538
D. schwackei (Diels) Rivadavia	AB072535
D. sessilifolia A.St.-Hil.	AB072551
D. spatulata Labill.	L19530
D. stenopetala Hook.f.	AB072539
D. stolonifera Endl.	L19531
D. trinervia Spreng.	AB072548
D. tomentosa A.St.-Hil.	AB072536
D. uniflora Willd.	AB072540
D. villosa A.St.-Hil.	AB072541
Drosophyllum lusitanicum Link	L01907
Limonium sinense Kuntze	FJ872106
Polygonum capitatum Korth. ex Meisn.	HM850243
Rheum delavayi Franch.	FJ872104

Akaike, 1974) to estimate the parameters. We used maximum likelihood (ML) and a Bayesian framework (BA) with Metropolis-coupled Markov chain Monte Carlo (MCMCMC; Geyer, 1991) inference to estimate the phylogenetic hypotheses to each data set. The ML analyses were run in PAUP* version 4b10, using individual models, and estimated parameters to each matrix and clade support were calculated with 2000 replicates (with 40 heuristic search replicates and random addition). MCMCMC analyses were performed in MrBayes version 3·1·2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) for each data set with 9×10^6 generations sampled every 100 generations, using the default parameters. For each analysis, four separate runs were carried out starting from random trees. The sample points prior to reaching stationarity were discarded as burn-in. The posterior probabilities (PPs) for each clade obtained from individual analyses were compared for congruence and combined for evaluating a 50 % majority-rule consensus tree.

RESULTS

Germination pattern

Seed germination occurred after approx. 3–4 weeks at 20–25 °C. *Drosera meristocaulis* exhibits a cryptocotylar (hypogaeous) germination pattern, with the cotyledons remaining in the testa (Fig. 5).

Pollen morphology

Drosera meristocaulis has pollen tetrads with the intermediate aperture type 7–8, following the terminology of Takahashi and Sohma (1982) (Fig. 6). The size measurements are based on our own LM observations and SEM micrographs, and on Duno de Stefano (1995): tetrahedral or frequently tetragonal tetrad, 90–130 μm in diameter (confirming Duno de Stefano, 1995), exine spicate, pollen inoperculate, aperture: one single central pore per grain (aperture type 7–8), with approx. 5–8 large channel openings with a thick exinous wall surrounding one proximal central pore, radial plaits poorly developed. Single grain 35–43 μm in diameter (45–55 μm by Duno de Stefano, 1995). Channel openings approx. 10 × 5 (–10) μm, standing alternate or opposite to those of adjoining grains. Spines up to 4 μm long, density of the spines 1·0–1·5 μm^{-2} (confirming Duno de Stefano, 1995), spinules absent (confirming Duno de Stefano, 1995).

Chromosome counts

In total, ten meristematic root tips were prepared, and numerous counts were made. However, due to the small chromosome size, and overall small size of the meristematic root cells of *D. meristocaulis* of about 10 μm in diameter, an evaluation of the exact karyotype was not possible. The chromosome counts for *D. meristocaulis* revealed numbers of 32, 34 and 36 with equal frequency of occurrence. Therefore, a karyotype of $2n =$ approx. 32–36 is given for *D. meristocaulis* here.

Molecular data

All three markers used in this study revealed *D. meristocaulis* in the Australian *Drosera* clade (*sensu* Rivadavia *et al.*, 2003), although the exact phylogenetic position differs between *rbcL* and the other two markers (Fig. 4; see Supplementary Data for sequence alignments). The plastid marker *rbcL* shows *D. meristocaulis* nested within the pygmy *Drosera* clade, as sister to the two sister pairs *D. occidentalis* and *D. nitidula*, and *D. barbigera* and *D. scorpioides* (Fig. 4). In both the ITS and *rps16* data sets, and the combined phylogenetic reconstruction using all three markers, *D. meristocaulis* is revealed as sister to the pygmy clade (Fig. 3), and the two are sister to the

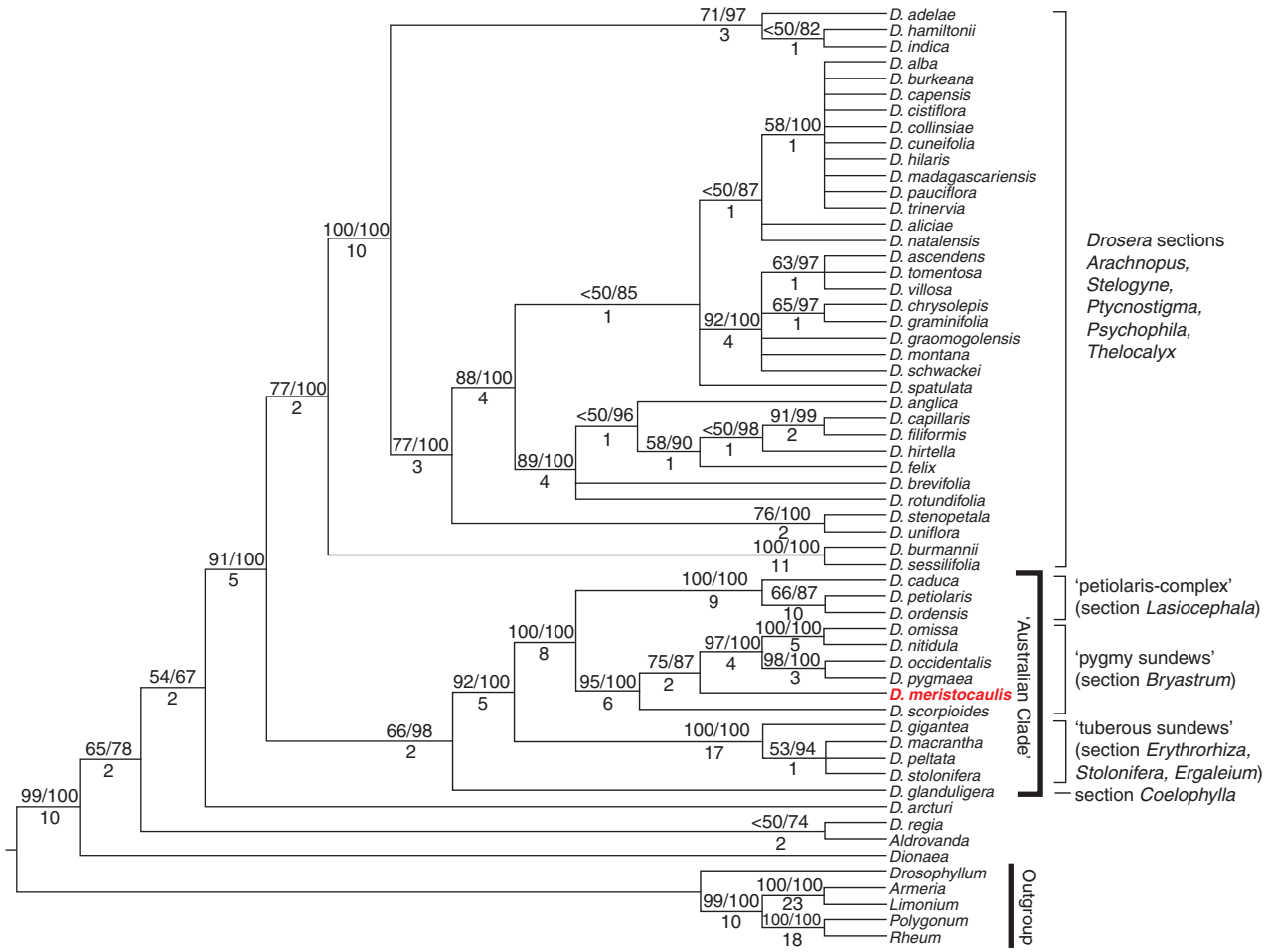

FIG. 2. Strict consensus from 576 most parsimonious trees (754 steps) of Droseraceae from Bayesian analysis of the *rbcL* data set. Numbers above branches show bootstrap (left), and Bayesian posterior probability (right) support values; numbers below branches are decay index values. The position of *Drosera meristocaulis* is highlighted. Taxonomic groups and clades are indicated, following Rivadavia *et al.* (2003).

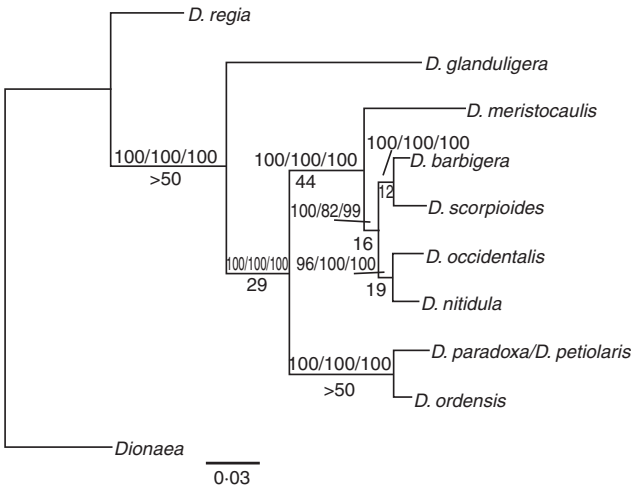

FIG. 3. Single most parsimonious tree (1331 steps) of the combined ITS, *rps16* and *rbcL* data sets. Numbers above branches show MP bootstrap (left), ML bootstrap (middle) and Bayesian posterior probability (right); numbers below branches are decay index values. Branch lengths represent genetic distance based on the scale at the bottom.

petiolaris clade (represented by *D. paradoxa*, *D. petiolaris* and *D. ordensis* in the present study). All nodes get high statistical support, both in the trees resulting from each single marker data set (see Fig. 2 for *rbcL*) and in the combined tree (Fig. 3).

DISCUSSION

Recently collected material of *D. meristocaulis* has revealed surprising new data supporting the placement of this species in the Australian *Drosera* clade, at the base of sect. *Bryastrum*. The most outstanding pattern recovered by the joint investigation of different biological aspects (seed germination pattern, pollen morphology, chromosome count and molecular phylogenetics) was the strong affinity of *D. meristocaulis* for the species from sect. *Bryastrum*.

Phylogenetic significance of germination pattern

Cryptocotylar germination in Droseraceae was thus far exclusively known from taxa belonging to a phylogenetic clade containing predominantly Australian *Drosera* spp., including

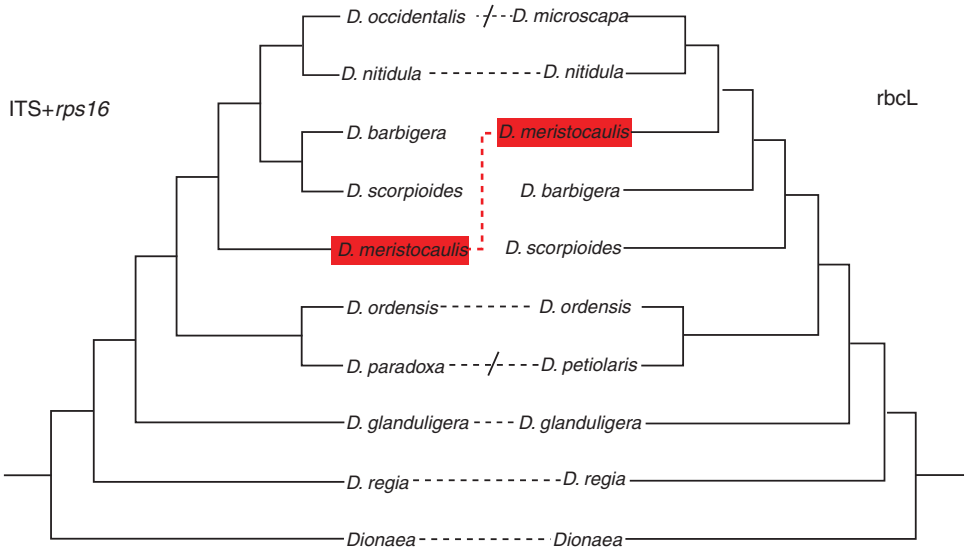

FIG. 4. Incongruence of the phylogenetic position of *Drosera meristocaulis* between ITS and *rps16* topology (left) and *rbcL* (right). Identical phylogenetic positions are indicated by dashed lines; a slash in the dashed line is for taxa equivalents used in the different data sets (see also Table 1).

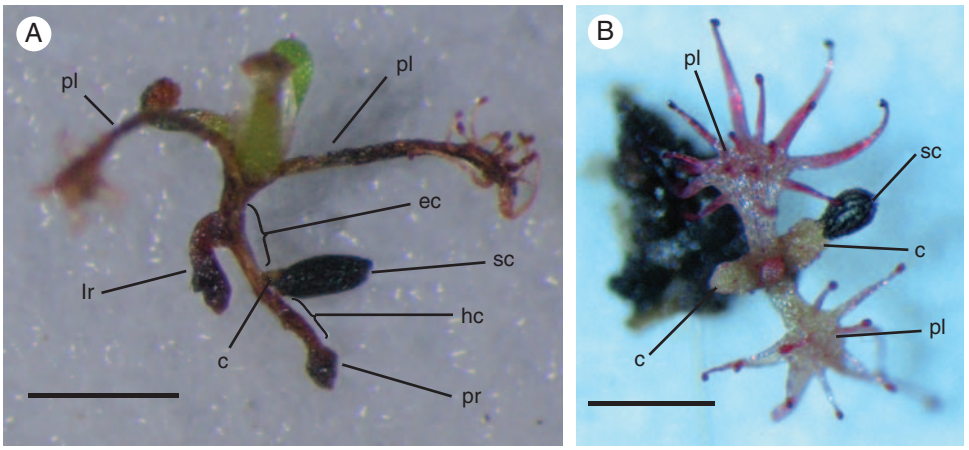

FIG. 5. Comparison of germination patterns of two different South American *Drosera* species. (A) Cryptocotyly in *Drosera meristocaulis*. (B) Phanerocotyly in *D. capillaris*. Abbreviations: c, cotelydons (hidden inside the testa in *D. meristocaulis*); ec, epicotyl; hc, hypocotyl; lr, lateral root; pl, primary leaf; pr, primary root; sc, seed coat (testa). Scale bars = 1 mm.

the pygmy sundews of sect. *Bryastrum* (Conran *et al.*, 1997, 2007). Thus, *D. meristocaulis* is the only New World *Drosera* species with this type of germination (Fig. 5), with all other species showing phanerocotyly (see *D. capillaris* in Fig. 5). Cryptocotylar germination in small-seeded plants like Droseraceae is rare (Clifford, 1984) and is usually associated with fluctuating ecological conditions and therefore interpreted as an adaptation to long-term seed dormancy which requires induced germination. Cryptocotyly has only evolved once in *Drosera*; it is a synapomorphy for the Australian clade (*sensu* Rivadavia *et al.*, 2003), but was lost in the monotypic sect. *Phycopsis* consisting of *D. binata*. Thus, it is most likely that this germination pattern evolved among *Drosera* in Australia as the continent moved northwards and became drier (Yesson and Culham, 2006), as an adaptation to the seasonal Mediterranean climate with a pronounced dry season, occasional summer fires and cool moist winters. The seed remains dormant until germination is triggered by changing seasonal conditions, an ecological strategy followed by a range of Australian plants, including numerous *Drosera* spp. (Bell *et al.*, 1993).

Although the summits of the Neblina massif are usually regarded as stable, wet tropical Amazonian habitats, *D. meristocaulis* occurs on the drier northern plateaus of these highlands, from where occasional fires have been reported (Givnish *et al.*, 1986; McPherson, 2006). At least a few endemic plants from this area seem to present morphological adaptations to avoid fire damage (Givnish *et al.*, 1986; Judziewicz, 1998). Seasonal droughts and wildfires are conditions reminiscent of the habitats occupied by *Drosera* sect. *Bryastrum* in Oceania, which may explain why cryptocotyly is maintained in *D. meristocaulis*.

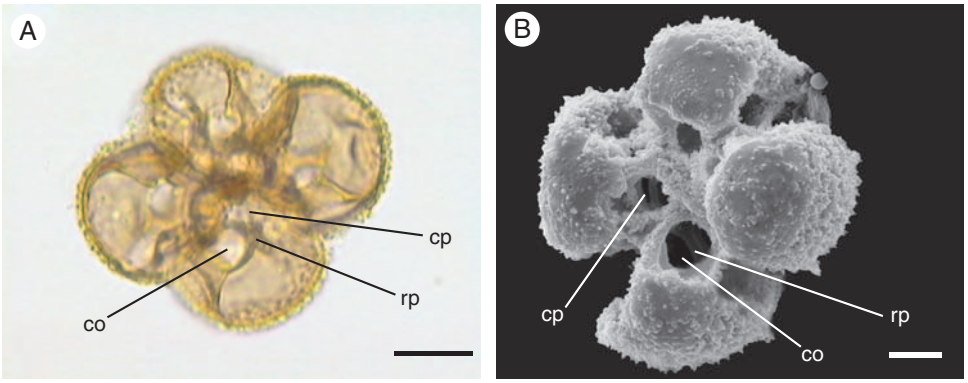

FIG. 6. Pollen of *Drosera meristocaulis*. (A) LM photograph, (B) SEM photograph. Abbreviations: co, channel opening; cp, central pore; rp, radial plait. Scale bars = 10 μm.

Phylogenetic significance of pollen morphology

Further morphological similarities between *D. meristocaulis* and members of sect. *Bryastrum* can be found in pollen. Duno de Stefano (1995) observed one single central pore as an aperture in pollen tetrads of *D. meristocaulis* and therefore assigned it to aperture type 7, which is confined to sect. *Drosera* (Takahashi and Sohma, 1982). However, he did not recognize that a single proximal pore is also found in aperture type 8 and the intermediate type 7–8 (Takahashi and Sohma, 1982).

The pollen tetrad of *D. meristocaulis* shares common features of aperture type (one proximal central pore in each pollen grain) and pollen structure (radial channel plaits poorly developed and channel openings surrounded by a thick exinous wall) with pollen known as type 8 or intermediate type 7–8, respectively (Takahashi and Sohma, 1982). These two pollen types are confined to species of the Australian *Drosera* clade (*sensu* Rivadavia *et al.*, 2003), except for *D. glanduligera* of the monotypic sect. *Coelophylla*, which exhibits a unique pollen tetrad of type 5 (Takahashi and Sohma, 1982). The ornamentation of the exinous wall of *D. meristocaulis* pollen is also distinct from that of all other South American *Drosera* spp. (Duno de Stefano, 1995), as it has no spinules and few rather large spines. This ornamentation is commonly found in Australian *Drosera* spp., especially in members of sect. *Bryastrum* and sect. *Lasiocephala* (Takahashi and Sohma, 1982).

Phylogenetic significance of leaf trichome characters

The leaves of members of the *Bryastrum* clade (including sect. *Lasiocephala* and sect. *Bryastrum*, *sensu* Seine and Barthlott, 1994) are characterized by the presence of biseriate sessile trichomes ('microglands') with elongated basal cells, which represent a synapomorphic character for this monophyletic group. These trichomes were called '*Rorella*-type glands' by Seine and Barthlott (1993) and classified as 'type 4 and 5 glands' by Länger *et al.* (1995). *Drosera meristocaulis* has type T2 biseriate and T11–12 multiseriate sessile trichomes (Seine and Barthlott, 1993; Länger *et al.*, 1995). Conran *et al.* (2007) stated that the trichome patterns found in *D. meristocaulis* are ambiguous, as they can be observed in members of both the *Drosera* and the *Bryastrum* clade (*sensu* Rivadavia *et al.*, 2003), and that only in combination with the germination pattern could the phylogenetic position of sect. *Meristocaulis* be verified. However, the stout, short yellow gland-like trichomes on the adaxial and abaxial petiole surface of *D. meristocaulis* (Seine and Barthlott, 1993) do also occur in some pygmy *Drosera* spp. (e.g. *Drosera nitidula* and related species, A. Fleischmann, pers. obs.). These trichomes have a four-celled peduncle and a glandular head consisting of about 20 cells. This type of glandular trichome produces a sub-cuticular yellow secretion and occurs on the leaf surface and also on the emergences (Fig. 7).

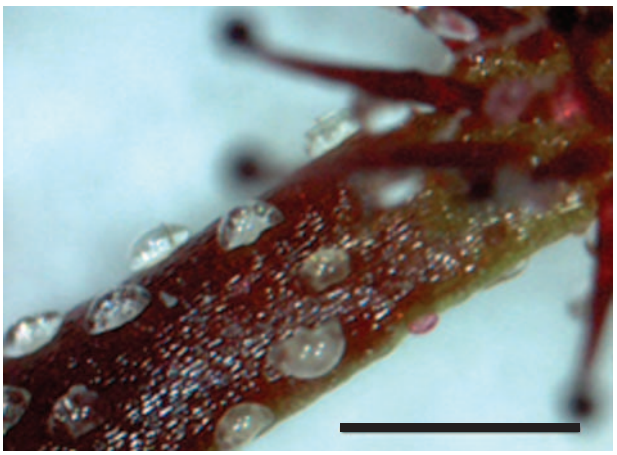

FIG. 7. Yellow glandular trichomes on a leaf of *Drosera meristocaulis*. Scale bar = 1 mm. Photograph by Daniel Olschewski, with kind permission.

Members of sect. *Bryastrum* all share a special, eight-celled biseriate type of microgland, so-called '*Rorella*-trichomes' (Seine and Barthlott, 1993), which are usually found on the abaxial surface of the petiole and lamina. Seine and Barthlott (1993) did not observe these *Rorella*-trichomes in the specimens of *D. meristocaulis* they studied, and we did not detect them in our study material. The absence of *Rorella*-trichomes is a morphological character that supports the phylogenetic position of *D. meristocaulis* as sister to sect. *Bryastrum*, not as a member of this section, and rejection of subgenus *Meristocaulis sensu* Schlauer (1996).

Phylogenetic significance of karyology

Chromosome numbers in *Drosera* range from $2n = 6$ to $2n = 80$, and are in strong phylogenetic accordance with the clades

revealed by Rivadavia *et al.* (2003). The Australian clade exhibits the greatest variability of karyotypes and forms extensive aneuploid and polyploid series, with relatively low chromosome numbers, ranging from $2n = 6$ to $2n = 40$, resulting from base numbers $x = 3, 5, 6, 7, 8, 9, 10, 11, 13, 14$ and 23 (Kondo and Lavarack, 1984; Sheikh and Kondo, 1995; Rivadavia *et al.*, 2003; Rivadavia, 2005; Lowrie and Conran, 2007). In contrast, all New World species of *Drosera* (belonging to sect. *Drosera* and sect. *Thelocalyx*) form a homogeneous group, with relatively conserved chromosome numbers of $2n = 20$ or 40 (i.e. polyploid series of the base number $x = 10$), suggesting at least two independent colonization events of a diploid and tetraploid group (Rivadavia *et al.*, 2003; Rivadavia, 2005). Although representing an approximate range, the newly inferred karyotype of $2n =$ approx. 32–36 for *D. meristocaulis* contrasts with the polyploid series found in all other South American species, but fits the aneuploid series found in *Drosera* spp. of the Australian clade.

Karyology can be a useful character in *Drosera* taxonomy for both species delimitation and infrageneric classification. An example for the latter was shown with the proposal to remove the enigmatic northern Australian *D. banksii* from sect. *Ergaleium* and to place it in sect. *Lasiocephala* (Kondo and Lavarack, 1984). This suggestion was later confirmed by further morphological (Seine and Barthlott, 1994) and molecular phylogenetic data (A. Fleischmann, unpubl. res.), which revealed that this species grouped with sect. *Lasiocephala*.

CONCLUSIONS

Molecular phylogenetic data and morphological characters, including germination pattern, pollen anatomy, karyotype and leaf trichome characters, support the placement of *D. meristocaulis* in the Australian clade in a monotypic section (sect. *Meristocaulis*), as sister to sect. *Bryastrum* (Figs 3 and 4), or even in this section in the case of the *rbcL* data set (Fig. 2). In contrast to the *Drosera* of sect. *Bryastrum*, *D. meristocaulis* does not reproduce asexually by gemmae. It is possible that the ancestors of *D. meristocaulis* lost the ability to produce gemmae after reaching South America, but it is also probable that gemmae production evolved in pygmy sundews after this lineage split from *D. meristocaulis*. Gemmae are a synapomorphy of the pygmy sundews and are likely to have evolved as an adaptation to a seasonal climate as the Australian continent became drier (Yesson and Culham, 2006). The fact that gemmae are found in all species of sect. *Bryastrum* suggests that it is not only a successful means of asexual reproduction, but also an essential ecological survival strategy in the Mediterranean climate of southwestern Australia. The production of gemmae requires a considerable allocation of resources (Karlsson and Pate, 1992) and is possibly an important mechanism for rapid clonal colonization of seasonally available habitats.

Cryptocotylar germination may represent an adaptation to fire, a common phenomenon in both regions (Oceania and the Neblina massif), playing an important role in the maintenance of morphological similarities between *D. meristocaulis* and species of sect. *Bryastrum*.

Any explanation for the presence on the Neblina massif of a plant species descended from an Australian lineage is sure to be at least controversial. A recent study estimated that sect. *Bryastrum* began its diversification about 13–12 Mya (Yesson and Culham, 2006), and therefore contradicts a Gondwanan origin for *D. meristocaulis*. Despite the lack of information on a dispersal route from Australia to northern South America, the evidence that this did in fact occur cannot be rejected. As a vector for this rare LDD event, birds or wind seem most conceivable, although no avian migratory pathways from Australia to northern South America have been reported (Lomolino *et al.*, 2006). An Australian to temperate South America disjunction is also known from a few plant families (Thorne, 1972), including Winteraceae. A strikingly similar biogeographic pattern is found in the three earliest branching members of Loranthaceae (showy mistletoes), namely the terrestrial monotypic genera *Nuytsia floribunda* from south-western Western Australia, *Atkinsonia ligustrina* from eastern Australia and *Gaiadendron* from Central and South America (also occurring on Neblina) (Vidal-Russell and Nickrent, 2008). In the case of Loranthaceae, a Gondawanan origin is assumed, which would explain the biogeography of the three taxa, which are successive sister taxa to all remaining Loranthaceae (Vidal-Russell and Nickrent, 2008).

Recent LDD from Australia (or southeast Asia) to South America has previously been proposed for the species sister pair *Drosera burmannii* and *D. sessilifolia* of sect. *Thelocalyx* (Rivadavia *et al.*, 2003). In accordance with phylogenetic and other evidence presented above, *D. meristocaulis* is most probably also descended from an LDD event from Australia to South America, and is probably not a supposed palaeoendemic species that descended from pygmy sundew-like plants previously widespread in Gondwana, and which also led to extant sect. *Bryastrum*.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of photographic vouchers for *Drosera barbigera*, *D. glanduligera*, *D. nitidula*, *D. occidentalis*, *D. ordensis*, *D. paradoxa* and *D. scorpioides*, and sequence alignments (.txt files) for *rbcL*, ITS and *rps16*.

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