

DNA sequencing supports the reinstatement of
Pycnolejeunea glauca from synonymy of *Cheilolejeunea*
intertexta, as *Cheilolejeunea glauca* comb. nov.
(Lejeuneaceae, Marchantiophyta)

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Abstract

Pycnolejeunea glauca, originally described by Stephani based on a collection in New Zealand by William Colenso, was placed by Grolle into the synonymy of the paleotropical species *Cheilolejeunea intertexta* with a type from Micronesia. In this study, we generated DNA sequences from a recently-collected sample of *P. glauca* and compared them with published sequences of *C. intertexta* from China. *Pycnolejeunea glauca* was recovered in the phylogenetic analyses as sister to *C. nipponica*, whereas *C. intertexta* grouped in another clade with *C. vittata* and *C. streimannii*. The analysis justifies the reinstatement of *P. glauca* as the new combination *Cheilolejeunea glauca*, a New Zealand endemic. Descriptions and illustrations are provided of key features of this species together with data on its position in the phylogeny of the genus.

Key Words: Liverwort, New Zealand, endemic, synonymy, phylogeny.

Introduction

Pycnolejeunea glauca Steph. was described and illustrated by Stephani (1892) based on a sample collected in New Zealand by William Colenso. Unfortunately the genus *Pycnolejeunea* was not validly published until the following year, so the species was invalid at first publication (Soderstrom *et al.* 2015), and until it was validly published in the fourth volume of Stephani's Species Hepaticarum (Stephani 1914). Grolle (1979) placed it in the synonymy of *Cheilolejeunea intertexta* (Lindenb.) Steph. with a type from Micronesia. Two observations suggested that this synonymy warranted investigation. An interrogation of the Australasian Virtual Herbarium (24 February 2017 and 23 August 2019), showed that in Australia *C. intertexta* is limited to tropical or subtropical Queensland with one record immediately south of the border in New South Wales, while records from New Zealand are all from temperate locations, in the North Island and from Stewart Island in the far south. Endemism in the New Zealand hepatics flora is about 50% (Engel and Glenny 2008) and almost all the non-endemic flora, 284 species of about 600, are shared with Australia. Given this close relationship between the New Zealand and Australian hepatic floras, the presence of a New Zealand temperate distribution for *C. intertexta*, together with its presence in tropical Australia but absence from temperate Australia was anomalous. It was also noted that the *P. glauca* orbicular underleaves, which are up to four times the stem width, are about twice the width of those cited for *C. intertexta* in the key to *Cheilolejeunea* in Australia (Thiers 1997).

Molecular tools provide a means to test the synonymy of *C. intertexta* and *P. glauca* but a lack of fresh material has prevented this analysis to date. Despite considerable recent interest and research into the New Zealand hepatic flora, of the fifteen specimens in New Zealand herbaria, most gatherings of *P. glauca* were made in 1960 and the first more recent collection was made in 2003. Following unsuccessful attempts to relocate it in Otari–Wilton’s Bush from which there are historical records, the recent finding of a *P. glauca* sample in Tararua Ecological District has provided fresh material for both DNA sequencing and for a description of the species.

Materials and Methods

Genomic DNA was extracted from a fresh sample *Pycnolejeunea glauca* using a modified-CTAB DNA extraction method (steps 1, 3–7 from Table 1 in Shepherd and McLay, 2011). Two chloroplast loci and one nuclear locus were PCR amplified and sequenced. The chloroplast trnL (UAA) 5’ exon - trnF (GAA) intergenic spacer (trnLF) was amplified using the C and F primers of Taberlet *et al.* (1991) and the transfer RNA^{Gly} (UCC) (trnG) was amplified with Pacak and Szweykowska-Kulinska’s (2000) trnGF and trnGR primers. The nuclear ribosomal internal transcribed spacer 1 and 2 including the 5.8S ribosomal subunit (ITS) was amplified using the primers of Hartmann *et al.* (2006).

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 was performed with the protocol of Ye *et al.* (2015) for ITS and the ‘slow and cold’ program of Shaw *et al.* (2005) for the two chloroplast loci. PCR products were purified with digestion at 37°C for 15 minutes using 0.5 U shrimp alkaline phosphatase (SAP, USB Corp.) and 2.5 U exonuclease I (ExoI, USB Corp.), 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 molecular study of the phylogeny of *Cheilolejeunea* (Ye *et al.*, 2015) provided a phylogenetic framework for establishing relationships of this species within *Cheilolejeunea*. The newly-generated *Pycnolejeunea glauca* sequences were aligned to the published sequences to create two alignments: dataset 1 included *Cheilolejeunea intertexta* and its close relatives, dataset 2 included sequenced New Zealand *Cheilolejeunea* sequences (Table 1). Alignments were performed with 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.

Mr Bayes v3.2.1 (Huelsenbeck and Ronquist, 2001) was used to perform Bayesian analyses (BA). Two concurrent analyses, each with four Markov chains of fifty million generations and sampling every 1000 generations, were run. For the combined dataset each locus was assigned as a separate partition and rates allowed to vary across partitions. The first 20% of samples were discarded as burn-in, after this point the standard deviation of split frequencies was below 0.01 and visual inspection of trace files with Tracer v.1.7.1 (Rambaut *et al.* 2018) confirmed that stationarity had been reached. The position of the root of the phylogenies was based on Ye *et al.*’s (2015) phylogeny.

Observations and measurements of gross structures were made using an Olympus TLE stereo microscope with ocular micrometer. Aqueous mounts were made for observation of oil-bodies, and for observation of other vegetative and gametangial structures and for hand-cut stem sections, with methylene blue added for contrast enhancement where appropriate. Observations were made using an Olympus CH compound microscope with ocular micrometer. Microscope images were captured with a Canon A630 digital camera, edited using Mac Photos software and printed as tracing table masters. Scanning Electron Microscope (SEM) photographs were taken with a Hitachi TM3030Plus desktop SEM. Specimens were sputter-coated with gold before examination.

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

Species	Voucher	Dataset	GenBank accession numbers		
			<i>trnL-F</i>	<i>trnG</i>	ITS
<i>Cheilolejeunea albovirens</i>	CHR 6238196	2	MN308478	MN308473	MN316699
<i>Cheilolejeunea aneogyna</i>	A. Schäfer-Verwimp 9751 (Herb. Schäfer-Verwimp)	1	-	KT190891	-
<i>Cheilolejeunea aurifera</i>	I. Holz CR00-0812 (GOET)	2	KT190825	KT190883	KT190949
<i>Cheilolejeunea beyrichii</i>	B. Allen 17393 (GOET)	1	DQ987387	-	DQ987271
<i>Cheilolejeunea campbelliensis</i>	CHR 638557	2	MN308477	MN308472	MN316698
<i>Cheilolejeunea ceylanica</i>	R.L. Zhu et al. 20050901-6 (HSNU)	2	-	KT190852	KT190914
<i>Cheilolejeunea clausa</i>	N.D. Santos et al. 400A (RB)	2	KT190819	KT190874	KT190940
<i>Cheilolejeunea clypeata</i>	B. Shaw 4714 (DUKE)	2	KT190807	KT190863	KT190928
<i>Cheilolejeunea comitans</i>	WELT H014163	2	MK294020	MK294018	MK294016
<i>Cheilolejeunea cordigera</i>	T. Pócs & A. Szabó 9878/FH (EGR)	1	KT190794	-	KT190910
<i>Cheilolejeunea glauca</i>	WELT H014290	1, 2	MT779803	MT779802	MT777620
<i>Cheilolejeunea filiformis</i>	S. Churchill et al. 23653 (GOET)	2	KT190822	KT190880	KT190946
<i>Cheilolejeunea inflexa</i>	A. Schäfer-Verwimp & I. Verwimp 22575 (GOET)	1	KT190790	KT190847	KT190906
<i>Cheilolejeunea insecta</i>	A. Schäfer-Verwimp & I. Verwimp 134471A (Herb. Schäfer-Verwimp)	1	KT190786	KT190843	KT190902
<i>Cheilolejeunea intertexta</i>	R.L. Zhu et al. 20050908-20 (HSNU)	1, 2	KT190792	KT190849	KT190908
<i>Cheilolejeunea krakammae</i>	R.L. Zhu 20070319-7 (HSNU)	2	KT190814	KT190869	KT190935
<i>Cheilolejeunea laevicalyx</i>	S.R. Gradstein 10104 (GOET)	2	KT190820	KT190875	KT190941
<i>Cheilolejeunea mimosa</i>	A. Schäfer-Verwimp & I. Verwimp 13664 (GOET)	2	KT190821	KT190877	KT190943
<i>Cheilolejeunea morgani</i>	WELT H014085	2	-	MG970148	MG970145
<i>Cheilolejeunea nipponica</i>	J. Wang et al. 20090801-5 (HSNU)	1, 2	KT190793	KT190850	KT190909
<i>Cheilolejeunea osumiensis</i>	R.L. Zhu 20090220-25B (HSNU)	1	KT190810	KT190866	KT190931
<i>Cheilolejeunea roccatii</i>	E. Fischer X-RWA-1120 (Herb. Schäfer-Verwimp)	2	KT190802	KT190858	KT190923
<i>Cheilolejeunea rodneyi</i>	WELT H014291	2	MN308476	MN308471	MN316697
<i>Cheilolejeunea ryukyuensis</i>	W. Ye & Y.M. Wei 20090715-4 (HSNU)	1	KT190791	KT190848	KT190907
<i>Cheilolejeunea streimannii</i>	H. Schneider V-2011-H-25-C (HSNU)	1	-	-	KT190920
<i>Cheilolejeunea subopaca</i>	J. Wang & T. Peng 20111018-48 (HSNU)	1	-	-	KT190921
<i>Cheilolejeunea trifaria</i>	A. Schäfer-Verwimp & I. Verwimp 22434 (GOET)	2	KT190817	KT190872	KT190938
<i>Cheilolejeunea vittata</i>	R.L. Zhu et al. 20050907-32 (HSNU)	1, 2	KT190798	KT190855	KT190917

Results

There were 127 substitutions and 29 indel events distinguishing *Pycnolejeunea glauca* from *C. intertexta* across the three loci (ITS: 100 substitutions and 18 indels, *trnL-F*: 8 substitutions and 4 indels, *trnG*: 19 substitutions and 7 indels).

The alignment of all loci for dataset 1, with ambiguously aligned nucleotide positions removed with Gblocks, was 2419 base pairs (bp). The maximum likelihood (ML) and Bayesian analyses (BA) of the combined dataset of all three loci (Fig. 1) plus the individual *trnG* and ITS datasets all recovered *Pycnolejeunea glauca* and *Cheilolejeunea nipponica* as sister taxa, with strong support (95% BS ML, 1.00 PP for the combined analysis, 98 BS ML, 1.00 PP for the *trnG* locus, 93 BS ML, 1.00 PP for the ITS locus). *Cheilolejeunea cordigera* was recovered as sister to *P. glauca* and *C. nipponica* (88% BS ML, 1.00 PP for the combined analysis and 97 BS ML, 1.00 PP for the ITS locus. *trnG* sequence was not available for *C. cordigera*). In the combined analysis *C. intertexta* was sister to *C. vittata* and *C. streimannii* (87 BS ML, 1.00 PP). For the *trnL-F* locus *P. glauca* grouped in a clade with *C. nipponica*, *C. ryukyuensis*, *C. cordigera*, *C. vittata*, and *C. intertexta* but the relationships between these taxa were not resolved.

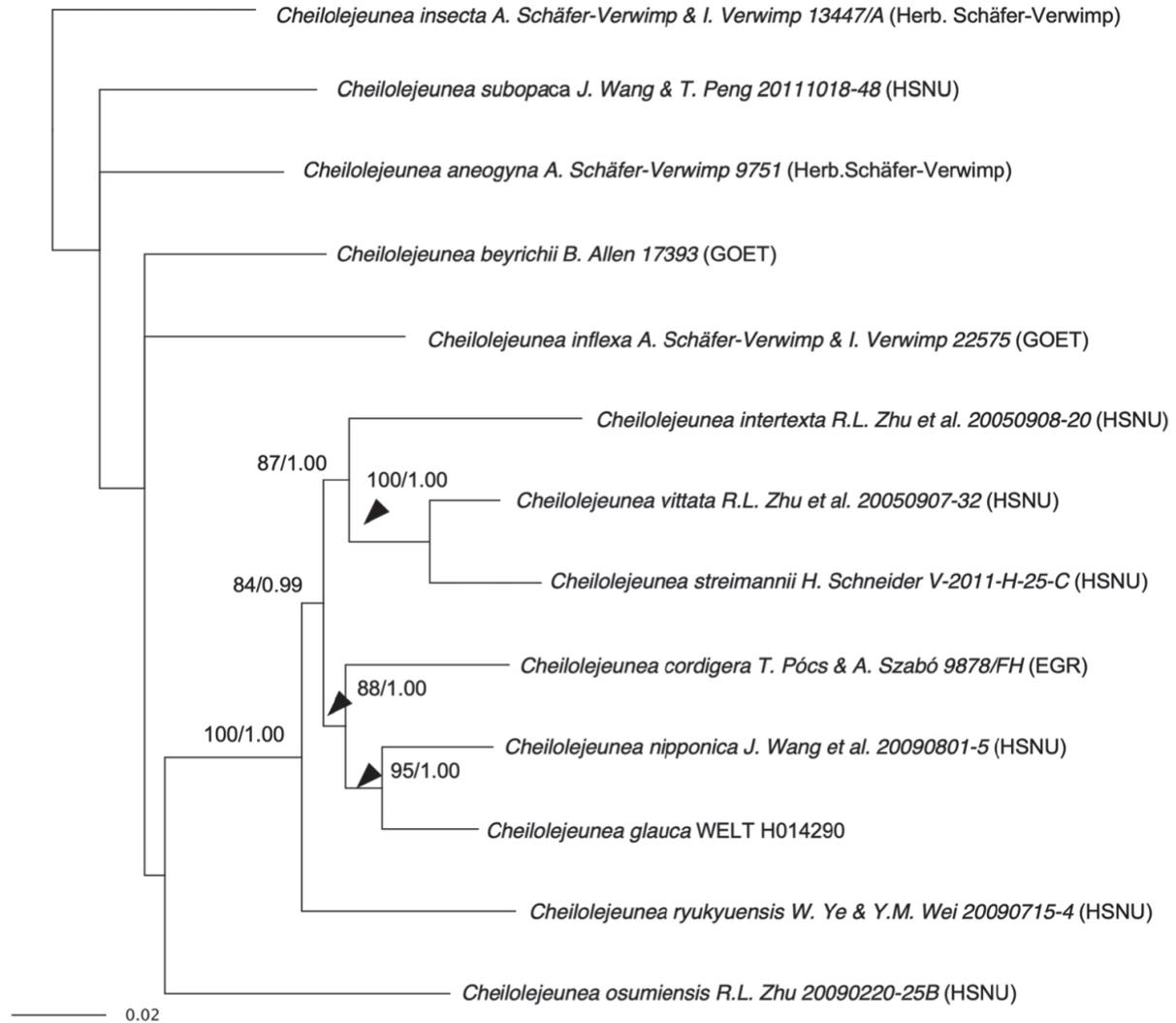


Fig 1. Bayesian phylogram illustrating the relative positions within *Cheilolejeunea* of *C. glauca*, *C. nipponica* and *C. intertexta*. The position of the root is inferred from Ye *et al.* (2015).

The alignment of all loci for dataset 2, with ambiguously aligned nucleotide positions removed, was 2357 bp in length. *Pycnolejeunea glauca* was recovered as sister to *C. nipponica* (100% BS ML, 1.00 PP) and was not closely related to any of the New Zealand species (Fig. 2).

Discussion and Conclusions

The results of the analyses justify the reinstatement of *Pycnolejeunea glauca* from the synonymy of *Cheilolejeunea intertexta* and confirm its position within the present broad concept of the genus *Cheilolejeunea*. It is accordingly designated as the new combination *Cheilolejeunea glauca* (Steph.) Bever. et Glenny *comb. nov.* The only available description of *Cheilolejeunea glauca* as *Pycnolejeunea glauca* is that of Stephani (1914), so we provide a re-description of the species below, based mainly on the following specimen: New Zealand, Tararua Ecological Region, Tararua Ecological District, Remutaka Forest Park, Graces Stream, 41° 20.62'S 174° 56.00' E, 23 Sep 2018, elevation 60 m, in mixed broadleaf forest with dominants *Nothofagus truncata* Colenso and *N. solandri* Hook.f, epiphytic on *Melicytus ramiforus* J.R.Forst. & G.Forst. growing on a stream bank, *P. Beveridge* QJ-2 (WELT H014290!).

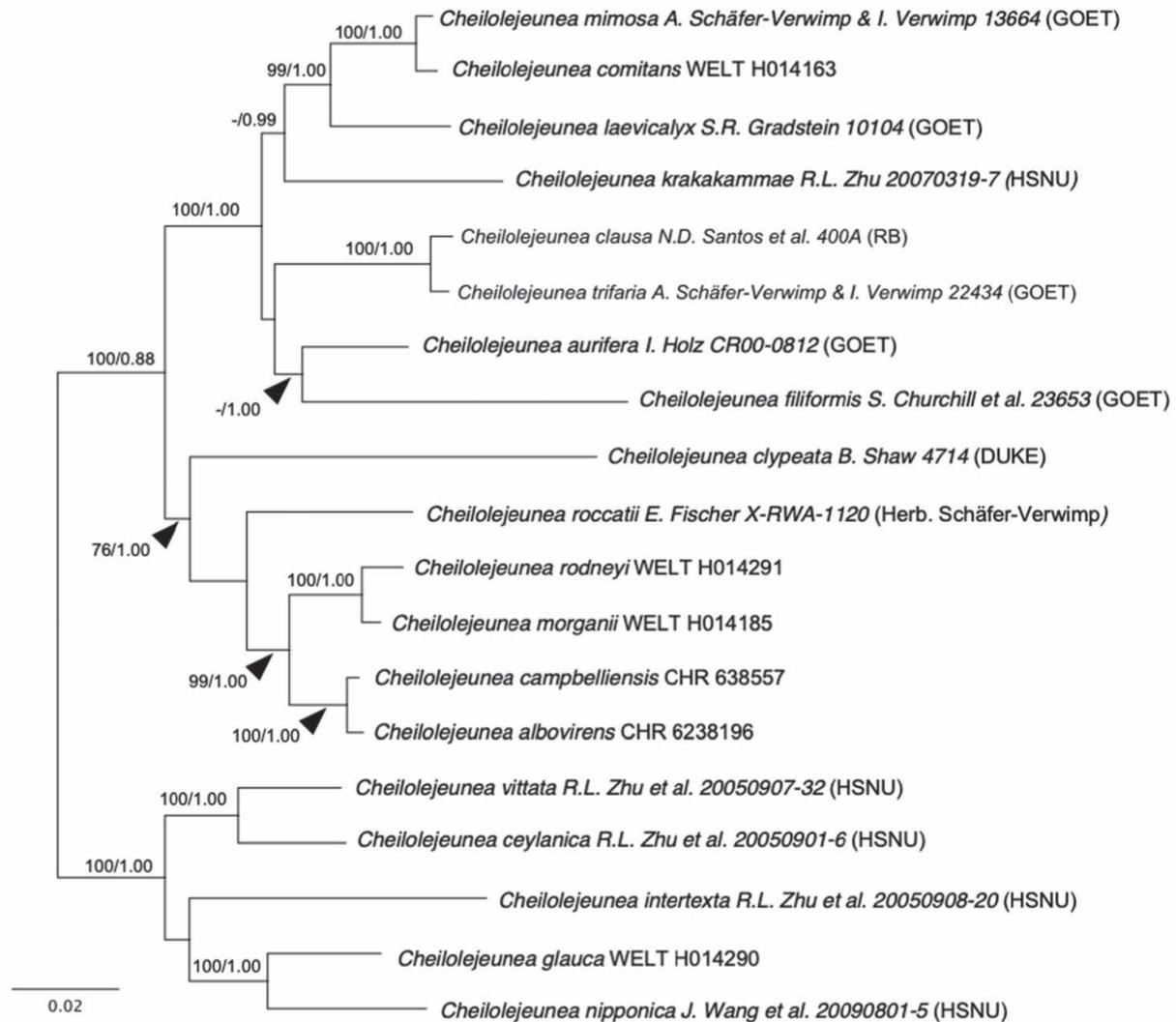


Fig 2. Bayesian phylogram illustrating relative positions within *Cheilolejeunea* of *C. glauca* and selected other sequenced NZ species of the genus. The position of the root is inferred from Ye *et al.* (2015). New Zealand-based collections are the source of the sequences for the following species: *Cheilolejeunea mimosa* (GOET), *C. albovirens*, *C. campbelliensis*, *C. comitans*, *C. glauca*, *C. morganii*, *C. rodneyi* with CHR or WELT numbers. In addition, *C. ceylanica* and *C. trifaria* occur in the New Zealand Botanical Region sense Allan (1961). Sequences for these species are taken from Ye *et al.* (2015).

Taxonomic Treatment

Cheilolejeunea glauca (Steph.) Bever. et Glenny **comb. nov.**

Basionym: *Pycnolejeunea glauca* Steph. Species Hepaticarum 5: 635 (1914)

Lectotype (chosen by Grolle, 1979): New Zealand, North Island, *Colenso a. 1694*, BM ex K (not seen). Isolectotypes: G 00121614 *ex herb.* Stephani, specimen label states in Stephani's hand "Pycno-Lej glauca St n sp, in cortice, monoica, N Zelandia, Colenso 1694 (*)"; BM (seen by Grolle); MPN 22660 *ex herb.* Hodgson 14402 (!).

Plants green, in a shallow procumbent growth of overlapping weakly convex shoots, closely attached to the bark substrate. *Branching* by frequent *Lejeunea*-type intercalary branches. Leading shoots (Fig. 3A) ca 0.75–1.0 mm wide × ca 1 cm long, sub-optimal lateral branches ca 0.5 mm wide. *Stem* (Fig. 3G) pale brown, ca 75 µm diameter, in cross section with 7 cortical cell rows, cells rounded-rectangular, ca 15 × 20 µm, outer wall ca 6 µm thick, medullary cell rows ca 10, isodiametric, 10–12 µm diameter, evenly to variably thick-walled. Stem cells in surface view rectangular, ca 20–25 µm wide, 30–35 µm long, longitudinal walls ca 7.5 µm wide, transverse walls 2.5 µm wide with some intermediate thickening, ca 10 cells intervening between successive underleaves. *Rhizoids* sporadically present in radiating clusters of 20–25, arising from underleaf cells adjacent to underleaf bases, hyaline, thick walled, ca 10 µm wide × ca 175 µm long. *Leaves* (Fig. 3F) incubous, alternate, lobes plane to very weakly convex, imbricate, erect-appressed, angled to stem axis at about 70°, the lobe apices not deflexed. In shoot dorsal-view, the distal imbricate leaf lobes usually completely cover the stem, the antical leaf margins extending across the stem and 0.5 to a full stem width beyond the opposite stem margin. Occasionally short sectors with stem dorsally exposed. *Lobes* (Fig. 3E) widely elliptic, 0.35–0.45 mm wide, 0.45–0.6 mm long, margins entire, weakly sinuose, without crenulation, weakly to moderately angled on postical margin at lobule apex. *Vitta* absent. *Mid-lobe cells* (Fig. 4) ca 15–17.5 µm wide × 15–25 µm long, cell walls ca 1.5–2.5 µm wide with weak to moderate concave or straight trigones, intermediate thickening sporadically present. Dorsal surface of lobe smooth or with weakly bulging cells. Marginal cells sub-quadrate to shortly rectangular, ca 10 µm wide × 12.5 µm long. *Oil-bodies* (Fig. 4) in lamina cells of distal leaves, wholly or mainly single, length ca 16–20 µm, botryoidal, arcuate, cashew-shaped, 6.0–7.5 µm wide at one end, 3.0–4.5 µm wide at the other, with spherules 2–3 µm diameter, 4 spherule rows across at the widest end, 2 at the narrowest end. In lamina cells of proximal leaves, oil-bodies often or mainly double. In cells of lobule and underleaves, oil-bodies mainly double, not arcuate. Dorsal leaf surface not or occasionally variably and weakly asperulate under light microscope, glaucous colour not observed. *Chloroplasts* peripheral in cell, appearing spindle-shaped, ca 2 µm wide × 3 µm long. *Lobules* (Fig. 3E) before flattening moderately inflated, ovate, with second tooth often visible and free margin obscured but not tightly deflexed, after flattening, ovate, ca 0.13–0.18 mm wide, ca 0.2 mm long, ca 0.36 lobe length, the keel weakly arched, the apical margin transverse to weakly oblique, of 5–6 subisodiametric cells including the single-celled first tooth, the second tooth a single spherical cell ca 15 µm diameter, the papilla, small, marginal, distal to the second tooth, in depression between second tooth and obsolete to slightly prominent first tooth. Free margin of lobule with ca 12 cells. Lobule carina cells weakly to moderately bulging. Lobules of suboptimal branch leaves relatively large, ca 0.5 × leaf length. *Underleaves* (Fig. 3A) appressed, attached to two-celled ventral merophyte by 6-celled weakly- to moderately-arched insertion, when flattened on well-developed shoots contiguous to shortly distant, occasionally slightly imbricate, orbicular, ca 3–4 × stem width, on leading shoots, ca 0.23–0.33 mm wide, 0.23–0.30 mm long, sinus on distal underleaves a narrow slit, occasionally V- or U-shaped in proximal underleaves, 0.15–0.25 × leaf length, lobe apices single-celled or two uniseriate cells, the apical lobe sectors bent inward in the plane of the underleaf and commonly crossing over each other, the lobe bases ca 10–14 cells wide, often differing in the same underleaf. *Asexual reproduction*, absent.

Monoicous. *Androecia* on abbreviated lateral branches from leading shoots and branches, diandrous, variable in length, the smallest androecia ovate, ca 0.40–0.45 mm wide, 0.45–0.50 mm long, spicate, projecting slightly beyond the adjacent leaves and visible dorsally, typically 2 male bracteoles associated proximally with a sterile bract pair, and with 2–3 pairs of fertile bracts, the proximal pair moderately larger than distal pairs. Longer androecia (Fig. 3C) as long as 1.5 mm long with up to 8 fertile bract pairs and a proximal sterile pair, the androecia ca 0.45–0.50 mm wide proximally tapering to 0.35–0.40 mm wide distally, the bracts loosely imbricate, with 1–2 proximal male bracteoles. Androecium apex occasionally innovating to generate a vegetative shoot. *Gynoecia* (Fig. 3A, B) terminal, on both leading shoots and short lateral intercalary branches, some with as few as 2 subgynoecial leaves, the subgynoecial underleaves on leading shoots progressively larger than shoot underleaves, female bracts free, the pair often asymmetric, bilobed, lobes ca. 0.27–0.3 mm wide, ca 0.55–0.65 mm long, variably falcate-spathulate, apices rounded, lobule broadly ligulate, deflexed, ca 0.07 mm wide, ca 0.3 mm long, sinus ca 0.05 mm deep, bracteole free, elliptic, 0.25 mm wide 0.42 mm long, sinus 0.1 mm long, narrow, obscured by overlapping lobes, lobe apices single-celled, curved towards midline. *Perianths* (Fig. 3B) ca 0.4–0.45 mm wide, ca 0.65 mm long, obovate, widest a little above mid-perianth, apex slightly retuse or truncate, cuneate proximally, lateral carinae sharply defined, the ventral carinae less sharply defined, with the perianth inflating between the ventral carinae before sporophyte enlargement, the dorsal carina a low profile ridge on a plane dorsal surface. *Rostrum* (Fig. 3D) ca 35 µm long, 3(–4) cell tiers. One or both innovations (Fig. 3A, B) pycnolejeuneoid, the other sometimes lejeuneoid. First innovation underleaf insertion ca. 2 stem cortical cell rows distal to first leaf insertion. Male and female gametangia often intermingled in close proximity.

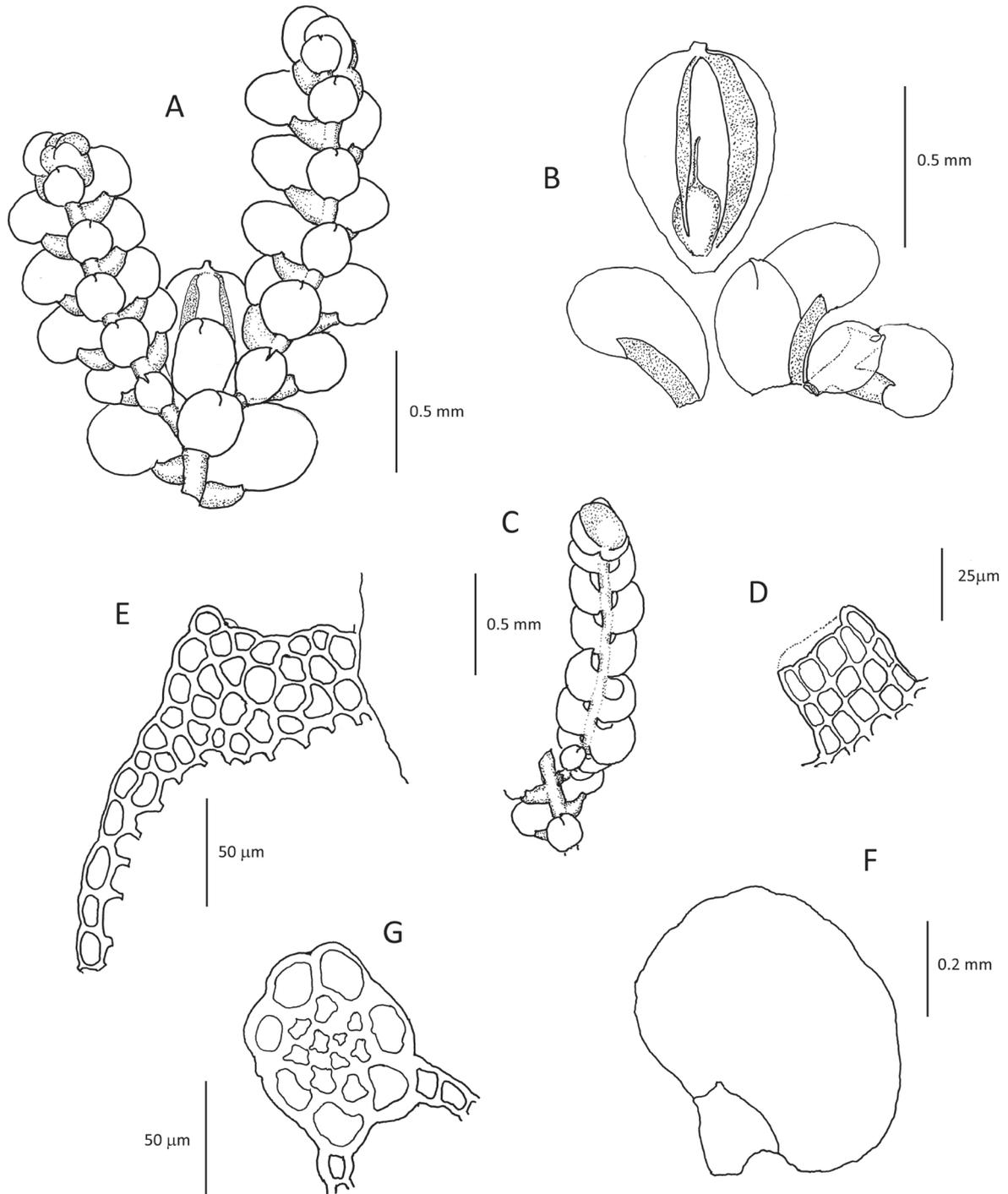


Fig 3. *Cheilolejeunea glauca* (Steph.) Bever. et Glenny comb. nov. A: Ventral view of leading shoot with gynoecium and pycnolejeuneoid innovations, B: Dissected gynoecium with perianth, female bracts, bracteole and innovation first underleaf, C: Androecium, D: Rostrum, E: Lobule with second tooth and papilla, F: Leaf, G: Stem section. All drawn from WELT H014290.

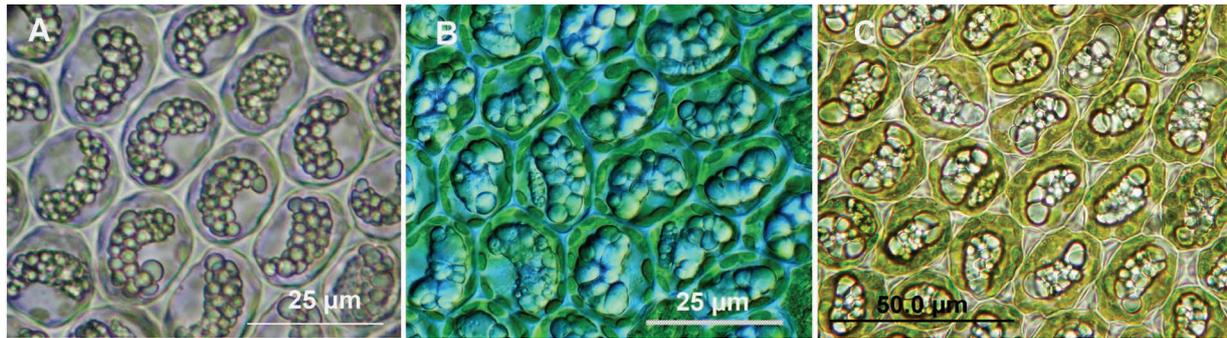


Fig 4. Oil-bodies at midleaf of A, *Cheilolejeunea glauca*; B, *C. nipponica*; and C, *C. intertexta*. *Cheilolejeunea glauca*: WELT H14290; *C. nipponica*: Wang & Han 20081010-4 (HSNU); *C. intertexta*: Zhu et al. 20050908-20 (HSNU); B and C courtesy of Prof. R.L.Zhu.

Additional Specimens Examined: New Zealand, *W. Colenso 1694*, Hodgson Collection, 14402, designated *Pycnolejeunea glauca* isoelectotype by Grolle 1978 (MPN 22660!); Herekino Forest, Okahu Stream, 35°10'S 173°16'E, 80 m, April 2003, *M. A. M. Renner 400b* (AK 282687); Auckland, 36°49'S 174°44'E, 1948, *E. A. Hodgson* (MPN 22675); Waitakere Range, Matuku Forest and Bird Reserve, 36°52.02'S 174°28.40'E, 40 m, June 2004, *J. E. Braggins 04044C* (AK 291005); Te Kuiti, Mapara Wildlife Management Reserve, 38°32.03'S 175°14.70'E, 340 m, June 2020, *L. Perrie & L. Shepherd* (WELT H014364); Te Kuiti, Mapara Wildlife Management Reserve, 38°32.03'S 175°14.70'E, 340 m, June 2020, *L. Perrie & L. Shepherd* (WELT H014365); Mahia Peninsula, Dec 1960, *E. A. Hodgson* (CHR 627383); Mahia Peninsula, 39°08'S 177°53'E, Dec 1960, *E. A. Hodgson* (MPN 22662); Rotorua Region, Sept 1941, *K. W. Allison* (CHR 627379); Taranaki, west of Ohura, 38°51'S 174°55'E, 516 m, July 1937, *E. A. Hodgson* (MPN 22670); Hawkes Bay, 39°28'S 176°55'E, undated, *E. A. Hodgson* (MPN 22349); Wellington, Wilton's Bush, Oct 1941, *R. Mason* (CHR 36721); Wellington, Wilton's Bush, 41°16'S 174° 45' E, Oct 1941 *R. Mason* (MPN 22669); Wellington, Wilton's Bush, 41° 16' S - 174° 45'E, Oct 1941 *R. Mason* (MPN 22649); Stewart Island, Pryce Peak, Feb 1947 *W. Martin* (CHR 627382); Stewart Island, Pryce Peak, 46°56'S 168°01'E, Feb 1947, *W. Martin* (MPN 22657).

Differentiation: Table 2 provides a summary of features of both *Cheilolejeunea nipponica* and *C. intertexta* that serve to distinguish them from *C. glauca*. The data on *C. nipponica* are derived from references for Japan (Mizutani 1982), and China including Hong Kong (So and Zhu 1996 and Zhu et al. 2002). The same references with addition of Asthana et al. (1995) provide the data for *C. intertexta*.

Table 2. Features for distinguishing between *Cheilolejeunea glauca*, *C. intertexta* and *C. nipponica*.

Character	<i>C. glauca</i>	<i>C. nipponica</i>	<i>C. intertexta</i>
shoot width	0.75–1.0 mm	0.40–0.55 mm	0.50–0.76 mm
lobule apex constriction	weak	strong	strong
second tooth	1 isodiametric cell	3-celled, 1 on base of 2, pointed	1 cell, obtuse
underleaf length x width	0.33 × 0.33 mm	0.15 × 0.18 mm	0.27 × 0.23 mm
ratio of underleaf to stem width	3–4:1	2–3:1	2–3:1
underleaf sinus	a narrow slit	widely obtuse	acute to obtuse
underleaf and female bracteole lobe tips	often crossing over	widely spreading	widely spreading
leaf oil-body number	distally single	1–2 (3)	unstated
oil-body shape and texture	in distal leaves cashew-shaped, botryoidal	elliptical, rarely cashew-shaped, botryoidal	unstated
leaf lobe length x width	0.60 × 0.45 mm	0.38 × 0.30 mm	0.40 × 0.40 mm

Distribution and Ecology: Basic information can be derived from the scant data available on older herbarium specimens in MPN and CHR with 12 samples including four duplicates and the isoelectotype which lacks a specified location in New Zealand. Specimens are from a number of North Island locations, from Auckland to Wellington. There are no records from the South Island but there are two collections from Pryse Peak on Stewart Island further south. All specimens are from forested habitats, and were bark epiphytes, either stated on the packet or confirmed from the specimen samples. Only one phorophyte from two locations is recorded

for the historical collections, *Lophomyrtus bullata* Burret to which can be added the recent collection from *Meliccytus ramiflorus* on which the description is based, and collections from Mapara Reserve near Te Kuiti on *Beilschmiedia tawa* (A.Cunn.) Benth. & Hook.f. ex Kirk and *Kunzea* sp., from the Waitakere Range on *Vitex lucens* Kirk, and from Herekino Forest on *Coprosma grandifolia* Hook.f. The Herekino Forest specimen was from *Beilschmiedia tarairi* (A.Cunn.) Benth. & Hook. f. ex Kirk, *Vitex lucens* Kirk and *Prumnopitys taxifolia* (Sol. ex D.Don) de Laub. forest with an understorey of *Hoheria populnea* A.Cunn., *Rhopalostylis sapida* H.Wendl. & Drude, *Ripogonum scandens* J.R.Forst. & G.Forst. and *Geniostoma rupestre* (J.R.Forst. & G.Forst.) J.R.Forst. & G.Forst. The Mapara Wildlife Reserve collection was from *Beilschmiedia tawa* forest. Inferred elevational range is from near sea level to about 500 m asl. Three collections were from tree trunks adjacent to or lying over a stream suggesting constant humidity is a requirement of the species.

Recognition: In the absence of perianths *Cheilolejeunea glauca*, with its green colour and closely appressed shoots, may initially be mistaken for a *Frullania* such as *F. monocera*. The presence of dorsio-ventrally compressed *Lejeunea*-type perianths, large, orbicular underleaves up to four times stem width with a narrow slit-like sinus, and frequently with overlapping lobe tips in underleaves and female bracteole, combined with distal leaf lobe cells with single arcuate cashew-shaped oil-bodies will confirm its identity. In the New Zealand context, the species can be identified with confidence by features of the underleaves alone. The orbicular underleaves, up to four times stem width with narrow sinus and with inwardly oriented acute apices, frequently overlapping, are usually present. The overlapping underleaf apices were illustrated for the female bracteole by Stephani (1892).

Key: In the most recent key to the New Zealand species of *Cheilolejeunea* (Beveridge *et al.* 2019), *C. glauca* replaces *C. intertexta*.

Conservation Status: Herbarium records show *Cheilolejeunea glauca* to have been present in seven ecological regions in the North Island and on Stewart Island. Until the recent collection on which this description is based, there were few recorded collections since 1960. A recent bulk collection from Mapara Reserve in *Beilschmiedia tawa* forest near Te Kuiti had five scant samples of *C. glauca*, one small with perianths, the others sterile and consisting of short, single leading shoots. This suggests that *C. glauca* may be much more common than collection records indicate. As both *Pycnolejeunea glauca* and *Cheilolejeunea intertexta*, it was listed as data deficient in the most recently published liverwort threat classification (de Lange *et al.* 2020). The evidence above suggests that its classification according to the New Zealand Threat Classification System (Townsend *et al.* 2008) should be naturally uncommon.

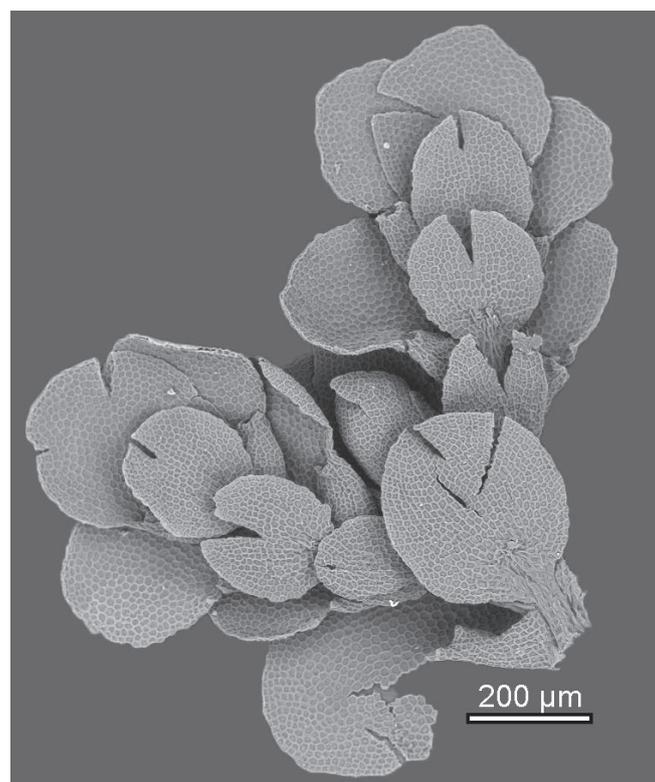


Fig 5. SEM ventral view of gynoecium and two innovations of *Pycnolejeunea glauca*, isoelectotype MPN 22660.

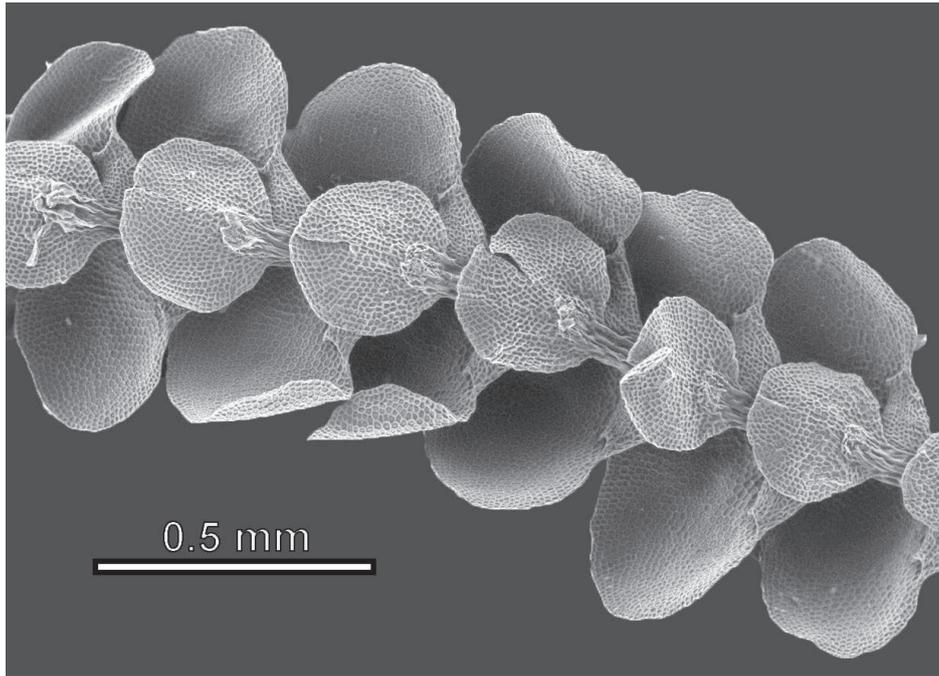


Fig 6. SEM ventral view of shoot of *Cheilolejeunea glauca*, WELT H14290.

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