



# Revisiting the bipolarity of *Roaldia revoluta* (Mitt.) P.E.A.S. Câmara & Carv.-Silva (Bryophyta, Pylaisiaceae)

PAULO E.A.S. CÂMARA <sup>1</sup>, MICHELINE CARVALHO-SILVA <sup>1</sup>, DAIANE VALENTE VALENTE <sup>2</sup>,  
DIEGO KNOP HENRIQUES <sup>1</sup>, EDUARDO TOLEDO DE AMORIM <sup>3</sup>, WELLINGTON SANTOS FAVA <sup>4</sup>,  
HANS (J.D.) KRUIJER <sup>5,6</sup> and MICHAEL STECH <sup>5,6</sup>

<sup>1</sup>Universidade de Brasília, Botany Dept, Brasília, Brazil

<sup>2</sup>Instituto Federal de Educação, Ciência e Tecnologia Farroupilha - Campus São Vicente do Sul. Rua 20 de Setembro,  
2616 - CEP 97420-000 - São Vicente do Sul - Rio Grande do Sul, Brazil

<sup>3</sup>Centro Nacional de Conservação da Flora/Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Brazil

<sup>4</sup>Laboratory of Ecology and Evolutionary Biology, Institute of Biosciences, Federal University of Mato Grosso do Sul,  
Campo Grande, Mato Grosso do Sul, 79070-900, Brazil

<sup>5</sup>Naturalis Biodiversity Center, PO Box 9517, 2300 RA Leiden, The Netherlands

<sup>6</sup>Leiden University, Leiden, The Netherlands

[paducamara@gmail.com](mailto:paducamara@gmail.com)

**Abstract:** The occurrence of species in both polar regions (bipolarity) is a common phenomenon in the Antarctic flora. Considering the high morphological variation in polar regions due to extreme conditions, the use of molecular tools is indispensable for testing whether Arctic and Antarctic populations indeed belong to the same species. However, few phylogeographic studies of bipolar bryophytes have been conducted so far, especially when comparing molecular and morphological variation. Here, we assess the bipolarity and intraspecific variation of *Roaldia revoluta*, a strictly bipolar species of pleurocarpous mosses. Phylogenetic analyses based on ITS sequences clearly resolve *R. revoluta* as monophyletic and confirm its bipolar distribution pattern. Low intraspecific molecular variation in the markers ITS/26S and *rpl16* was observed, and most specimens from both polar regions belong to a single haplotype, making it difficult to infer the origin and dispersal routes between both polar regions of *R. revoluta*. Morphometric analysis furthermore suggests that there are no significant morphological differences among populations from both polar regions and that morphological variation is mainly influenced by local environmental conditions. Our data do not unequivocally support the recent separation of the former intraspecific taxon *Hypnum revolutum* var. *dolomiticum* at the species level as *Roaldia dolomitica*.

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**Key words:** Antarctica, Arctic, genetic diversity, haplotype, *Hypnum revolutum*, Pylaisiaceae

## Introduction

Apart from two native and one invasive flowering plant species, the Antarctic land plant flora is composed solely of bryophytes, including more than 116 species of mosses (Bryophyta; Ochyra *et al.* 2008, Ellis *et al.* 2013a, 2013b, Sollman *et al.* 2015, Câmara *et al.* 2019). The Antarctic bryophytes are grouped into six geographical elements: endemic, sub-Antarctic, south temperate, bipolar, cosmopolitan and tropical (Ochyra *et al.* 2008). Bipolarity (i.e. the occurrence of species in both polar (Arctic and Antarctic) and cool-temperate regions, with or without intermediate occurrences in tropical mountain areas) is quite a common phenomenon in Antarctica, with ~45% of its flora being bipolar, including some of the most common species (Ochyra *et al.* 2008).

Three main hypotheses have been proposed to explain bipolar distribution patterns, namely vicariance,

stepping-stone migration via tropical mountains or direct long-distance dispersal (Lamb 1970, Ochyra *et al.* 2008, Lewis *et al.* 2017). Three main migration routes have been proposed, the so-called 1) American pathway, with dispersal via the Neotropics and Patagonia to Maritime Antarctica, 2) African pathway, via East African mountains to sub-Antarctic islands such as Kerguelen, and 3) Indomalayan-Malesian pathway via south-east Asia to south-east Australia, New Zealand and associated nearby islands (Ochyra *et al.* 2008).

However, the possibility that Arctic and Antarctic populations of presumed bipolar species actually belong to different species cannot be ruled out. Our current understanding of morphological variation and taxonomical knowledge may be insufficient to correctly delimit and identify supposedly bipolar species. Many polar bryophyte species are characterized by high levels of morphological variability (e.g. Longton 1988,

Buryová & Shaw 2005, Hebel *et al.* 2012, Hesse *et al.* 2012, Daniëls *et al.* 2013). On the other hand, bipolarity could represent an artifact due to extreme environmental conditions in both polar regions, with non-related taxa displaying similar morphologies in the Arctic and the Antarctic (Câmara *et al.* 2018b). The use of molecular data is therefore indispensable for investigating bipolarity, along with a (re)assessment of morphological variation.

A recent example of insufficiently understood bipolarity is found in the pleurocarpous moss genus *Drepanocladus*, with molecular data indicating that the presumed bipolar *Drepanocladus longifolius* (Wilson ex Mitt.) Broth. ex Paris comprises two geographically separated species, of which only one is bipolar (Holarctic and Australia; Saługa *et al.* 2018). Other recent molecular phylogenetic analyses confirmed the monophyly of bipolar moss species in their current morphological circumscriptions (*Polytrichastrum alpinum* (Hedw.) G.L. Sm. and three *Polytrichum* species, Biersma *et al.* 2017; *Schistidium rivulare* (Brid.) Podp., Biersma *et al.* 2018b), and they suggested a Holarctic origin for all of them except *Polytrichum juniperinum* Hedw. In the earlier studied moss genera *Cinclidium* (Piñeiro *et al.* 2012) and *Sanionia* (Hedenäs 2012), the (molecular) species delimitations were less clear, but Northern Hemisphere origins of Southern Hemisphere populations of the bipolar species *Cinclidium stygium* Sw. and *Sanionia uncinata* (Hedw.) Loeske were inferred as well, as was also the case in *Tetraplodon fuegianus* Besch. (Lewis *et al.* 2017).

To better understand the geographical origins and diversification of the Antarctic bryophyte flora, further taxa from all main phytogeographical elements, including bipolar species, should be studied (Câmara *et al.* 2021), combining molecular and morphological data in an integrative approach (e.g. Bijlsma *et al.* 2020). Pleurocarpous mosses of the order Hypnales are yet understudied in this respect. According to Ochyra *et al.* (2008), eight out of the 17 pleurocarpous moss species that occur in Antarctica are bipolar, but apart from the aforementioned *D. longifolius* and *S. uncinata*, only preliminary data of a third species, *Roaldia revoluta* (Mitt.) P.E.A.S. Câmara & Carv.-Silva, are available. *R. revoluta*, formerly known as *Hypnum revolutum* Mitt., has an arctic-boreal-montane distribution in the Northern Hemisphere. In the Southern Hemisphere, *Roaldia revoluta* is present in Antarctica (the South Orkney Islands, the South Shetland Islands and the Antarctic Peninsula), Patagonia and New Zealand (South Island). The distribution of *R. revoluta* is considered strictly bipolar (Ochyra *et al.* 2008, Câmara *et al.* 2018a), despite three occurrences in tropical mountain areas in Mexico (Ando 1973), Guatemala (Câmara *et al.* 2018a) and Uganda (Hedenäs & Watling 2005).

The taxonomy and phylogenetic position of *R. revoluta* as a monospecific genus in the family Pylaisiaceae were discussed by Câmara *et al.* (2018a) based on molecular and morphological data. Formerly distinguished intraspecific taxa in *H. revolutum*, in particular *H. revolutum* var. *dolomiticum* (Milde) Moenk. and fo. *pumilum* (Husn.) Ando (Ando & Matteri 1982), were considered as mere morphological variations in that study. In contrast, Schlesak *et al.* (2018) considered *H. revolutum* var. *dolomiticum* sufficiently separated at the species level, for which they made the combination *Roaldia dolomitica* (Milde) Hedenäs, Schlesak & D. Quandt; their view was taken over by Kučera *et al.* (2019). Câmara *et al.* (2018b) showed the absence of genetic variation among populations of *R. revoluta* in Antarctica and concluded that the observed morphological plasticity was probably caused by environmental conditions acting upon this species. Morphological and molecular variability and relationships between northern and southern populations of *R. revoluta*, however, still need to be investigated.

In this paper, we aim to 1) test the monophyly and bipolarity of *R. revoluta* based on samples from its entire distribution area, 2) re-evaluate the status of *R. dolomitica*, 3) re-evaluate the morphological variation in Antarctica reported in Câmara *et al.* (2018b) in comparison with morphological variation in the Northern Hemisphere and the molecular variation worldwide and 4) compare intraspecific molecular and morphological variation with that in other bipolar species.

## Material and methods

### Sampling

The sampling of *R. revoluta* specimens was based on Câmara *et al.* (2018a,b) but was much expanded to include representatives from the Northern Hemisphere. In the present study, we were able to include representatives from all main areas where *R. revoluta* is known to occur, except the South Orkney Islands, New Zealand and Uganda. For phylogenetic studies, 69 specimens were sequenced from a wide range of geographical locations. Voucher data and GenBank accession numbers are listed in Table 1. In addition, ITS sequences of seven *Roaldia* samples, two samples each of three other species of Pylaisiaceae, namely *Calliergonella curvifolia* (Hedw.) B.H.Allen, *Calliergonellopsis dieckii* (Renauld & Cardot) Jan Kučera & Ignatov and *Pseudostereodon procerrimus* (Molendo) M.Fleisch., as well as of *Stereodon callichrous* (Brid.) Lindb. (Stereodontaceae), as outgroup representatives, were downloaded from GenBank and included in the analysis, following Câmara *et al.* (2018a), Schlesak *et al.* (2018) and Kučera *et al.* (2019). A total of

**Table I.** Voucher information and GenBank accession numbers (Acc. no.) of the sequenced *Roaldia revoluta* specimens.

DNA no.	Country	Location	Collector or herbarium number	Herbarium	Acc. no. <i>rpl16</i>	Acc. no. ITS	Acc. no. 26S	Morphometric analysis
H24	Antarctica	Alexander Island	Smith 11070	AAS	OP925007	OQ216646	OQ216695	+
H28	Antarctica	Alexander Island	Harris 11092	AAS	OP963392	OQ216648		+
H37	Antarctica	Graham Coast, Darboux Islands	Smith 0335	AAS		OQ225728		+
H21	Antarctica	James Ross Island, Ulu Peninsula	Smith 07498	AAS		OP700848		+
H78	Antarctica	James Ross Island, Ulu Peninsula	Kitaura 2828	UB	OP963400	OQ216670	OQ216712	+
H79	Antarctica	James Ross Island, Ulu Peninsula	Kitaura 2950	UB	OP963401	OQ216671	OQ216713	+
H80	Antarctica	James Ross Island, Ulu Peninsula	Kitaura 3052	UB	OP963402	OQ216673	OQ216715	+
H83	Antarctica	James Ross Island, Ulu Peninsula	Kitaura 2939	UB	OP963403	OQ216674	OQ216716	+
H84	Antarctica	James Ross Island, Ulu Peninsula	Kitaura 3106	UB	OP963404	OQ216675	OQ216717	+
H85	Antarctica	James Ross Island, Ulu Peninsula	Kitaura 3105	UB	OP963405	OQ216676	OQ216718	+
H118	Antarctica	James Ross Island, Ulu Peninsula	Kitaura 3136	UB		OQ230912		+
H120	Antarctica	James Ross Island, Ulu Peninsula	Kitaura 2933	UB		OQ230913		+
H4	Antarctica	King George Island, Admiralty Bay	Ochyra 2284/80	S		OQ216650		+
H18	Antarctica	King George Island, Fildes, Two Summit	Costa & Vandeira 6164	UB, RB			OQ225233	+
H29	Antarctica	King George Island, Keller Peninsula	Ochyra 442/80	AAS		OQ216649		+
H33	Antarctica	King George Island, Fildes, Two Summit	L.X.D. 890213	AAS				+
H64	Antarctica	King George Island, Ore Point	Carvalho-Silva 2015	UB	OP925013	OP700858	OQ216703	+
H69	Antarctica	King George Island, Keller Peninsula	Carvalho-Silva 2052	UB	OP925014	OQ216660	OQ216704	+
H70	Antarctica	King George Island, Keller Peninsula	Carvalho-Silva 2102	UB	OP925015	OQ216662	OQ216705	+
H72	Antarctica	King George Island, Keller Peninsula	Carvalho-Silva 2053	UB	OP925017	OQ216664	OQ216707	+
H71	Antarctica	King George Island, Keller Peninsula	Carvalho-Silva 2058	UB	OP925016	OQ216663	OQ216706	+
H99	Antarctica	King George Island, Duffayel	Dantas 603	UB	OP963412	OQ230905	OQ216728	+
H122	Antarctica	King George Island, Duffayel	Dantas 603B	UB		OQ216640		+
H123	Antarctica	King George Island, Ore Point	Carvalho-Silva 2106	UB		OQ230914		+
H124	Antarctica	King George Island, Ore Point	Carvalho-Silva 2111	UB		OQ230915		+
H25	Antarctica	Marguerite Bay	Smith 4637	AAS		OQ216647		+
H111	Antarctica	Ostrov Geologov	Henriques 233	UB	OQ230919	OQ230909	OQ216689	+
H109	Argentina	Ushuaia, Parque Nacional Tierra del Fuego	Peralta 20260	UB		OQ216635		+
H15	Canada	British Columbia, Marble Canyon Prov. Park	Schofield 121192	MO		OP700830	OQ216691	+
H113	Canada	New Foundland	Hedderson 12019	CANM	OQ225458	OP700832		+
H115	Canada	Quebec, Lac Leau Claire	Ireland 20892	CANM		OQ230911		+
H114	Canada	Quebec, Lac Guillaume	Ireland 21223	CANM		OQ230910		+
H106	Finland		TUR 119987	TUR		OQ230908	OQ225234	
H76	Greenland	Zackenbergl	Stech & Kruijjer 13-136	L	OP963398	OQ216668	OQ225239	
H17	Guatemala	Huehuetenango	Laeger 3654	NY	OQ225459	OP700833	OQ216692	
H19	Iceland		Erikson 41112	ICEL	OP925006	OQ216644	OQ216693	+
H20	Iceland		Erikson 44279	ICEL	OP963391	OQ216645	OQ216694	+
H52	Iceland		Erikson 41188	ICEL	OP925008	OQ216651	OQ216696	+
H100	Kyrgyz Republic		Lommi 3281	H	OP963413	OQ230920	OQ216686	
H1	Norway	Oppland	Hedenäs B193247	S	OP925002	OP700842	OQ225240	+
H58	Norway	Troms	Hedenäs B138918	S	OP963393	OQ216654	OQ216698	+
H59	Norway	Finnmark	Hedenäs B63161	S	OP925010	OQ216655	OQ216699	+
H73	Norway	Svalbard, Kongsfjorden	Stech & Kruijjer 08-233	L	OP963395	OQ230902	OQ216708	

(Continued)

TABLE I. (continued).

DNA no.	Country	Location	Collector or herbarium number	Herbarium	Acc. no. <i>rp116</i>	Acc. no. ITS	Acc. no. 26S	Morphometric analysis
H74	Norway	Svalbard, Kongsfjorden	Stech & Kruijer 09-152	L	OP963396	OQ216666	OQ216709	
H75	Norway	Svalbard, Kongsfjorden	Stech & Kruijer 10-213	L	OP963397	OQ216667	OQ216710	
H77	Norway	Svalbard, Barentsøya	Stech & Kruijer 15-308	L	OP963399	OQ216669	OQ216711	
H86	Norway	Svalbard, Edgeøya	Stech & Kruijer 15-122	L	OQ230917	OQ216677	OQ216719	+
H87	Norway	Svalbard, Edgeøya	Stech & Kruijer 15-182	L	OQ230918	OQ216678	OQ216720	+
H88	Norway	Svalbard, Edgeøya	Stech & Kruijer 15-155	L	OP963406	OQ216679	OQ216721	+
H89	Norway	Svalbard, Barentsøya	Stech & Kruijer 15-303	L		OQ216680	OQ216722	+
H90	Norway	Svalbard, Kongsfjorden	Stech & Kruijer 10-212	L	OP963407	OQ216681	OQ216723	+
H91	Norway	Svalbard, Kongsfjorden	Stech & Kruijer 08-234	L	OP963408	OQ230904	OQ216724	+
H101	Russia	Chukotka	Afonina s.n.	H	OP925018	OQ230906	OQ216687	+
H102	Russia	Kamchakta	Czerdnyadjeva 114	H	OP963414	OQ230907	OQ225235	+
H7	Sweden	Jämtland	B164564	S	OP925004	OQ216672	OQ216714	+
H60	Sweden	Härjedalen	B122924	S	OQ230916	OQ216657	OQ225237	+
H12	Switzerland	Graubünden	B184393	S	OP925005	OQ216643	OQ216690	+
H54	Switzerland	Graubünden	B184356	S	OP925009	OQ216652	OQ216697	+
H55	Switzerland	Graubünden	B84802b	S		OQ216653	OQ230900	+
H16	USA	Alaska, near Alaska Highway	Schofield 118260	MO	OP963390	OP700831	OQ225232	
H93	USA	Alaska, Tetlin National Reserve	Schofield 118328	DUKE	OP963409	OQ216683	OQ216725	+
H94	USA	Alaska, Aleutian Islands, Dutch Harbour	Schofield 127044	DUKE	OP963410	OQ216684	OQ216726	+
H95	USA	Alaska, Selawick Wildlife Refuge	Schofield 122007A	DUKE	OP963411	OP700877	OQ216727	+
H6	USA	California, Sierra Nevada	Shevock <i>et al.</i> 14800	UB	OP925003	OQ230901	OQ225238	+
H63	USA	Colorado	King B552	DUKE	OP963394	OQ230902	OQ216702	+
H108	USA	Colorado	B141512b	S	OP925019	OQ216634	OQ216688	+
H5	USA	Minnesota, Pine County	Shevock <i>et al.</i> 24249	UB		OQ216656	OQ216700	+
H96	USA	Montana, Missoula	Schofield 121018	DUKE		OP700878	OQ225241	
H62	USA	Wyoming	Kosovich 11989	DUKE	OP925012	OQ216658	OQ216701	+

95 specimens (46 from the Antarctic and 49 from the Arctic and other regions), including a selection of sequenced specimens (Table I) and further specimens not molecularly analysed, were subjected to morphometric analysis.

Material was obtained from herbaria (AAS, CANM, H, DUKE, ICEL, L, MO, NY, RB, S, SP, TUR, UB; acronyms follow <http://sweetgum.nybg.org/science/ih>) and from fieldwork. Fieldwork in Antarctica (South Shetland Islands, James Ross Island and Antarctic Peninsula region) was carried out by PEASC, MC-S, DVV, DKH, ETdA and WSF during the summers of 2015/2016 and 2016/2017 with support of the Brazilian Antarctic Program (PROANTAR). Material in the High Arctic was collected by HK and MS during fieldwork in Greenland (2013) and Svalbard (2008, 2009, 2010 and during the Netherlands Scientific Expedition Edgeøya Spitsbergen (SEES) in 2015).

#### *DNA extraction, polymerase chain reaction, sequencing and sequence editing*

Total genomic DNA was extracted using the CTAB protocol (Doyle & Doyle 1987). We amplified and sequenced the chloroplast marker *rp116* intron as well as the nuclear ribosomal markers ITS (ITS1-5.8S-ITS2) and partial 26S gene using the primers published in Hedenäs (2012), Pisa *et al.* (2013) and Cox *et al.* (2004), respectively. The polymerase chain reaction (PCR) amplification mixture had a total volume of 50 µl and contained 5 µl of 5× thermophilic buffer, 5 µl of 50 mM MgCl<sub>2</sub>, 0.5 µl Taq (Promega), 2 µl of BSA (10 mg/ml), 4 µl of 1 mM dNTPs, 2.5 µl of each primer (10 µM) and 2.0 µl of DNA; the remaining volume was filled with water. The PCR profile was: 1 min at 94°C, 1 min at 56–58°C, 1 min at 72°C for 35 cycles, always preceded by an initial melting step of 2 min at 94°C, and with a final extension of 7 min at 72°C. The PCR products

were purified and bidirectionally sequenced by Macrogen, Inc. (Seoul, Korea).

Sequences were assembled using *Geneious* v. 6.1.6 (Biomatters 2010), initially aligned using Clustal X (Higgins & Sharp 1988), manually adjusted in *PhyDE* (Müller *et al.* 2006) and exported as Nexus files. All polymorphisms were validated by visually checking of the original electropherograms.

#### *Phylogenetic and haplotype analyses*

Phylogenetic analyses of the ITS sequences, which could be obtained for all 69 specimens, were carried out using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). MP analyses were carried out using *PAUP* v. 4.0b10 for Macintosh (Swofford 2002). Heuristic searches were performed with 1000 random addition replicates and tree-bisection-reconnection branch swapping, saving a maximum of 10,000 trees. All characters were unordered and equally weighted, and gaps were either treated as missing data or coded as informative by a simple indel coding (SIC) strategy (Simmons & Ochoterena 2000) as implemented in *SeqState* (Müller 2004). ML analyses were carried out using *GARLI* v. 0.951 for Macintosh (Zwickl 2006). Clade support for MP and ML was assessed from bootstrap analyses with 1000 replicates (Felsenstein 1985). For ML and BI analyses, the best-fit model of evolution (GTR + I +  $\Gamma$ ) was obtained based on the Akaike information criteria using *jModeltest* 3.06 (Posada 2008). BI analyses were carried in *MrBayes* v. 3.2.5 (Ronquist *et al.* 2012). Two runs with four Markov chain Monte Carlo chains were run for (Piñeiro *et al.* 2012). Chains were sampled every 1000 generations and the respective trees were written to a tree file. Convergence of runs was verified by ensuring that the average standard deviation of split frequencies was  $< 0.01$ . *Tracer* 1.5 (Rambaut & Drummond 2013) was used to determine when the tree sampling stabilized. The first 25% of the trees were discarded as 'burn-in'. A majority rule consensus tree and posterior probabilities (PPs) were calculated from the resulting trees.

Haplotype analyses of 44 *Roaldia* specimens for which all three markers could be sequenced were performed with two datasets: 1) the chloroplast marker *rp16* intron and 2) the nuclear ribosomal markers ITS (ITS1-5.8S-ITS2) and partial 26S gene concatenated. Haplotypes were inferred based on statistical parsimony using *TCS* v.1.21 (Clement *et al.* 2000).

#### *Morphometric analysis*

As sporophytes of *R. revoluta* are unknown from Antarctica, only gametophytic characters were scored. Furthermore, leaf characters were restricted to stem leaves, as plants from King George Island (Antarctica)

usually produce very small branches, making it almost impossible to measure their branch leaves. Microscopic slides with several leaves taken from the median region of the gametophyte were prepared under a dissecting microscope and mounted using Hoyer's solution (Anderson 1954).

Nine quantitative characters of the stem leaves were selected (leaf length, leaf width, acumen length, acumen base width, acumen width, length of longer costa branch, length of shorter costa branch and length and width of five cells at mid-leaf). Each character was measured on five leaves taken from one stem per specimen using an optical microscope (Leica DM750) and a video camera (MC 170 HD) to capture the images to a computer. *Leica Application Suite* software (Piñeiro *et al.* 2012) was used for image analysis.

A matrix was constructed with the median values obtained from the five leaves measured from each specimen for four characters (leaf length, leaf width, acumen length and acumen width) and subjected to principal component analysis (PCA) in *PAST* 3.15 (Hammer *et al.* 2001). Linear discriminant analysis (LDA) was performed to ascertain the significance of the groups formed. The cell and costa characters were excluded from these analyses due to low significance in the analysis.

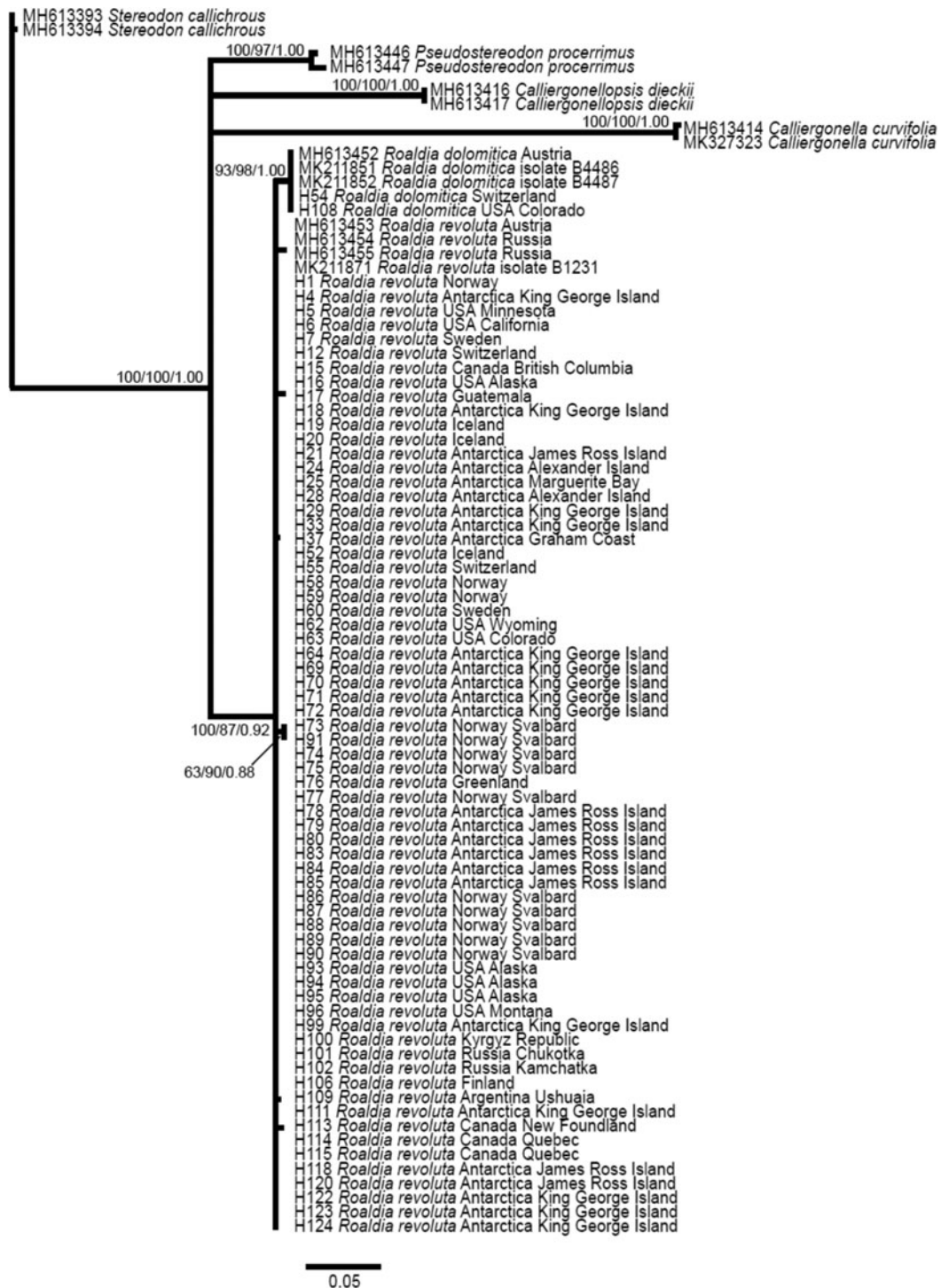
## Results

#### *Phylogenetic analysis*

The ITS alignment for phylogenetic analysis comprised 789 positions, of which 100 were parsimony-informative. SIC yielded another 86 characters, 81 of which were parsimony-informative. The Bayesian phylogenetic reconstruction is shown in Fig. 1, with PPs and bootstrap support from the respective MP (MP-BS) and ML (ML-BS) analyses shown at the branches. All analyses revealed a monophyletic *Roaldia* clade with high bootstrap support (MP-BS 100%, ML-BS 87%) and a PP of 0.92. Within the *Roaldia* clade, one clade comprising all included specimens of *R. dolomitica* formed a subclade with high support (PP1, MP-BS 93%, ML-BS 98%), and two *R. revoluta* specimens from Svalbard formed a subclade with lower bootstrap support (MP-BS 63%, ML-BS 90%). These subclades and all other *R. revoluta* specimens formed a large polytomy. The clades of the species of the other included genera all received maximum support except ML-BS 97% for *P. procerrimus*. Relationships between these clades and the *Roaldia* clade remained unresolved.

#### *Haplotype analysis*

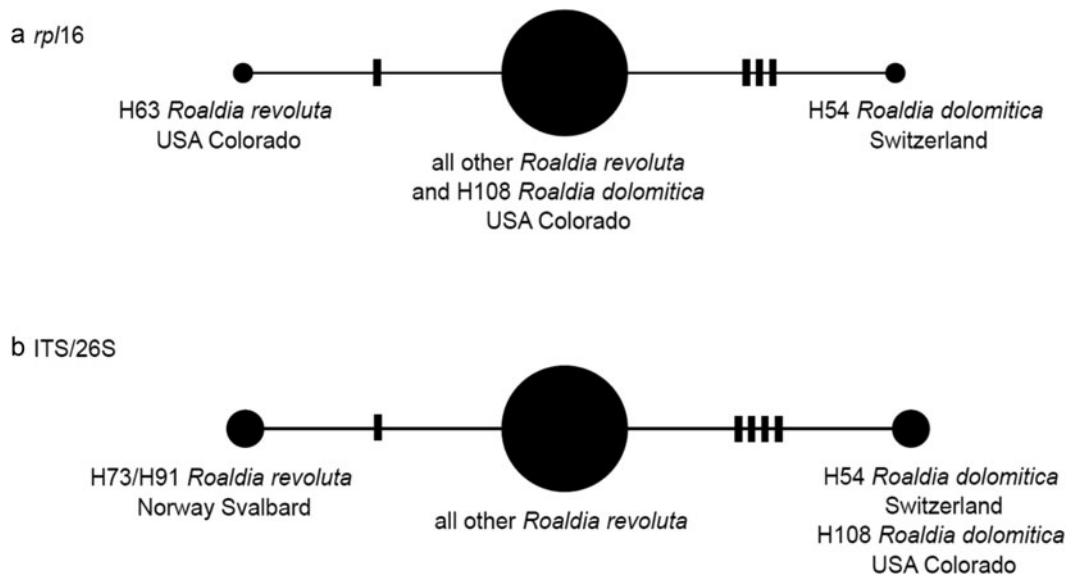
The alignments of the chloroplast *rp16* intron and nuclear ribosomal ITS/partial 26S sequences of the 44 included



**Fig. 1.** Bayesian phylogenetic reconstruction from ITS sequences of *Roaldia revoluta*, *Roaldia dolomitica* and related taxa of the Hypnales, with two samples of *Stereodon callichrous* as outgroup representatives. Posterior probabilities (PPs) > 0.95 and bootstrap support > 60% from respective maximum parsimony (MP-BS) and maximum likelihood (ML-BS) analyses are shown at the branches in the order MP-BS/ML-BS/PP.

*Roaldia* specimens comprised 684 and 1633 positions, respectively, without any length variation. Three *rp116* haplotypes were observed, one in one of the two

included samples of *R. dolomitica* (H54), the second in (Piñeiro *et al.* 2012) sample H63 from the USA and the third in all remaining samples (Fig. 2a).



**Fig. 2.** Haplotype networks of **a.** the chloroplast marker *rpl16* and **b.** the nuclear ribosomal ITS/partial 26S of 44 specimens of *Roaldia revoluta*/*Roaldia dolomitica*.

In the nuclear region three haplotypes were observed as well, one in both included samples of *R. dolomitica*, the second in the two *R. revoluta* samples from Svalbard that were separated in the phylogenetic analysis as well and the third in all remaining samples (Fig. 2b).

#### Morphometric analysis

Biplots of PCA and LDA are presented in Figs 3 & 4, respectively. Table II shows the coefficient and correlation values of the variables with the components. Leaf length was most closely related to the first axis, while leaf width and length of the leaf apex were more related to the second axis. The scree plot (Fig. 5) showed that the first component was sufficient to explain the variation. The first component explained 90.8% of the variation and the second component explained 6.7% of the variation (with eigenvalues of 0.10 and 0.008, respectively). The results of the morphometric analyses show a continuous range of morphological variation, with a tendency of higher variation in the Northern Hemisphere and some differentiation, but also considerable overlap, between specimens from both hemispheres (Figs 3 & 4).

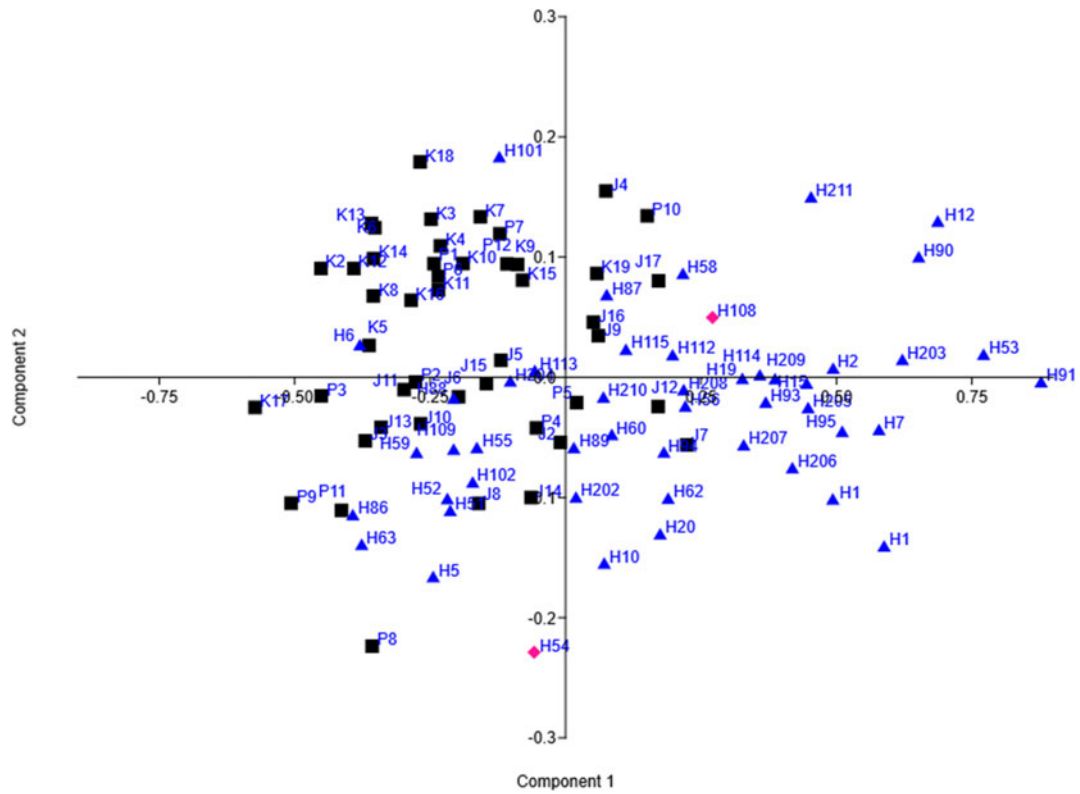
#### Discussion

Our phylogenetic analyses based on ITS sequences clearly resolve *Roaldia* as monophyletic, confirming the preliminary results of previous studies (Câmara *et al.* 2018b, Schlesak *et al.* 2018, Kučera *et al.* 2019). The lower support for the *Roaldia* clade in our ML and BI

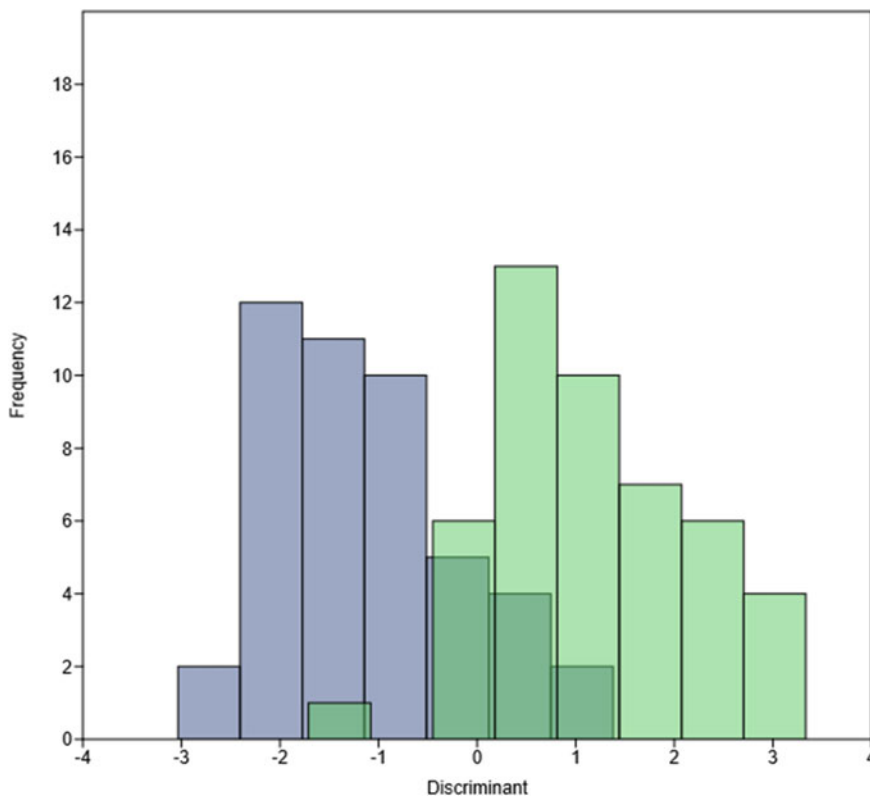
analyses compared to the phylogenetic trees in Schlesak *et al.* (2018) and Kučera *et al.* (2019) probably results from the single-marker (ITS) vs multiple-marker approaches. Furthermore, our extended taxon sampling supports *R. revoluta* as a truly bipolar species, as specimens from both hemispheres and both polar regions comprise the same clade. Intraspecific molecular variation is low, and most specimens from both polar regions belong to a single haplotype in each investigated marker.

The only well-distinguished subgroup within the ITS-based phylogeny corresponds to *H. revolutum* var. *dolomiticum*. In the plastid marker *rpl16*, in contrast, only one of the two newly analysed specimens (H54) forms a separate haplotype. This specimen also appears more distinct in the PCA, whereas specimen H108 is indistinguishable from other Northern Hemisphere specimens both morphologically and by its *rpl16* sequence. More extensive analyses of molecular data and possibly sporophytic characters may give more insight into the relationship between *R. dolomitica* and *R. revoluta*, but based on the available data we propose to continue distinguishing the former as a variety of *R. revoluta*, for which we provide a new combination.

The low intraspecific genetic diversity of *R. revoluta* corroborates the findings of Câmara *et al.* (2018b) for *R. revoluta* and Biersma *et al.* (2018a) for the acrocarpous moss *Chorisodontium aciphyllum* (Hook. f. & Wilson) Broth. (Dicranaceae). According to these studies there is very low genetic variability - even in markers that are often variable at the species and population levels (particularly ITS; Stech & Quandt 2010) - between populations of mosses from Antarctica and South America or, as shown



**Fig. 3.** Principal component analysis of *Roaldia revoluta* samples from the Northern Hemisphere (blue triangles; specimens identified as *Roaldia dolomitica* are displayed as red diamonds) and the Southern Hemisphere (black squares).



**Fig. 4.** Graphic of the linear discriminant analysis showing the intersection between the two groups of Antarctic (grey) and Arctic (green) *Roaldia revoluta* specimens.



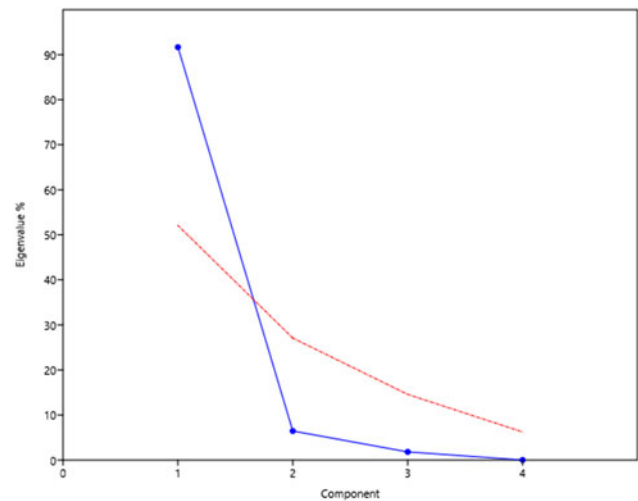
**Table II.** The coefficient and correlation values of the variables with the first and second axes of the principal component analysis.

	Coefficients		Correlation	
	PC1	PC2	PC1	PC2
Length leaf	0.90	0.14	0.93	-0.03
Wide_apex	0.01	-0.002	0.76	-0.01
Wide_leaf	0.10	0.84	0.47	0.87
Length_apex	0.41	-0.51	0.89	-0.31

PC = principal component.

here, across the whole bipolar distribution area. In the case of *Roaldia* and *Chorisodontium*, this may reflect taxon-related patterns: the pleurocarpous order Hypnales is considered to have evolved by rapid diversification that resulted in generally low genetic variation, short branches and consequently low support in phylogenetic trees (Shaw *et al.* 2003), while several closely related genera of Dicranaceae have also been shown to exhibit low molecular variation, complicating species delimitation and phylogeographic analysis (Lang *et al.* 2014, 2015). In other pleurocarpous species, low variation was observed as well, such as within the bipolar *Drepanocladus capillifolius* (Warnst.) Warnst. and the South American-Antarctic *D. longifolius* (Sařuga *et al.* 2018), among Antarctic populations of *S. uncinata* (Hebel *et al.* 2018) and in Arctic/Scandinavian populations of *Buckia voucheri* and *Campylium bambergeri* (Hedenäs 2014a). In bipolar *Polytrichum* species (Polytrichaceae), however, ITS exhibited higher variation, in particular compared to the plastid marker *trnL-F* (Biersma *et al.* 2017).

*Buckia voucheri*, *C. bambergeri* and *R. revoluta* are all segregates of the formerly broadly treated genus *Hypnum* that rarely produce sporophytes. Ando (1972) assumed that the contemporary populations of such *Hypnum* s.l. species are relicts of a once more continuous distribution that lost the ability of sexual reproduction. Relict populations could then have undergone a gradual loss of haplotypes, as was inferred for populations from Scandinavian lowland glacial refugia in *Rhytidium rugosum*, a pleurocarpous moss species that does show significant intraspecific genetic variation (Hedenäs 2014b). It might thus be that only the current main Northern Hemisphere haplotype of *R. revoluta* survived in Antarctica. Another possible explanation could be one or multiple long-distance dispersal events of the same haplotype. As sexual reproduction in *Roaldia* is unknown in Antarctica but does occur rarely in the Northern Hemisphere (seemingly more frequently in North America than in Europe; Ando 1973), under this scenario it is most probable that spores were transported from the Northern Hemisphere into Antarctica by means of birds or wind (cf. Lewis *et al.* 2014). However, as Hedenäs (2014a)



**Fig. 5.** Scree plot showing the line above broken stick values (red dashed line) for the first component. The percentages of eigenvalues for all components are represented by the blue line.

already concluded for *B. voucheri* and *C. bambergeri*, possible migration patterns cannot be inferred when haplotype variation is too low. In cases of such low molecular variation, sequencing approaches such as RAD sequencing (Lewis *et al.* 2017) or hybrid capture sequencing should be applied, which may provide more genetic information than adding further single markers. Such an approach may also help us to infer the distribution history and possible dispersal routes between both polar regions of *R. revoluta*.

The morphometrics presented by Câmara *et al.* (2018b) suggested the existence of two different morphotypes in Antarctica, one being more frequent in King George Island, the other more common in James Ross Island and both present in the Antarctic Peninsula. This differentiation (samples K, J and P in Fig. 3) is here extended by a broader range of morphological variation in the Northern Hemisphere, which may not be surprising considering that samples originate from a much larger area and from habitats with various environmental conditions. On the other hand, the present results suggest that there are no significant morphological differences among populations from both polar regions. Furthermore, 'extreme' morphotypes seem to occur at both poles (e.g. sample P8 vs H91; Fig. 3), but the large intersection of points in the middle of the graph suggests that such morphotypes are not common. Altogether, the extended sampling seems to corroborate the conclusion of Câmara *et al.* (2018b) that intraspecific morphological variation is mainly influenced by environmental regulators at small spatial scales (microhabitats), as is also inferred in other studies (McDaniel & Shaw 2003, Pereira *et al.* 2013, Medina *et al.* 2014, Amorim *et al.* 2017).

## Taxonomic and nomenclatural implications

*Roaldia revoluta* (Mitt.) P.E.A.S. Câmara & Carv.-Silva var. *dolomitica* (Milde) P.E.A.S. Câmara, Carv.-Silva & M. Stech, comb. nov. - Basionym: *Hypnum dolomiticum* Milde, *Botanische Zeitung (Berlin)*, **22** (Beilage zu No. 17–19): 21. 1864.

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## Author contributions

PEASC, MC-S, DVV, DKH, ETdA and WSF performed the fieldwork in Antarctica. PEASC and MC-S conducted the laboratory work. MC-S, WSF and MS performed the bioinformatic analysis. WSF and MS worked on the haplotype study. DVV, DKH and ETdA performed the statistical analysis. HK and MS conducted the fieldwork in the Arctic. All authors contributed to the previous and final versions of the manuscript.

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## Conflicts of interest

The authors declare none.

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