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Molecular and morphological evidence support the reinstatement of *Cheilolejeunea comitans* from synonymy of *C. krakakammae*.

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Abstract

In 2006 *Cheilolejeunea comitans* (Hook.f. & Taylor) R.M.Schust., previously treated as endemic to New Zealand and Australia, was placed in synonymy of *C. krakakammae* (Lindenb.) R.M.Schust., a species with an African type. Here we generated DNA sequences from samples of New Zealand *C. comitans* and compared them with published sequences for *C. krakakammae* from China. The molecular evidence, together with a new analysis of morphological evidence from the isolectotype and samples of *C. comitans*, and from the holotype, isotype and descriptions of *C. krakakammae,* justify the reinstatement of *C. comitans*.

Key Words: Liverwort, New Zealand, Australia, *Cheilolejeunea krakakammae, C. khasiana, C. comitans,* synonymy, phylogeny.

Introduction

Cheilolejeunea comitans (Hook.f. & Taylor) R.M.Schust., previously treated as endemic to New Zealand and Australia, was placed, together with the Asian *C. khasiana*, in synonymy of *C. krakakammae* (Lindenb.) R.M.Schust., a species with an African type (Zhu 2006). That study, undertaken at the time without the benefit of molecular evidence, proposed a pan-paleotropical, including Oceania, distribution for *C. krakakammae*. A common factor in the species synonymised under *C. krakakammae* was the usual or frequent presence of eplicate perianths. In order to test this broad circumscription for *C. krakakammae* proposal with respect to *C. comitans*, we compare newly-generated DNA sequences from *C. comitans* from New Zealand with published sequences for *C. khasiana as C. krakakammae* from China. Samples of *C. krakakammae* from Africa were not available for DNA sequencing for this study. In addition, we compared the morphology of herbarium samples of *C. comitans* from New Zealand and Tasmania including the isolectotype, to the holotype and isotype of *C. krakakammae*, and compared available descriptions.

Materials and Methods

Genomic DNA was extracted from two samples of fresh material of *Cheilolejeunea comitans* from New Zealand with a modified-CTAB DNA extraction method (steps 1, 3–7 from Table 1 in Shepherd and McLay, 2011). The nuclear ribosomal internal transcribed spacer 1 and 2 including the 5.8S ribosomal subunit (nrITS) was amplified with the primers of Hartmann *et al.* (2006). Two chloroplast loci were also amplified: the trnL (UAA) 5´exon - trnF (GAA) intergenic spacer (*trn*LF) was amplified using the primers of Taberlet *et al.* (1991) and the transfer RNA$^{\text{Gly}}$ (UCC) (*trn*G) was amplified with Pacak and Szweykowska-Kulinska's (2000) primers.

Table 1. Specimens used for phylogenetic analysis in this study, including herbarium voucher information and GenBank accession numbers.

Species	Voucher	GenBank accession numbers		
		trnL-F	*trnG*	ITS
Cheilolejeunea aurifera	*I. Holz CR00-0812* (GOET)	KT190825	KT190883	KT190949
Cheilolejeunea comitans	WELT H014163	MK294020	MK294018	MK294016
Cheilolejeunea comitans	WELT H014265	MK294021	MK294019	MK294017
Cheilolejeunea ecarinata	*A. Vojtko 9427/AS* (EGR)	-	KT190878	KT190944
Cheilolejeunea krakakammae	*R.L. Zhu 20070319-7* (HSNU)	KT190814	KT190869	KT190935
Cheilolejeunea laevicalyx	*S.R. Gradstein 10104* (GOET)	KT190820	KT190875	KT190941
Cheilolejeunea laevicalyx	*S.R. Gradstein & A. Velasquez s.n.* (GOET)	-	KT190876	KT190942
Cheilolejeunea laeviuscula	*A. Schäfer-Verwimp & I. Verwimp 23802A* (HSNU)	KT190813	KT190868	KT190934
Cheilolejeunea mimosa	*A. Schäfer-Verwimp & I. Verwimp 13664* (GOET)	KT190821	KT190877	KT190943
Cheilolejeunea pluriplicata	*A. Schäfer-Verwimp & I. Verwimp 23883B* (HSNU)	KT190811	KT190867	KT190932
Cheilolejeunea pocsii	*T. Pócs et al. 04011/AW* (EGR)	KT190812	-	KT190933
Cheilolejeunea roccatii	*E. Fischer X-RWA-1120* (Herb. Schäfer-Verwimp)	KT190802	KT190858	KT190923
Cheilolejeunea trifaria	*A. Schäfer-Verwimp & I. Verwimp 22434* (GOET)	KT190817	KT190872	KT190938

PCR amplifications were performed in 12 μl reactions with 1× Mytaq reagent buffer (Bioline, Australia), 5 pmol of each primer and 1 M betaine. PCR thermocycling conditions followed Ye *et al.* (2015) for ITS and Shaw *et al.* (2005) for the two chloroplast loci. PCR products were purified by adding 0.5 U shrimp alkaline phosphatase (SAP, USB Corp.) and 2.5 U exonuclease I (ExoI, USB Corp.) and digested at 37°C for 15 minutes, followed by inactivation of the enzymes at 80°C for 15 minutes. Sequencing was performed with the ABI Prism Big Dye Terminator cycle sequencing kit version 3.1 on an ABI 3730 DNA sequencer (Macrogen, Seoul, Republic of Korea) and sequences were edited in Sequencer 5.2.3 (Gene Codes Corporation).

The newly-generated sequences were aligned to published sequences (Table 1) from Ye *et al.* (2015) using MAFFT 6.849 (Katoh and Toh, 2008), at the EMBL-EBI online server, with default settings. Regions of low homology at each locus were detected and removed using Gblocks (Talavera and Castresana 2007), with the least restrictive settings.

Maximum likelihood (ML) analyses were performed on the individual and combined datasets with the PhyML v3.0 web server (http://www.atgc-montpellier.fr/phyml/; Guindon *et al.* 2010), with the best-fit model of sequence evolution determined with Smart Model Selection (Lefort *et al.* 2017). Heuristic searches were performed with 10 random addition sequence replicates and SPR branch-swapping and branch support was assessed with 1000 bootstrap pseudoreplicates.

Bayesian analyses (BA) was performed with MrBayes v3.2.1 (Huelsenbeck and Ronquist 2001). Two concurrent analyses were run, each with four Markov chains of five million generations and sampling every 1000 generations. Each locus was assigned as a separate partition and rates allowed to vary across partitions. For each locus the best-fit model of sequence evolution was determined using the Akaike information criterion (AIC) in jModelTest v2.1.7 (Darriba *et al.* 2012). The first 20% of samples were discarded as "burn-in", after this point the standard deviation of split frequencies was below 0.01. Tracer v.1.6 (Rambaut and Drummond 2009) was used to confirm that stationarity had been reached. Trees were rooted with *Cheilolejeunuea roccatii*, based on Ye *et al.*'s (2015) phylogeny.

Herbarium specimens from throughout the range of *Cheilolejeunea comitans* in New Zealand and Australia were examined and compared with the isolectotype of *C. comitans*, (S-B47667) and with the types and descriptions of the types of *C. krakakammae* (holotype S-B30191 and isotype S-B30192). For the purposes of examination of (S) type materials, digital images of selected features were made available online, courtesy of Lars Hedenäs.

Results

The two *Cheilolejeunea comitans* specimens had identical *trn*LF and *trn*G sequences and differed at the ITS locus by specimen WELT H014265 having one more T in a mononucleotide run than WELT H014163. All sequences have been deposited in GenBank (Accession numbers MK294016- MK294021), and sequences used in the analysis are provided in Table 1. The initial MAFFT alignment across all loci was 2622 bp in length. The removal of ambiguous alignment positions by Gblocks resulted in a final alignment of 2386 bp, of which 304 characters were parsimony informative. The maximum likelihood (ML) and Bayesian analyses (BA) of the individual and combined datasets resulted in trees with similar topologies (Fig. 1) In all analyses the *C. comitans* specimens did not cluster with the *C. khasiana* as *C. krakakammae* sequence from Ye *et al.* (2015). Instead *C. comitans* formed a clade with *C. laevicalyx* and *C. mimosa* with strong support (100% BS ML, 1.00 PP). The *C. khasiana* sequence was sister to *C. laeviuscula* (100% BS ML, 1.00 PP) with these forming a clade with *C. pluriplicata* and *C. pocsii* (99% BS ML, 1.00 PP).

Morphological differences between, *Cheilolejeunea comitans, C. mimosa* and *C. laevicalyx*

Although the three species, *Cheilolejeunea mimosa* (Hook.f. & Taylor) R.M.Schust., *C. comitans* and *C. laevicalyx* (J.B.Jack & Steph.) Grolle grouped together in the same clade in the phylogeny (Fig. 1), they are clearly morphologically distinct. Two Australasian species, *C. mimosa* and *C. comitans* were both included by Grolle in his study of the Lejeuneaceae of Tasmania, based on an examination of types and of herbarium records (Grolle 1982). Detailed description and illustrations of *C. laevicalyx*, a species from Bolivia, Columbia and Equador are included by Zhu (2006) in his study of *C. krakakammae* and treated as distinct from that species. These sources have been drawn on, together with images of the isolectotype (S-B47667) of *C. comitans*, for the following analysis with additional notes on *C. mimosa* from images of the *C. mimosa* lectotype (FH 00079610). What follows is a summary of the morphology of these three species.

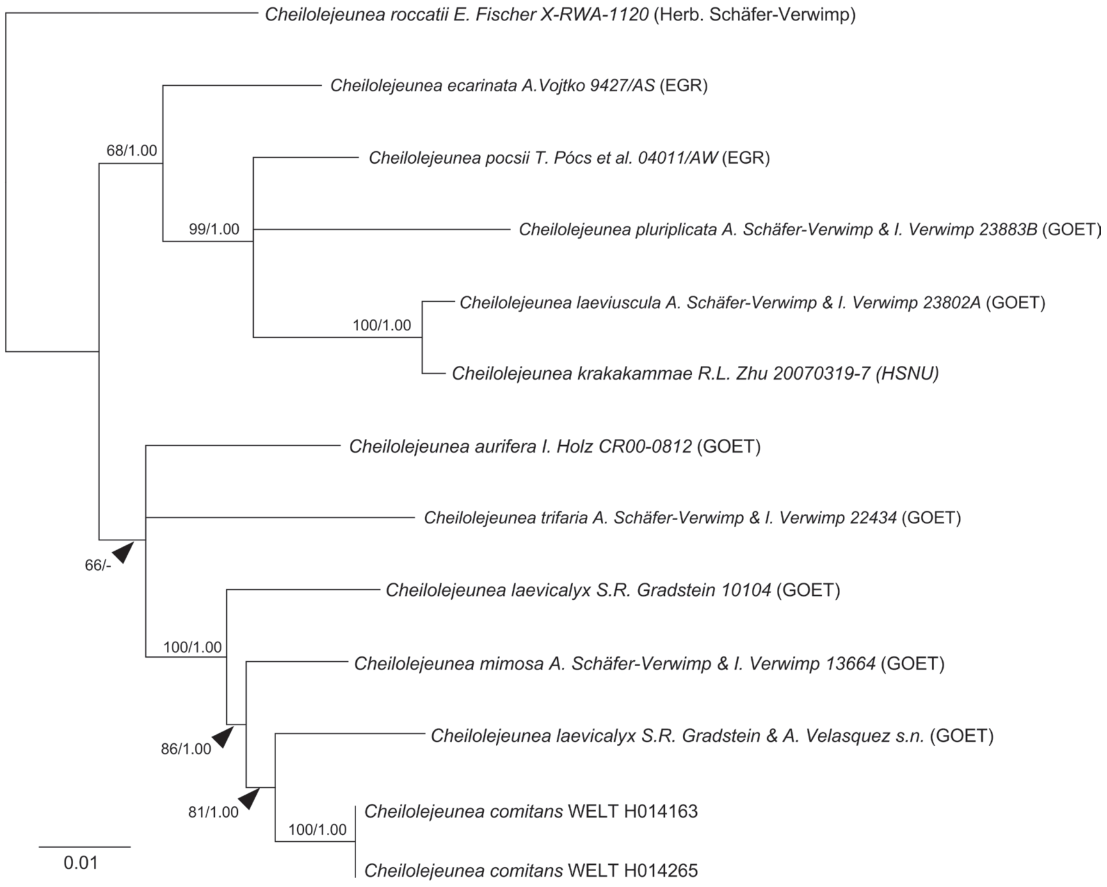

Fig. 1. Phylogram from the maximum likelihood phylogenetic analysis showing *C. comitans* does not group with the Asian *C. khasiana* (labelled as *C. krakakammae* in this tree). Support values are given in the order of: maximum likelihood bootstrap support and posterior probability in the Bayesian inference.

Cheilolejeunea comitans: Perianth mostly eplicate, exceptionally with 'rounded to obtuse keels', with circular apical depression, rostrum 4–)5–7(–8) cell tiers with distal dilation, second lobule tooth sharp single celled, first tooth single-celled, rectangular, and is not prominent, ventral merophyte 2-cells wide.

Cheilolejeunea mimosa: Perianth sharply pentacarinate, obovate, without circular apical depression, rostrum 2 or 3 cell tiers without distal dilation, second lobule tooth sharp single-celled, first tooth single cell, rectangular, and is not prominent, ventral merophyte 2 cells wide.

Cheilolejeunea laevicalyx: Perianth obovoid or pyriform. 0.83–1.8 mm long, eplicate or with 3–4 keels, truncate or retuse, rostrum indistinct or absent, lobule second tooth rounded single cell or is inconspicuous, first tooth usually a prominent two or three-celled bulge, ventral merophyte 3–5 cells wide.

Morphological comparison of *C. comitans* and *C. krakakammae*

Morphological evidence for distinguishing between *C. comitans* and *C. krakakammae* is most readily obtained from the gynoecium. What follows is a summary of the gynoecium of *C. comitans* and *C. krakakammae*, highlighting the differences between them, also presented in Table 2.

Table 2. Summary comparison of gynoecial morphology of *C. comitans* and *C. krakakammae*, with additional notes on *C. khasiana* based on Zhu *et al.* (2002), and including the synonym of *C. khasiana*, *C. giraldiana* (Mass.) Mizut. based on Asthana *et al.* (1995) and Mizutani (1982).

	C. comitans	**C. krakakammae**	**C. khasiana**
Perianth shape	pyriform-turbinate	obovoid	obovoid
Perianth length	0.47–0.55 mm	0.6–0.7 mm	0.75–0.9 mm
Perianth width	0.35–0.4 mm	ca 0.35–0.5 mm	0.45–0.5 mm
Perianth widest	>0.7 from base	<0.7 from base	<0.7 from base
Bracteole/perianth ratio	(0.8–) 1	ca 0.6	ca 0.55
Perianth circular apical depression	yes	no	no
Rostrum cell tiers	(4–) 5–7 (–8)	3–6 (–12)	4–5
Rostrum distal dilation	yes	no	no (inferred)
Plication	Rare, not observed	Mostly ecarinate or with 3–4 broad keels	Mostly ecarinate or with 3–4 broad keels

Gynoecium of *Cheilolejeunea comitans*: *Cheilolejeunea comitans* has usually been recognised by the features of the gynoecium, which are distinctive and well-illustrated in the images of the isolectotype (Figs 2 and 3). The perianth is pyriform-turbinate, eplicate, tumid, widest just below the apex, with a shallow apical depression from which the rostrum arises. The rostrum (4–)5–7(–8) tiers of cells, which terminates with a distal dilation, the diameter at the apex greater than the median diameter. The bracteole is large, approximately the length of the perianth to which it is closely appressed, completely obscuring the perianth in ventral view, save for the gap between the bracteole lobes. The perianth in dorsal view is similarly largely obscured by the overlapping female bracts leaving only the apical section of the perianth visible between the leaves.

Twenty herbarium specimens of *C. comitans* were examined, three from Tasmania, and 17 from New Zealand, all of which were epiphytic. Except for rostrum distal dilation, the characteristic gynoecial features described were evident in the perianths of all specimens examined. The distal dilation of the rostrum showed variation from flanged to infundibulate to weak, occasionally absent. Expression was mixed within the collections. Of the 20 specimens examined, seven had some perianths with flanges, 12 had perianths with funnels, 13 had perianths with weak dilation and four collections had perianths without rostrum dilation. Only one scant collection had perianths with no distal dilation. All the perianths observed were eplicate.

In the protologue of *Lejeunea comitans* Taylor (1846), described the perianth as obcordate triquetrous. In his treatment of the Lejeuneaceae of Tasmania Grolle (1982) dismissed the protologue description of the perianth as triquetrous as being an artefact arising from pressing. He described the perianth as cylindrical and completely without carinae or with apex wide with three weak keels ('oder apical breit schwach dreifaltig'). In her key to the species of *Cheilolejeunea* in Australia Thiers (1997) characterised *C. comitans* as completely lacking keels. In contrast, a later key to the species of *Cheilolejeunea* in Australia (Zhu and Lai 2002), the perianth is described as mostly ecarinate or sometimes with 3-4 rounded to obtuse keels, 2 lateral keels and 1-2 ventral ones. In Zhu (2006) there is a single image of the *C. comitans* perianth, Fig. 1 n, which shows the pyriform-turbinate shape, without plicae or apical depression, and with a rostrum without distal dilation.

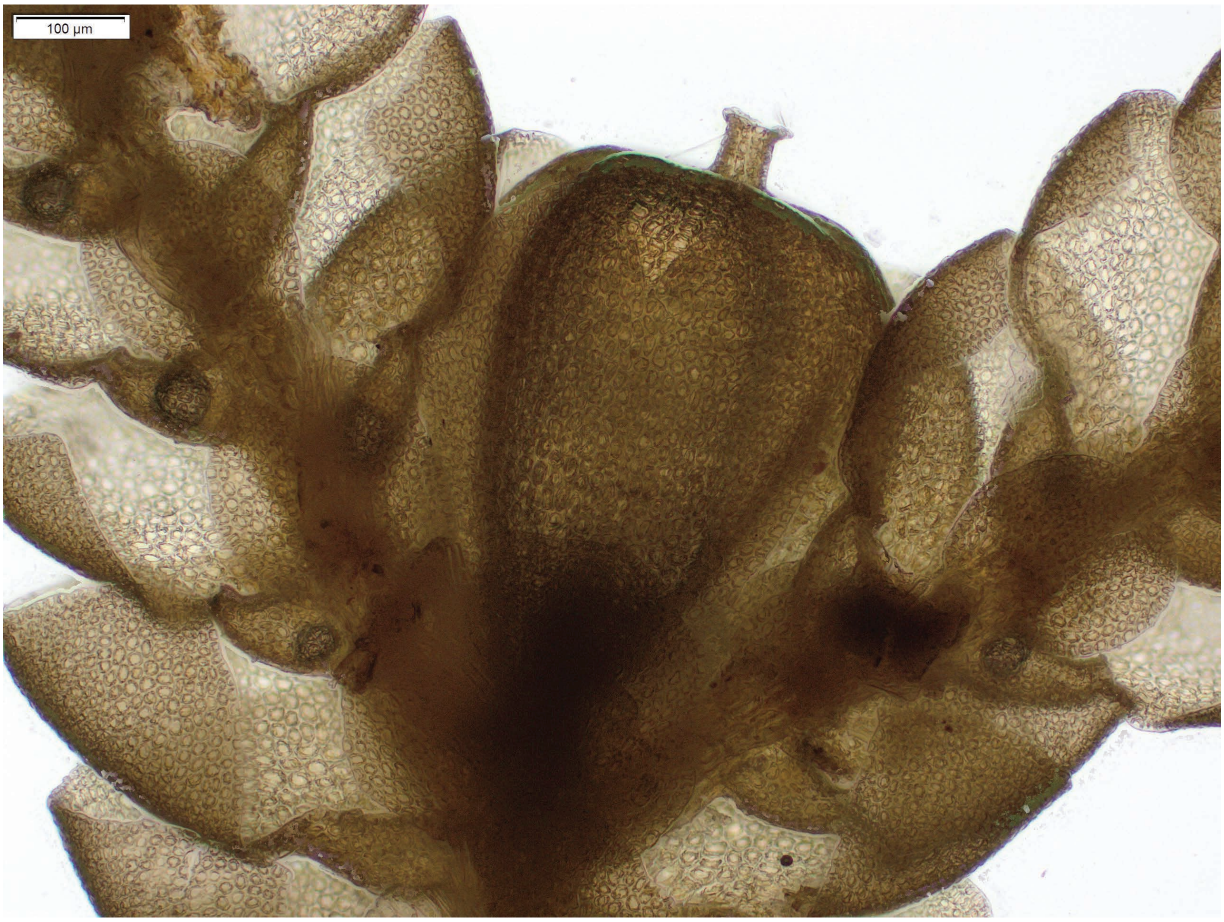

Fig. 2. Ventral view of *C. comitans* type (S) B47667 gynoecium with rostrum and with visible perianth apex between bracteole lobes.

Our observation of the herbarium specimens in this study found all to be ecarinate We conclude the reported plication of the *C. comitans* gynoecium to be uncommon. Examination of the morphology of the *C. comitans* gynoecium, including the isolectotype, shows it to be relatively constant. Perianth dimensions exhibit some variation with perianth width 0.34–0.4 mm and perianth length 0.47–0.55 mm. Rostrum cell tier numbers are (4–)5–7(–8) and length 60–75 µm. Very occasionally, perianths at maturity extend beyond the bracts and bracteole by 0.2× perianth length.

Gynoecium of *Cheilolejeunea krakakammae* types: The perianth, if not the full gynoecium, of *C. krakakammae* (Figs 4 and 5) has been described by Jones (1988) and by Zhu (2006) based largely on or including type materials. Zhu (2006) included an illustration of a *C. comitans* perianth but not a *C. khasiana* perianth. Zhu's description of *C. krakakammae* combines observations of *C. krakakammae, C. comitans, C. khasiana* and other synonyms using types and other material, and cannot be used to find distinguishing characters of the three. Jones' (1988) descriptions have a small number of *C. krakakammae* synonyms and limited geographic range in Africa , Kivu Volcan Ninagongo (Kivu Nyiragongo DR Congo),Tanzania, Natal (RSA), Transvaal (RSA),Cape Province (RSA Type locality), and Madagascar and are, perhaps, more informative. Perianth illustrations drawn from the types are provided by both authors, Jones (1988: p. 151, Fig. 1 a–d), and Zhu (2006: p. 192 Fig. 1d, h–m, o–q). These provide relevant evidence.

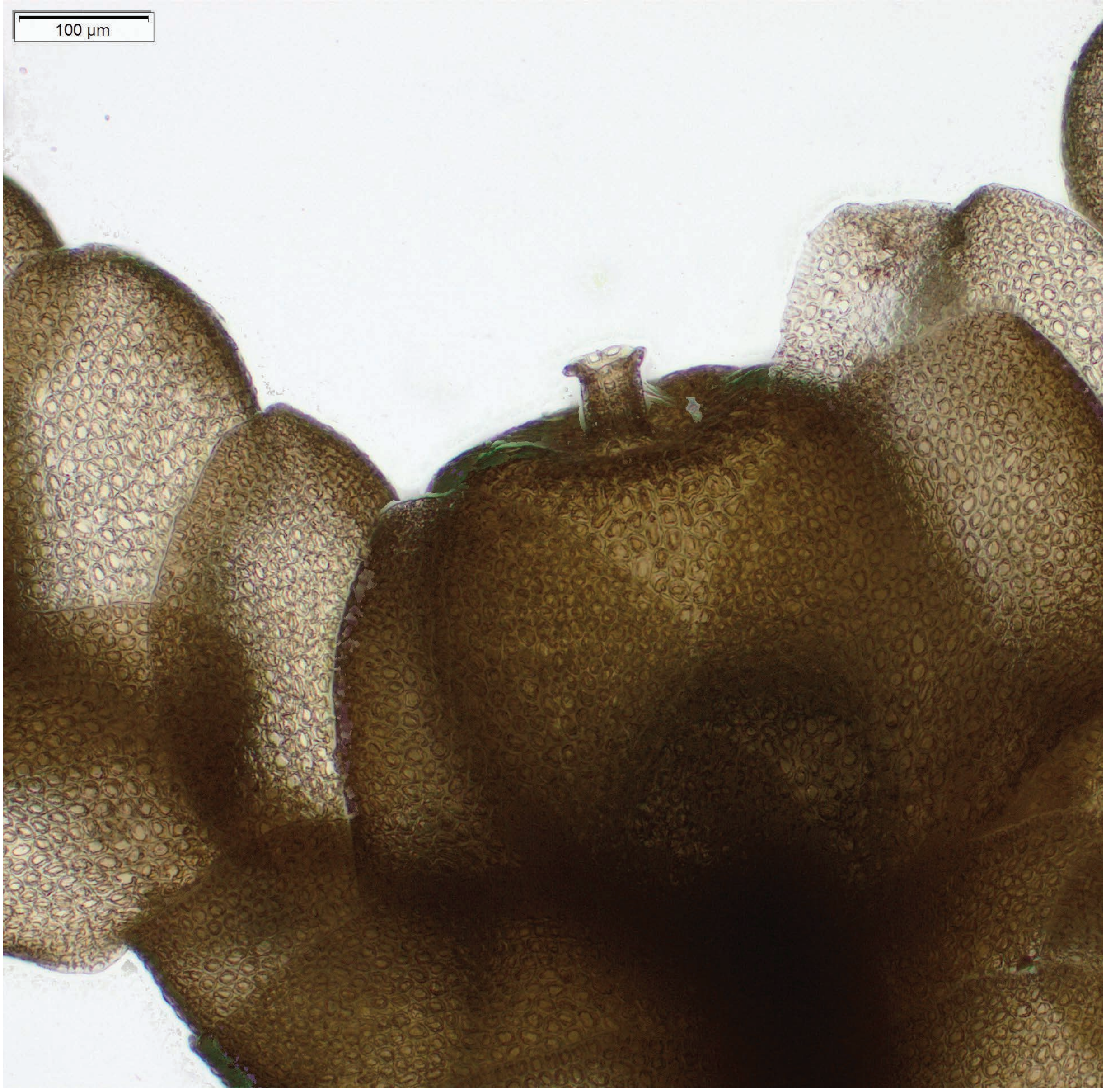

Fig. 3. Dorsal view of *C. comitans* type (S-B 47667) gynoecium with perianth largely obscured by bracts.

Jones' (1988: Fig. 1c) illustrates a ventral view of a type gynoecial stem with innovations, bracteole and perianth but without bracts. In the accompanying description including synonyms, perianths are described as '0.6–0.7 mm long, pyriform, varying from obscurely 5-carinate to almost terete and ecarinate when mature, dorsally more or less convex with median keel usually absent, less often weak and low; lateral keels around the apex distal parts of the flanks are very narrow to moderately wide at least the median part of the ventral surface, and often the whole of the ventral surface, strongly and broadly inflated, either ecarinate or distally with two obtuse ill-defined, more or less confluent keels, thus bluntly angled rather than carinate. Rostrum slender, often conspicuously long (c), up to 50–80 microns long'. Measurements derived from Fig.1 (c) show the perianth to be 0.65 mm long and the bracteole 0.43 mm long, ca. 70% perianth length. Zhu's Fig. 1 also provides a ventral view of a type gynoecial branch (c) with a bracteole ca. 0.6 mm long but the perianth is post discharge and dimensions uncertain. Variation in type perianth form and size, including plication is shown in Fig. 1 (o, p, and q), while variation in plication is further illustrated in the perianth cross sections, Fig. 1 (h–m).

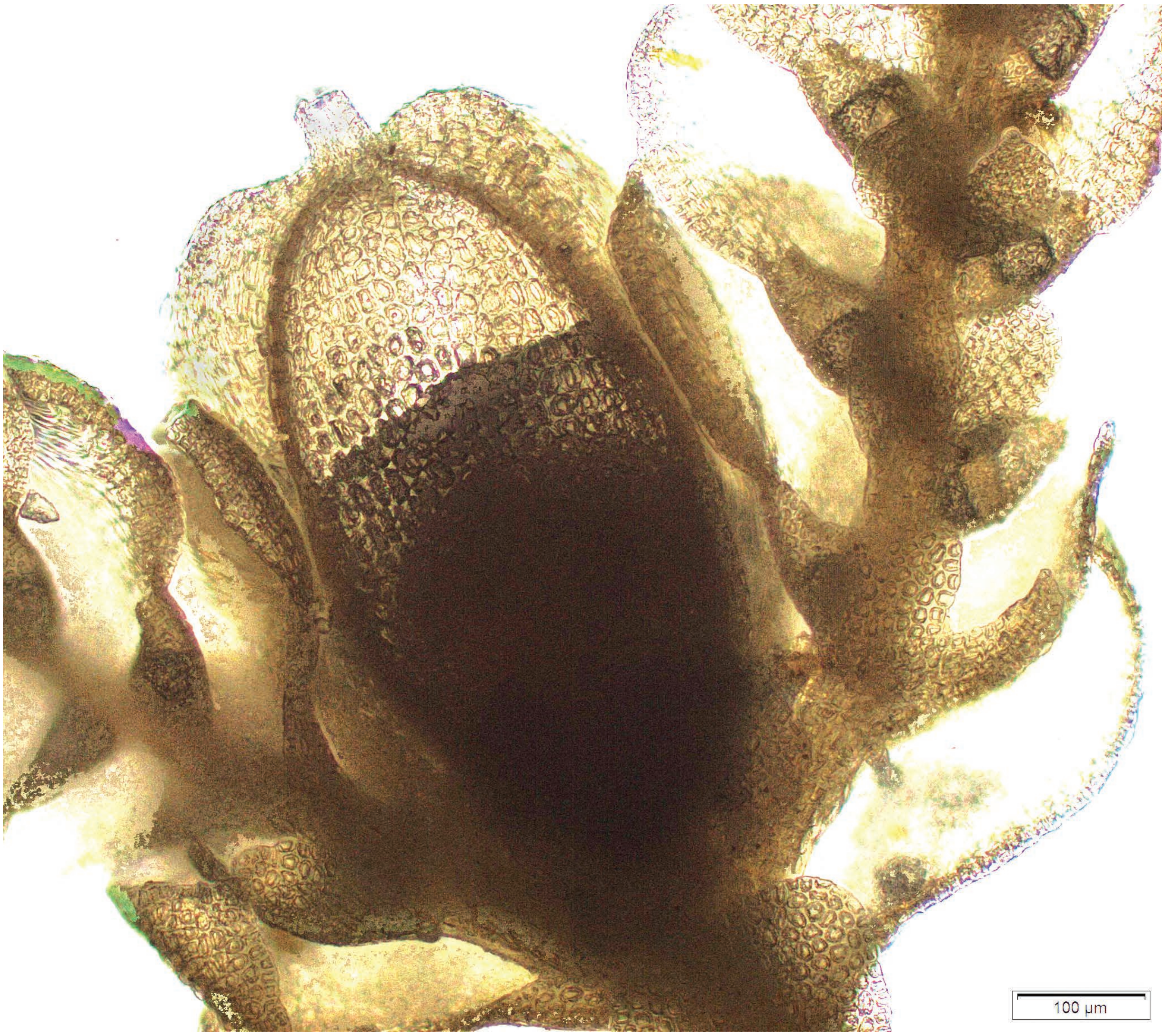

Fig. 4. Ventral view of a selected *C. krakakammae* holotype (S-B30191) gynoecium with rostrum and plication.

Distal rostrum dilation was present in almost all *C. comitans* specimens examined in this study and in the isolectotype (S-B 47667) images (Figs 2, 3). Jones (1988) and Zhu (2006) in their treatments and illustrations of the *C. krakakammae* type make no mention of rostrum dilation and we infer that it was absent in the specimens they examined. This is consistent with our observation of absence of dilation in three different *C. krakakammae* S type images viewed online, including in Figs 4 and 5. Further, we infer absence of dilation in C. khasiana, as noted in Table 2, on the grounds it was not mentioned by Zhu *et al.* (2002), Asthana *et al.* (1995) or Mizutani (1982). It is curious, however, that neither Grolle (1982) nor Zhu (2006) made reference to the rostrum in their descriptions of *C. comitans*.

Notes on additional specimens. Three additional Australian specimens are referred to by Zhu, CANB 551228 (Duplicates in NICH, NY, JE), *Engel 14608* (JE), and *Norris 33495* (JE). *Engel 14609* and *Norris 33495*, not seen as part of this study, were identified as *C. comitans* by Grolle (1982), prior to their placement by Zhu in *C. krakakammae*. CANB551228! is cited by Zhu as an example of eplicate and plicate perianths on the same plant. Apart from a small eplicate paper-fold sample separated by Zhu, the plant has sharply plicate ventral and lateral carinae. It does not have any of the characteristic features of *C. comitans*, and thus lies outside the scope of this study.

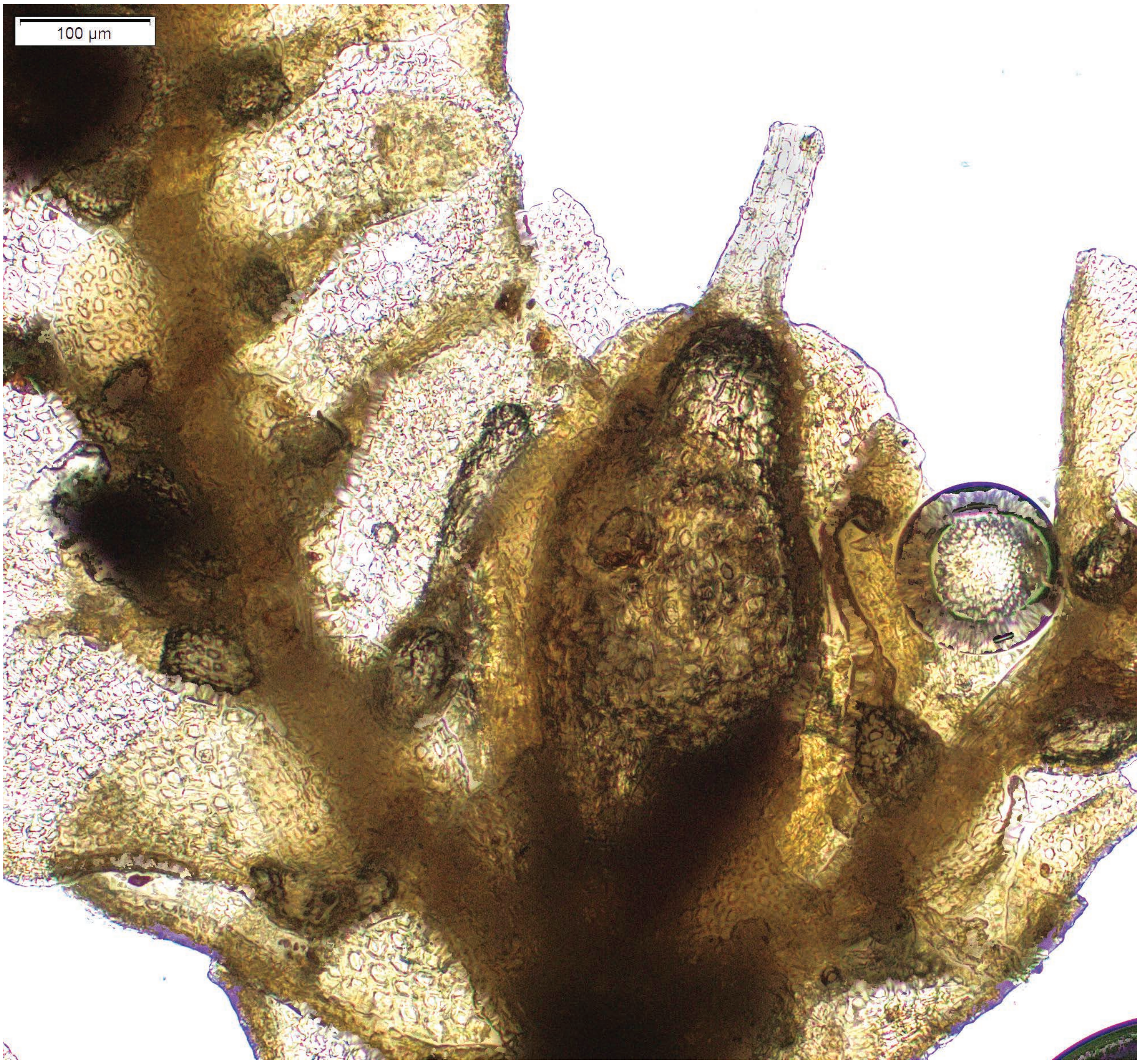

Fig. 5. Ventral view of a selected *C. krakakammae* isotype (S-B30192) gynoecium with rostrum and plication.

Discussion

Relationships inferred from molecular data refute the hypothesis that *Cheilolejeunea comitans* from New Zealand, and *C. krakakammae* from China included in the phylogeny presented by Ye *et al.* (2015) are the same species. It follows that the proposal of a pantropical and Pacific distribution for *C. krakakammae* including the Australasian *C. comitans* is not tenable. The *C. krakakammae* sequences from China were from the former *C. khasiana* (R-L Zhu *pers. comm.*), a taxon widespread in Asia. This outcome raises interesting questions about the status of *C. khasiana* and other species placed in synonymy of *C. krakakammae*, unfortunately *C. krakakammae* from Africa has not been included in any molecular study. In the absence of African *C. krakakammae* samples for sequencing for this study we have assembled morphological evidence to support the argument that *C. comitans* is not the same species as *C. krakakammae*, based on the distinctive gynoecial morphology of *C. comitans* from throughout its range in Australasia. When compared with the gynoecium of the *C. krakakammae* type material, the *C. comitans* gynoecium is consistently smaller, with a terete pyriform-turbinate perianth, a circular apical depression from which a rostrum arises with variable distal dilation, and the perianth is largely or wholly enclosed by bracts and a relatively large bracteole. Plication has been reported, but not seen in the herbarium material examined. The *C. krakakammae s.s.* gynoecium, by contrast, is both described and seen in the illustrations as variable in size, shape, degree of plication or eplicate, and in rostrum length. No gynoecia with the distinctive combination of features of *C. comitans* have been described for *C. krakakammae* or for *C. khasiana*. The distinctive gynoecium, together with the molecular evidence support the reinstatement of *C. comitans,* which we propose.

Specimens Examined: Australia: Tasmania: Pieman River, S bank, 41°38'57.8"S 145°03'48.8"E, 10 m, 4 Feb 2015, *L.H. Cave 2763*, (HO 579926); Granville harbour, 41°49'S 145°03'E, 50 m, 16 May 2013, *J. Jarman s. n.*, (HO 570624); Manuka Road, 43°06'S 146°41'E, 100 m, 5 May 1998, *J. Jarman s.n.* (HO 588715). **New Zealand**: North Island: Waipoua, Lookout Track, 35°39.80'S 173°34.30'E, 250 m, 28 Nov 1994, *D.S. Glenny 5471*, (WELT H010449); Waipoua, Waipoua River Road, 35° 39.0'S 173°29.9'E, 20 m, 27 Nov 1994, *D.S. Glenny 5471*, (WELT H010446); Coromandel Peninsula, Coroglen, 36°56.62'S, 175°40.20'E, 50 m, 6 Dec 2016, *P. Beveridge PE-3a,* (WELT H014163); Kaimai Ranges, Kaimai Summit, Loop Track, 37°52.42'S, 175°55.70'E, 460 m, 21 Nov 2013, *P. Beveridge NW-47b*, (WELT H013351); Kaimai Ranges, Dickey Flat Road, bank of Waitawheta River 37°26.25'S - 175°44.85'E, 100 m, 1 Dec. 2014, *P. Beveridge NX-22a*, (WELT H013369); Katikati, end of Hot Springs Road off SH 2, Tuahu Track**,** 37°35.92'S - 175° 55.55'E, 180 m 2 Dec 2014, *P. Brownsey s.n.*, (WELT H013550); 20 km S of Katikati, Te Tuhi Track, 37°45.77'S 175°57.60'E, 380 m, *P. Beveridge NV-72a*, (WELT H013455); Mangatoa Scenic Reserve, 38°25.68'S 174°42.85'E, 160 m, 4 Dec 2014, *P. Beveridge OA-50a*, (WELT H013574); Pureora Forest Park, Karamarama Stream, 38°28.60'S 175°33.83'E, 540 m, 2 Dec 2016, *P. Beveridge PA-10*, (WELT, H014196); 30 km W of Otorohanga, Appletree Rd, near Kokakoroa, 38°18.13'S 174°58.10'E, 280 m, *P. Beveridge NZ-18* (WELT H013556); 2 km S of Waikawau, Manganui Gorge Scenic Reserve, 38°29.22'S 174°41.70'E, 80 m, *P. Beveridge OB-36a*, (WELT H013406); 8 km W of Ohura on Tongaporutu Mangaroa Rd (SH 40) near Waitanga Saddle track toTV mast, 38°51.82'S 174°55.60'E, 550 m, *P. Beveridge MW-32a*, (WELT H012960); Tangarakau Gorge, Mangapapa Stream track, 38°58.88'S - 174°49.22'E, 40 m, 4 Feb 2014, *P. Beveridge NG-20a*, (WELT H013283); Whangamomona Scenic Reserve, 39°8.70'S 174°44.35'E, 180 m, 19 Nov 2013, *P Beveridge MU-76*, (WELT H012969); Karioi Station Road, Rotokura Ecological Reserve, 39°26.25'S 175°31.17'E, 680 m, 25 Nov 2013, *P Beveridge NC-1*, (WELT H013268); South Island: Nelson, Pelorus Bridge, (derived coordinates 41.29297° S 173.57164°E, 80 m) Jan 1945, *A.R. Hodgson s.n.,* (CHR 627378); Greymouth, Omoto forest, 43°31.13'S 171°16.37'E, 293m, 6 May 2002, *M.A.M. Renner s.n.* (CHR 583696).

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References

Asthana G, Srivastava SC, Asthana AK (1996) [1995] The genus *Cheilolejeunea* in India. - *Lindbergia* 20: 125–143.

Beveridge P, Glenny D. Shepherd L (2019) *Cheilolejeunea morganii,* (Lejeuneaceae, Marchantiopsida), a newly described species from lowland indigenous forest sites in New Zealand. *Journal of Bryology* 41: 157–165. https://doi.org/10.1080/03736687.2019.1584432

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. https://doi.org/10.1038/nmeth.2109

Grolle R (1982) Ubersicht der Lejeuneaceae in Tasmanien. *Wissenschaftliche Zeitschrift der Friedrich-Schiller-Universitaet Jena. Naturwissenschaftliche Reihe* 31: 207–227.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59: 307–321. https://doi.org/10.1093/sysbio/syq010

Hartmann FA, Wilson R, Gradstein SR, Schneider H, Heinrichs J (2006) Testing hypotheses on species delimitations and disjunctions in the liverwort *Bryopteris* (Jungermanniopsida: Lejeuneaceae). *International Journal of Plant Sciences* 167: 1205–1214. https://doi.org/10.1086/508023

Huelsenbeck JP, Ronquist FR (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Katoh K, Toh H (2008) Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. *BMC Bioinformatics* 9: 21. https://doi.org/10.1186/1471-2105-9-212

Lefort V, Longueville J-E, Gascuel O (2017) SMS: Smart model selection in PhyML. *Molecular Biology and Evolution* 34: 2422–2424. https://doi.org/10.1093/molbev/msx149

Jones EW (1988) African hepatics. XXXVIII. *Cheilolejeunea* subgenus *Strepsilejeunea* (Spruce) Schust., with special references to East Africa. *Journal of Bryology* 15: 149–160. https://doi.org/10.1179/jbr.1988.15.1.149

Mizutani M. (1982) Notes on the Lejeuneaceae. 6. Japanese species of the genus *Cheilolejeunea*. – *Journal of the Hattori Botanical Laboratory* 51: 151–173.

Pacak A, Szweykowska-Kulinska Z (2000) Molecular data concerning alloploid character and the origin of chloroplast and mitochondrial genomes in the liverwort species *Pellia borealis*. *Journal of Plant Biotechnology* 2: 101–108.

Rambaut A, Drummond AJ (2009) Tracer, version 1.6, available at: http://tree.bio.ed.ac.uk/software/tracer/ (accessed 23 July 2019).

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL (2005) The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142–166. https://doi.org/10.3732/ajb.92.1.142

Shepherd LD, McLay TGB (2011) Two micro-scale protocols for the isolation of DNA from polysaccharide-rich plant tissue. *Journal of Plant Research* 124: 311–314. https://doi.org/10.1007/s10265-010-0379-5

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109. https://doi.org/10.1007/BF00037152

Taylor T (1846) New Hepaticae. *London Journal of Botany* 5: 258–284, 365–417.

Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* 56: 564–577. https://doi.org/10.1080/10635150701472164

Thiers BM (1997) *Cheilolejeunea* in Australia: description of new taxa and key. *Journal of the Hattori Botanical Laboratory*. 82: 321–328.

Ye W, Gradstein SR, Shaw J, Shaw B, Ho B-C, Schäfer-Verwimp A, Pócs T, Heinrichs J Zhu R-L (2015) Phylogeny and classification of Lejeuneaceae subtribe Cheilolejeuneinae (Marchantiophyta) based on nuclear and plastid molecular markers *Cryptogamie, Bryologie* 36: 313–333. https://doi.org/10.7872/cryb/v36.iss4.2015.313

Zhu R-L (2006) Taxonomy and distribution of *Cheilolejeunea krakakammae* (Lejeuneaceae, Jungermanniopsida, Marchantiophyta), with a description and illustrations of *Cheilolejeunea laevicalyx* from Bolivia, Colombia and Ecuador. *Nova Hedwigia* 83: 187–198. https://doi.org/10.1127/0029-5035/2006/0083-0187

Zhu R-L, So ML Wang YF (2002) The genus *Cheilolejeunea* (Hepaticae, Lejeuneaceae) in China. *Nova Hedwigia* 75: 387–408. https://doi.org/10.1127/0029-5035/2002/0075-0387

Zhu R-L, Lai M-J (2005) *Cheilolejeunea decursiva* (Sande Lac.) R.M.Schust. and C. ventricosa (Schiffn.) X.L.He (Lejeuneaceae. Hepaticae) new to Australia. *Journal of the Hattori Botanical Laboratory* 98:193–203.

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