

Cheilolejeunea rodneyi Bever. & Glenny (Lejeuneaceae, Marchantiopsida), a new species from lowland indigenous forests in New Zealand.

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Abstract

Cheilolejeunea rodneyi Bever. & Glenny, a new species of liverwort from lowland indigenous forest reserves in the Tararua Ecological District near Wellington in the North Island of New Zealand is described and illustrated. In both morphology and phylogeny, it is closest to the recently-described *C. morganii* Bever. & Glenny, with which it shares monoicy, pycnolejeuneoid innovation leaf sequences, a pentacariniate perianth with long rostrum and absence of large botryoidal oil-bodies, but differs in having a single or two-celled uniseriate second tooth, a large papilla oriented over the lobule exterior surface, a large cylindrical rostrum, the dorsal perianth carina comprising a low-profile ridge, oil-bodies with large, usually homogeneous segments, and the common presence of cladia. Its addition to the New Zealand flora brings the total species of *Cheilolejeunea* to 11, four of which are endemic.

Keywords: *Cheilolejeunea*, Lejeuneaceae, endemic, Marchantiopsida, liverwort, phylogeny, New Zealand, new species

Introduction

Cheilolejeunea (Spruce) Steph. is a pantropical genus of about 200 species (Söderström *et al.* 2016), including a number distributed in sub-tropical and temperate regions (Thiers 1997; Ye *et al.* 2015). The characters by which *Cheilolejeunea* is distinguished within the Lejeuneaceae include the elaboration of a second lobule tooth oriented away from the stem toward the leaf apex, and overtopping a reduced first tooth, enlarged stem cortical cells, a ventral merophyte two cells wide, the presence of bifid underleaves, cells with 1–5 large usually coarsely botryoidal oil-bodies, absence of vegetative reproductive organs, and (0)1–2 lejeuneoid or pycnolejeuneoid gynoecial innovations (Schuster 1980; Zhu and So 2001; Ye *et al.* 2015). Exceptions to each of these characters are known to occur. *Cheilolejeunea* is, after *Cololejeunea* (Spruce) Steph. and *Lejeunea* Lib., the third largest genus in Lejeuneaceae, the largest family of liverworts with over 1800 species in about 70 genera (Söderström *et al.* 2016; Zhu *et al.* 2017, 2018; Ye and Zhu 2018).

The most recently-published listing for the genus *Cheilolejeunea* in the New Zealand Botanical Region, sensu Allan (1961), (Beveridge *et al.* 2019) includes ten species: *C. albovirens* (Hook.f. & Taylor) E.A.Hodgs., *C. campbelliensis* (Steph.) R.M.Schust., *C. ceylanica* (Gottsche) R.M.Schust. & Kachroo, *C. hamlinii* Grolle,

C. intertexta (Lindenb.) Steph., *C. krakammae* (Lindenb.) R.M.Schust., *C. mimosa* (Hook.f. & Taylor) R.M.Schust., *C. morganii* Bever. & Glenny, *C. novaezelandiae* R.M.Schust. and *C. trifaria* (Reinw., Blume & Nees) Mizut. As a consequence of recent research (Beveridge and Shepherd 2019), *C. comitans* has been reinstated as an Australasian endemic species from the synonymy of *C. krakammae* (Zhu 2006).

The New Zealand hepatic flora is recognised for its high species diversity as a liverwort hot spot (von Konrat *et al.* 2008) and for its high degree of endemism at about 50% (Engel and Glenny 2008). Work on a variety of families over recent years has been informing the production of a four-volume New Zealand liverwort flora, three volumes of which have now been published (Engel and Glenny 2008, 2019a and 2019b). The production of the final volume in the next decade will place New Zealand in the select group of countries with a comprehensive liverwort floral treatment. However, most genera in the Lejeuneaceae including *Cheilolejeunea* have not been the subject of a comprehensive national or regional revision. Instead there has been an intermittent publication of new species and new records (Grolle 1973; Glenny 1996; Renner 2010; Renner 2013; Renner *et al.* 2009, 2010; Renner and Pócs 2011; Renner and de Lange 2011; Braggins *et al.* 2014; Lewington *et al.* 2013; Beveridge *et al.* 2019) or inclusion as part of studies of other regions (e.g. Grolle 1982). Within the Lejeuneaceae, the genus *Cheilolejeunea* may be regarded as particularly neglected with 10 species, including only three endemics, the recent publication of *C. morganii* (Beveridge *et al.* 2019) being the first endemic addition since that of *C. novaezelandiae* (Schuster 1985). A more detailed analysis of the status of the Lejeuneaceae and the genus *Cheilolejeunea* in the New Zealand hepatic flora may be found in Beveridge *et al.* (2019). In this study we report a new endemic species of *Cheilolejeunea* for New Zealand. It was initially recognised as morphologically distinct by comparison with fresh and herbarium material of other New Zealand species. The addition of *Cheilolejeunea rodneyi* brings the total number of species of *Cheilolejeunea* in New Zealand to 11, four of which are regarded as endemic.

Materials and Methods

Specimens from three locations were available for this study. The type specimen of the new species (WELT H014288) was collected in the Horoeka Reserve, Stokes Valley, close to Wellington during a bioblitz diversity survey. Field collection had previously located similar material in two other Wellington reserves, at Graces Stream in the Remutaka Forest Park (WELT H014291) and Tane's Track, Pakuratahi Regional Park (WELT H014289). 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 hand-cut stem sections, with methylene blue 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. A subsequent collection from the same tree in the Horoeka Scenic Reserve provided material for description of sporophyte and spores, and for SEM images.

The molecular study of the phylogeny of *Cheilolejeunea* (Ye *et al.* 2015) has provided a phylogenetic framework for establishing relationships of the new species within *Cheilolejeunea*. Genomic DNA was isolated from three accessions of *C. rodneyi*, including the type specimen, and one accession each of the previously unsequenced species *C. albovirens* and *C. campbelliensis* using a modified-CTAB DNA extraction method (steps 1, 3–7 from Table 1 in Shepherd and McLay 2011). The two chloroplast loci and one nuclear locus used by Ye *et al.* (2015) were PCR amplified. The chloroplast transfer RNA^{Gly} (UCC) (*trnG*) and the *trnL* (UAA) 5' exon-*trnF* (GAA) intergenic spacer (*trnLF*) were amplified using the primers of Pacak and Szweykowska-Kulinska (2000) and Taberlet *et al.* (1991), respectively. The nuclear ribosomal internal transcribed spacer 1 and 2 with the intervening 5.8S ribosomal subunit (nrITS) was amplified using the primers of Hartmann *et al.* (2006).

PCR amplifications were performed in 12 µl reactions with 1× Mytaq buffer (Bioline, Australia), 5 pmol of each primer and 1 M betaine. PCR thermocycling conditions followed Shaw *et al.* (2005) for *trnG* and *trnLF* and Ye *et al.* (2015) for ITS. Purification of PCR products was performed by digestion with 0.5 U shrimp alkaline phosphatase (SAP, USB Corp.) and 2.5 U exonuclease I (ExoI, USB Corp.) at 37°C for 15 minutes, followed by enzyme inactivation at 80°C for 15 minutes. Sequencing was undertaken on an ABI 3730 DNA sequencer (Massey University Genome Service, Palmerston North, New Zealand) with the ABI Prism Big Dye Terminator cycle sequencing kit version 3.1.

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

Species	Voucher	GenBank accession numbers		
		<i>trnL-trnF</i>	<i>trnG</i>	ITS
<i>Cheilolejeunea albovirens</i>	CHR 6238196	MN308478	MN308473	MN316699
<i>Cheilolejeunea aurifera</i>	<i>I. Holz</i> CR00-0812 (GOET)	KT190825	KT190883	KT190949
<i>Cheilolejeunea campbelliensis</i>	CHR 638557	MN308477	MN308472	MN316698
<i>Cheilolejeunea clausa</i>	<i>N.D. Santos et al.</i> 400A (RB)	KT190819	KT190874	KT190940
<i>Cheilolejeunea clypeata</i>	<i>B. Shaw</i> 4714 (DUKE)	KT190807	KT190863	KT190928
<i>Cheilolejeunea filiformis</i>	<i>S. Churchill et al.</i> 23653 (GOET)	KT190822	KT190880	KT190946
<i>Cheilolejeunea intertexta</i>	<i>R.L. Zhu</i> 20050908-20 (HSNU)	KT190792	KT190849	KT190792
<i>Cheilolejeunea krakammae</i>	<i>R.L. Zhu</i> 20070319-7 (HSNU)	KT190814	KT190869	KT190935
<i>Cheilolejeunea laevicalyx</i>	<i>S.R. Gradstein</i> 10104 (GOET)	KT190820	KT190875	KT190941
<i>Cheilolejeunea mimosa</i>	<i>A. Schäfer-Verwimp & I. Verwimp</i> 13664 (GOET)	KT190821	KT190877	KT190943
<i>Cheilolejeunea morganii</i>	WELT H014086	-	MG970147	MG970144
<i>Cheilolejeunea morganii</i>	WELT H014185	-	MG970148	MG970145
<i>Cheilolejeunea nipponica</i>	<i>J. Wang et al.</i> 20090801-5 (HSNU)	KT190793	KT190850	KT190909
<i>Cheilolejeunea roccatii</i>	<i>E. Fischer</i> X-RWA-1120 (Herb. Schäfer-Verwimp)	KT190802	KT190858	KT190923
<i>Cheilolejeunea rodneyi</i>	WELT H014288	MN308474	MN308469	MN316695
<i>Cheilolejeunea rodneyi</i>	WELT H014289	MN308475	MN308470	MN316696
<i>Cheilolejeunea rodneyi</i>	WELT H014291	MN308476	MN308471	MN316697
<i>Cheilolejeunea turgida</i>	<i>W. Ye & Y.M. Wei</i> 20090720-20 (HSNU)	KT190801	-	KT190922
<i>Cheilolejeunea vittata</i>	<i>R.L. Zhu et al.</i> 20050907-32 (HSNU)	KT190798	KT190855	KT190917

The sequences were edited in Sequencer 5.2.3 (Gene Codes Corporation). The edited sequences were then aligned with selected sequences from Ye *et al.* (2015), Beveridge *et al.* (2019) and Beveridge and Shepherd (2019) using MAFFT 6.849 (Kato 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.

Phylogenetic analyses were conducted on both the individual and combined datasets using the PhyML v3.0 web server (<http://www.atgc-montpellier.fr/phyml/>; Guindon *et al.*, 2010) with maximum likelihood (ML) and MrBayes v3.2.1 (Huelsenbeck and Ronquist 2001) for Bayesian analyses (BA). For the ML analyses, the best-fit model of sequence evolution for each dataset was determined using Smart Model Selection (Lefort *et al.* 2017). Heuristic searches were performed with 10 random addition sequence replicates and SPR branch-swapping, and branch support assessed with 1000 bootstrap pseudoreplicates. For the BA on each dataset two concurrent analyses were run, each with four Markov chains of five million generations and sampling every 1000 generations. For the combined dataset, each locus was assigned as a separate partition and rates allowed to vary across partitions. For each partition in the combined dataset and for the individual datasets 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 and Tracer v.1.6 (Rambaut and Drummond 2009) also confirmed that stationarity had been reached.

Results

The three *Cheilolejeunea rodneyi* specimens shared identical DNA sequences across the three loci, which differed from other published sequences. The sequences detected in *C. albovirens* and *C. campbelliensis* differed from each other and also any other published sequences. All newly-generated sequences have been deposited in GenBank (Accession numbers MN308469–MN308478, MN316695–MN316699).

The final unambiguous alignment of the three loci was 2335 base pairs (bp) in length, of which 275 bp were parsimony informative. The phylogenies estimated by the different tree building methods and for the individual loci were very similar, differing only in nodes that received low support. The Bayesian phylogeny is shown in Figure 1, with both the ML and BA support values reported. The *Cheilolejeunea rodneyi* specimens were recovered as sister to *C. morganii* with strong support (100% BS ML, 1.00 PP). The newly-sequenced *C. albovirens* and *C. campbelliensis* were strongly supported as sister taxa (100% BS ML, 1.00 PP), and were in

turn sister to *C. morgani* and *C. rodneyi* (95% BS ML, 1.00 PP). *Cheilolejeunea turgida* and *C. roccatii* grouped with the above species with this clade, which corresponds to Section Paroicae of Ye *et al.* (2015), also receiving strong support (99% BS ML, 1.00 PP).

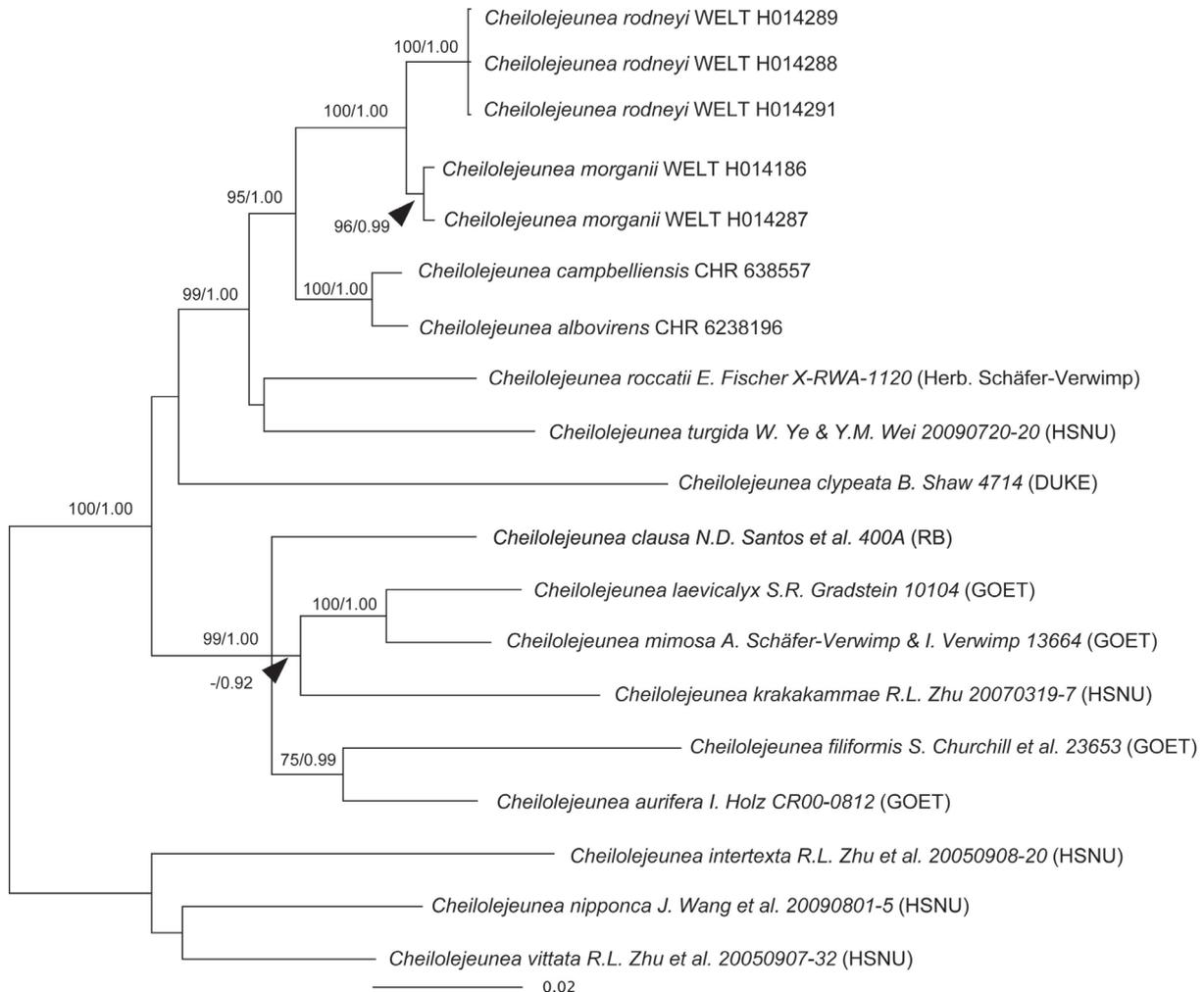


Fig. 1. Bayesian phylogram indicating the position of the new species within *Cheilolejeunea* based on comparison with the position of selected indicative species in the phylogeny of Ye *et al.* (2015), plus the newly sequenced *C. albovirens* and *C. campbelliensis*. Support values higher than 75% maximum likelihood bootstrap (ML BS) and 0.90 posterior probability in the Bayesian analysis (BA PP) are reported in the following order; ML BS / BA PP.

Discussion

Both the morphological comparison and molecular analyses demonstrate the distinctiveness of *Cheilolejeunea rodneyi*. The species is closest to the New Zealand endemic *C. morgani* but is not that species, see the recognition section below for details. It does not match any of the currently accepted New Zealand species and does not match species reported for Australia (Thiers 1997; Zhu and Lai 2005; Renner 2012), China, (Zhu *et al.* 2002) Japan (Mizutani 1982), and North America (Schuster 1980). The New Zealand hepatic flora is closest to that of Australia (Engel and Glennly 2008). About 50% of the c. 640 species are endemic and c. 280 (44%) species are shared with Australia. As all New Zealand non-endemic *Cheilolejeunea* species are found also in Australia (McCarthy 2003), and *C. rodneyi* does not match any of the species described for Australia by Thiers (1997) with additions by Zhu and Lai (2005) and Renner (2012), we propose it as a new species. However, we have not conducted an exhaustive examination of types and synonyms. Thus, the possibility remains that *C. rodneyi* has been previously described. However, in that case, a question of nomenclatural priority will be readily resolved for well circumscribed taxa. It follows that its current provisional status is that of a New Zealand Botanical Region endemic.

Cheilolejeunea rodneyi and the recently-described *C. morganii* are resolved in Section *Paroicae* of the *Cheilolejeunea* phylogeny. *Cheilolejeunea albovirens* and *C. campbelliensis*, whose DNA sequences are newly reported here, also group in this section. Further research into the genus *Cheilolejeunea* in New Zealand is planned to investigate species in the clade including *C. mimosa* to which most undescribed New Zealand species diversity appears to belong.

Taxonomic Treatment

Cheilolejeunea rodneyi Bever. & Glenný sp. nov.

Diagnosis: *Cheilolejeunea rodneyi* is most similar to *C. morganii* Bever. et Glenný with which it shares monoicy, pycnolejeuneoid innovation leaf sequences, obovate pentacarinatè perianth and a long rostrum, oil-bodies mainly or frequently with uniseriate segments, and moderately bulging leaf lamina and lobule cells; but differs in having a one- or two-celled lobule second tooth, oil-bodies with few large homogeneous segments, a large lobule papilla oriented over the lobule external surface, usually distant underleaves, a perianth with low profile dorsal carina, and asexual reproduction by cladia.

Type: New Zealand, Tararua Ecological Region, Tararua Ecological District, Stokes Valley, Horoeka Scenic Reserve 41°10.30'S, 174°59.27'E, 12 Dec 2017, *P. Beveridge* QC-2 (Holotype: WELT H014288!)

Plants pale green, grey-green in herbarium, in procumbent, often parallel, overlapping growth over bark. *Branching* by *Lejeunea*-type intercalary branches. *Leading shoots* c. 0.75–1.0 mm wide by 4–9 mm long, intermingled with a range of suboptimal shoots down to c. 0.2–0.35 mm wide, of variable length, apparently derived from cladia. *Stem* pale brown, c. 60–70 µm diameter, in cross section (Fig. 2E) with 7 cortical cells, the cells more or less rectangular-elliptical, c. 10 µm wide × 20 µm long, the outer wall 3–7 µm thick, medullary cell rows c. 9–12, variably thick-walled. Stem cells in surface view quadrate to short rectangular, 15.0–22.5 µm wide, 17.5–25.0 µm long with walls c. 2.5 µm thick, c. 10 cells intervening between successive leaves. *Rhizoids* mostly absent or traces only, sporadically present in clusters of up to 30, arising from underleaf cells adjacent to underleaf bases, hyaline, thick walled, c. 7.5 µm wide × 150 µm long. *Leaves* (Fig. 2H) incubous, alternate, lobes convex dorsally, imbricate, patent, obliquely spreading, angled to stem axis at about 45°, the lobe apices usually deflexed and obscured in dorsal view. In dorsal view of leading shoots, imbricate leaf lobes completely covering the stem, the antical leaf margin usually extending c. 15–30 µm across stem width. *Lobes* in leading shoots ovate to elliptic, c. 0.3–0.4 mm wide × 0.5 mm long, usually rounded at the leaf apex, occasionally narrowing to an obtuse-angled point, margins entire or with weak crenulation, weakly to moderately angled at lobule apex. *Vitta* absent. *Mid-lobe cells* (Fig. 3) c. 12.5–20.0 µm wide × 17.5–27.5 µm long, walls 1.5–2.0 µm wide, trigones absent or small and concave, usually without intermediate thickening. Dorsal surface of lobe with weakly to moderately bulging cells. *Marginal cells* c. 15 µm wide × 12.5 µm long. *Oil-bodies* (Fig. 3) 2–4, colourless or grey, homogeneous, occupying 25–75% of cell lumen 3–5 µm wide × 7.5–20 µm long, usually deeply segmented, segments variable in length, 2.5–8 µm long, segmentation in linear sequences or occasionally in short double rows. *Chloroplasts* peripheral in cell, appearing spindle-shaped, c. 2.5 µm wide × 3 µm long. *Lobules* (Fig. 2 I, J) before flattening triangular, antical margin inrolled, and the lobule apex constricted with the second tooth normally obscured, but with inflated papilla often visible in profile. Lobule after flattening, ovate triangular, c. 0.08–0.12 mm wide, c. 0.14–0.18 mm long, c. 0.3× lobe length, the keel weakly arched, the free margin of c. 9 cells between stem and second tooth, the lobule apex oblique, the second tooth unicellular or two celled and uniseriate; in the type unicellular c. 20 µm long, 10 µm wide, otherwise two celled, c. 25.5 µm long, 12.5 µm wide, narrowing distally, the papilla when fully expressed c. 8–10 µm diameter, situated at the marginal depression between the second tooth and the slightly prominent rectangular first tooth and oriented over the lobule external surface. Cells of the lobule, including keel, usually weakly bulging. *Underleaves* (Figs 2A,B, 5) weakly to moderately patent, attached to two-celled ventral merophyte by 4–6-celled sub-transverse insertion, typically distant by about one underleaf length, contiguous only close to shoot apices or gynoecea, very occasionally imbricate, obovate to suborbicular, 0.18 mm wide × 0.18 mm long, sinus V-shaped, 0.05 mm deep. Lobe apices rounded, or pointed with a single apical cell. Lobe base c. 6–8 (–9) cells wide.

Asexual reproduction by cladia, present in all collections, abundant in the type. *Cladia* (Fig. 2 AD) short slender fragile branches with reduced leaves and underleaves, produced by *Lejeunea*-type branching from main stem or branches, by pycnolejeuneoid single or double innovations in the form of cladia, or by shoots narrowing to apical cladia.

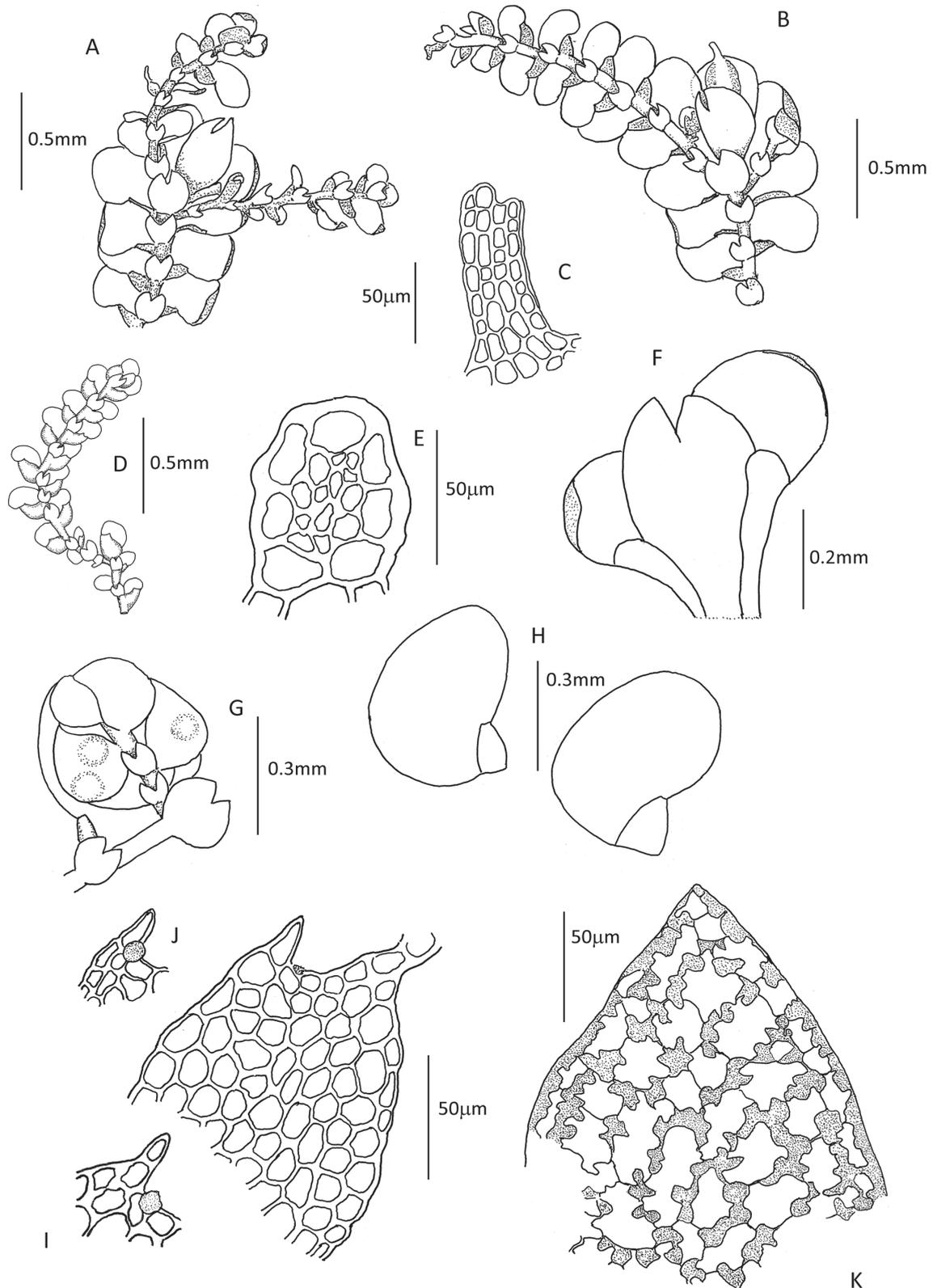


Fig. 2. *Cheilolejeunea rodneyi* Bever. & Glenny sp. nov. (A) Ventral view of leading shoot with unfertilised gynoecium and innovations as cladia. (B) Ventral view of leading shoot with gynoecium and innovation narrowing to lost cladium. (C) Rostrum (D) Cladium (E) Stem section (F) Female bracts and bracteole (G) Androecium (H) Leaves (I) Two-celled second tooth and papilla with exterior orientation (J) Detail of lobule with single celled second lobule tooth and papilla. (K) Sporophyte valve apex, outer layer. (I) Drawn from WELT H014291 (K) Drawn from WELT H014328, the remainder drawn from the type, WELT H014288.

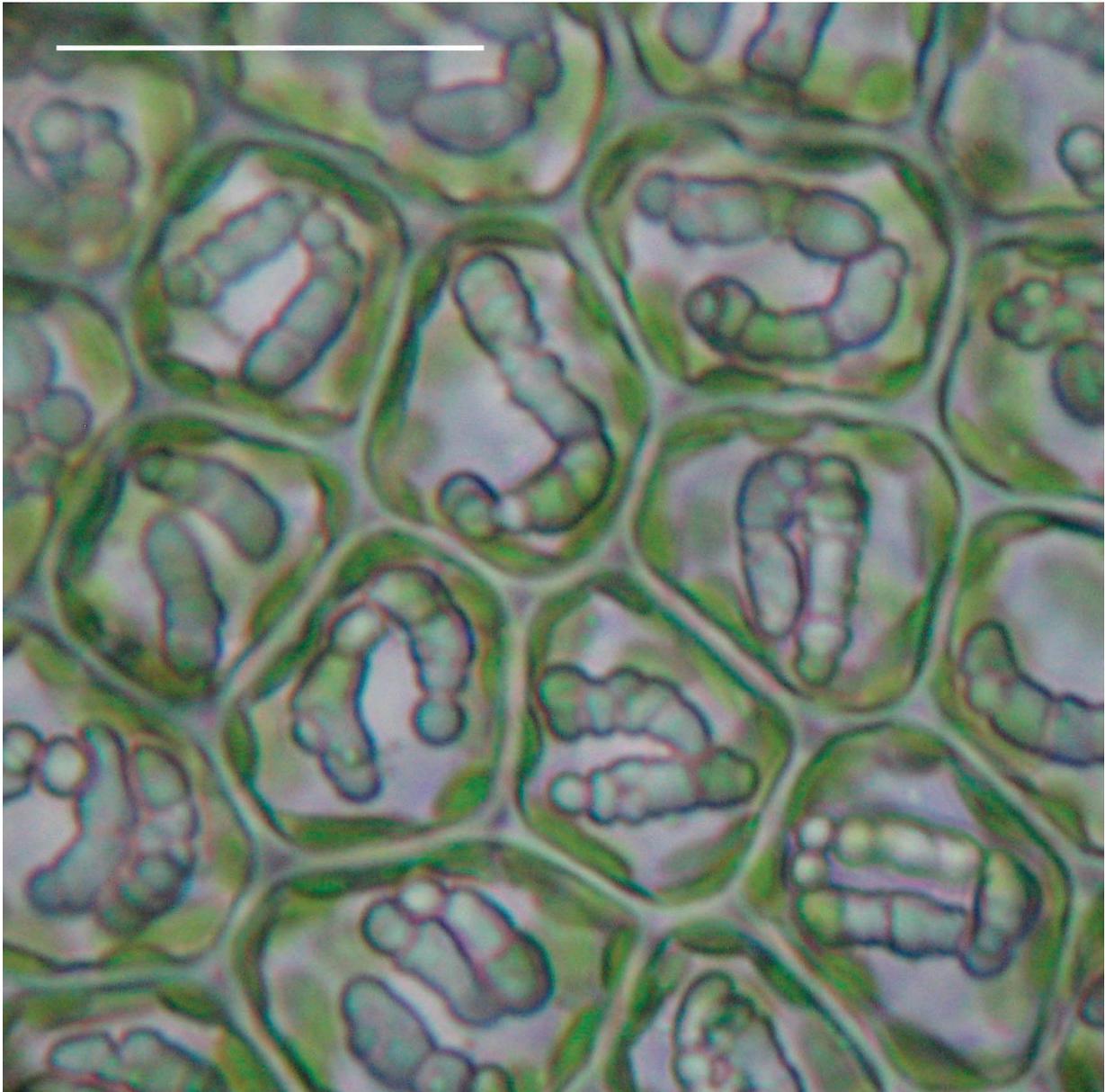


Fig. 3. Oil-body image from WELT HO14328, scale bar 25.5 μm .

Monoicous. Androecia (Fig. 2G) typically short lateral branches from leading shoots and branches, usually proximal on shoots, ovate, c. 0.4 mm wide, 0.5–0.6 mm long, spicate, determinate, projecting beyond the adjacent leaves and visible dorsally, typically 1 male bracteole associated proximally with a sterile bract pair, occasionally with a second bracteole associated with the proximal pair of 2–4 pairs of fertile bracts, the proximal pair of bracts moderately larger than distal pairs, the bracts typically imbricate to contiguous with moderately exposed stems. Androecia diandrous, antheridia c. 12.5 μm diameter, stalk uniseriate, 10 μm diameter. *Gynoecia* (Figs 2F, 4, 5) mostly terminal on long branches with the ultimate subgynoecial underleaf larger than the shoot underleaves not obscuring the innovation first leaf. Female bracts free, bilobed, lobes c. 0.2–0.35 mm wide, 0.45–0.55 mm long, entire, falcate-spathulate, apices rounded, lobule explanate, narrow to moderately broad and lingulate, deflexed, c. 0.07 mm wide, 0.3 mm long. Bracteole free, convex, obovate, cuneate proximally, in distal sector gradually rounded to apex, c. 0.24 mm wide, c. 0.45 mm long, sinus narrow, V-shaped, 0.1 mm deep, with usually single-celled lobe apices. *Perianths* (Figs 2B, 5) terminal on leading or lateral shoots, obovate, rounded to apex, widest close to apex, pentacarinatae, the lateral and ventral carinae sharply carinate, the dorsal carina a low profile ridge on a more or less plane dorsal surface. *Rostrum* (Fig. 2C) subcylindrical, often slightly arcuate, (60–) 75–100 μm long of 5–7 cell tiers, the cells sub-quadrate or rectangular with length up to 2 \times width, with more or less uniform cell thickening. Rostrum apparently fragile. Androecia and gynoecia not often in close proximity. Pycnolejeuneoid innovations, (Figs 2A,B, 5) 1–2 from fertilized and unfertilized gynoecia.

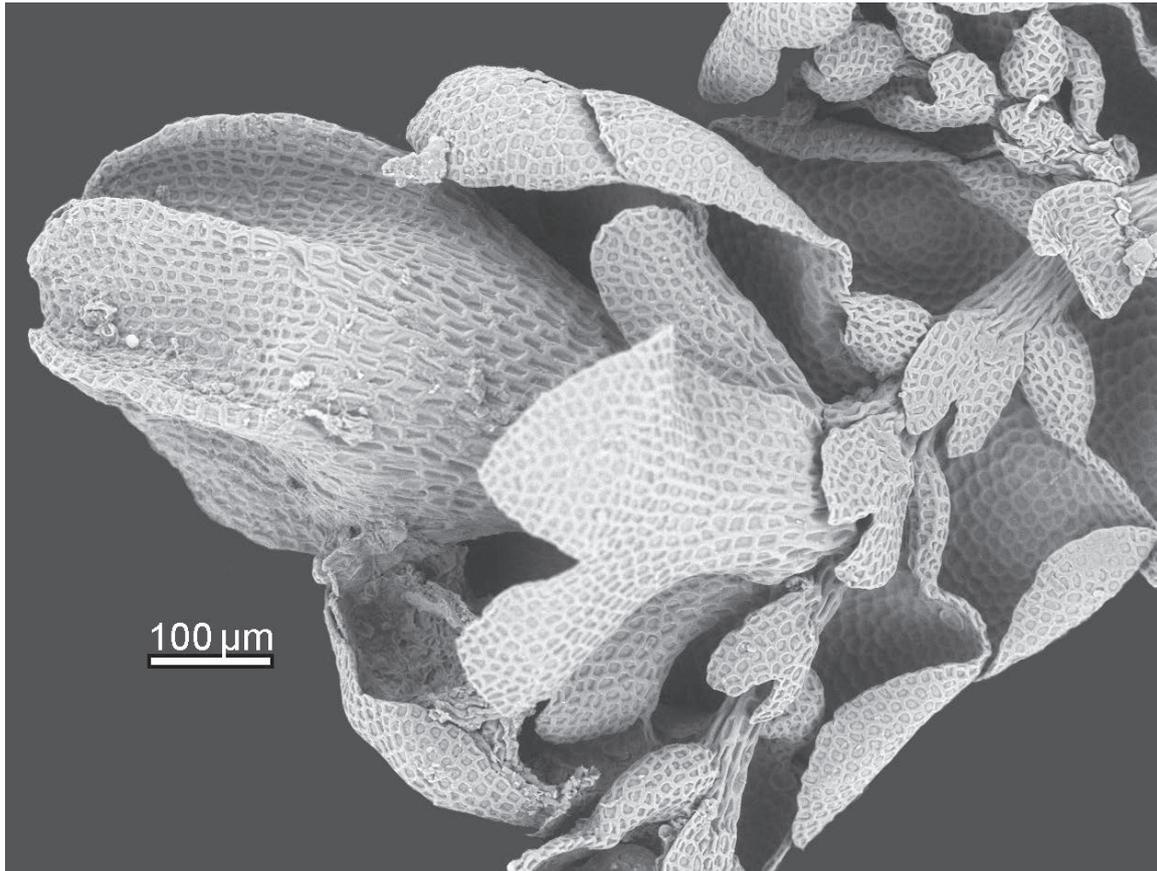


Fig. 4. Ventral view SEM image of WELT HO14328, gynoecium and pycnolejeuneoid innovation.

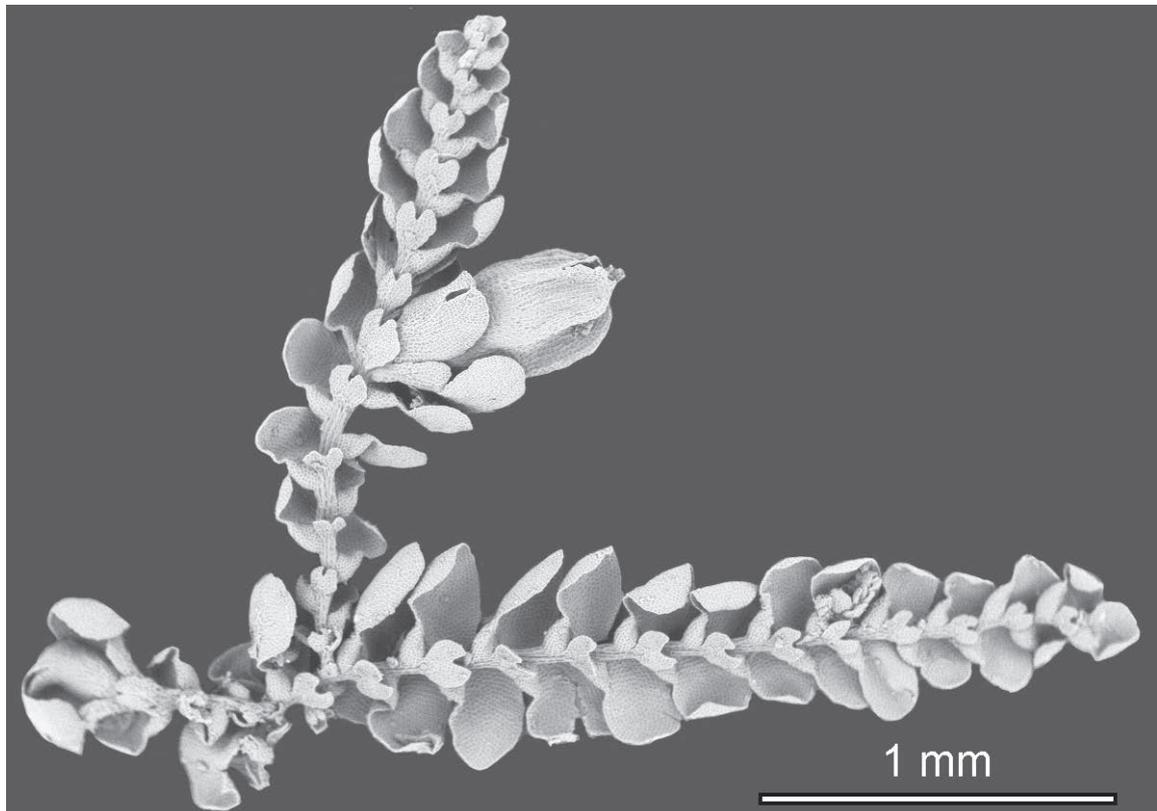


Fig. 5. Ventral view SEM image of WELT HO14328 of shoot, gynoecium and innovation.

Sporophyte (based on *P. Beveridge* QY-1 WELT H014328) shortly emergent from perianth, diameter *c.* 260 μm , seta tiered, width 150 μm , tiers 0.1 mm long, *c.* 0.6 mm between rostrum tip and capsule. Capsule dehiscent into four incompletely separated valves, valve sinus *c.* 0.70 \times valve length. Valves and valve thickenings pale grey to medially golden. *Outer layer* (Fig. 2K) cells in the upper half of the valve quadrate at valve margin with marginal dome-shaped thickenings. Upper valve medial cells larger, obscurely hexagonal, 25 μm \times 25 μm , with conspicuous thickenings, mainly irregular dome-shaped thickenings, occasionally becoming confluent and irregularly linear along cell boundaries. Below mid-valve a transition to a field of *c.* 40 rounded rhomboidal to subrectangular thin-walled cells, the cells *c.* 17.5 μm wide \times 30 μm long or smaller. *Inner layer* cells near apex and margins larger than outer layer cells, *c.* 25 μm wide \times 45 μm long, each with a small dome-shaped thickening, cells near valve midline becoming rectangular in 3–4 (–5) rows parallel to the mid-line, thin-walled with dome-shaped thickening along longitudinal walls, the thickening becoming continuous, sinuose and with a golden colour. *Elaters* *c.* 12.5 μm wide, 250 μm long, with a 2.5 μm wide mono-helical rudimentary thickening, one valve observed with an apical elater and two lateral elaters attached near valve margin, elater complement incomplete and precise pattern of attachment not established. *Spores* flattened, variable in shape, sub-rectangular 75 μm long \times 50 μm wide with transverse to rounded ends, or shorter and ovate, papillose-granular with 2 or 3 rosettes.

Additional specimens examined: New Zealand: North Island: Upper Hutt, Pakuratahi Regional Park, Tunnel Gully, Tane's Track 41°06.17'S, 174°09.30'E, 220 m, 11 Jun 2017, *P. Beveridge* PT-1 (WELT H014289); Wainuiomata, Remutaka Forest Park, Catchpool, Graces Stream, 41° 20.62'S 174° 56.00' E, 60 m, 9 Apr 2017, *P. Beveridge* PR-1 (WELT H014291)

Distribution and Ecology: *Cheilolejeunea rodneyi* has been collected in the Tararua Ecological District, in three lowland forest reserves along the western foothills of the Remutaka Range, east of Wellington and the Hutt Valley. The most southern, Graces Stream in the Remutaka Forest Park, is approximately 20 km S of the type locality, Horoeka Scenic Reserve in Stokes Valley. Tane's Track, Tunnel Gully in the Pakuratahi Regional Park is a further 15 km ENE of the Horoeka Scenic Reserve. At all the sites, *C. rodneyi* was present as an epiphyte on the trunk of *Nothofagus truncata* Colenso.

At the Horoeka SR, the mixed podocarp–broadleaf forest composed of *Dacrycarpus dacrydioides* (A.Rich.) de Laub., *Nothofagus truncata*, *Beilschmiedia tawa* (A.Cunn.) Benth. & Hook.f. ex Kirk, *Pseudopanax crassifolius* (Sol. ex A.Cunn.) K.Koch, *Pseudopanax arboreus* (L.f.) K.Koch, *Knightia excelsa* R.Br., *Olearia rani* (A.Cunn.) Druce, *Cyathea dealbata* (G.Forst.) Sw., with *Dicksonia squarrosa* (G.Forst.) Sw., growing in a confined stream gully with closed canopy.

Cheilolejeunea rodneyi was growing there as a luxuriant trunk epiphyte from tree base to at least two metres and apparently up to the lowest branches over a metre higher. This growth pattern is likely to be a response to relatively high humidity and asexual reproduction by cladia.

At Tane's Track, Tunnel Gully, at an elevation of 220 m, a mixed podocarp–broadleaf forest of *Dacrydium cupressinum* Sol. ex G.Forst., *Nothofagus truncata*, *Beilschmiedia tawa*, *Knightia excelsa* and *Pseudopanax crassifolius* is located on a steep slope with a western aspect and moderate to strong insolation. *Cheilolejeunea rodneyi* was found there on the lower trunk and base of *N. truncata*.

At Graces Stream, Remutaka Forest Park, at 60m elevation, the mixed broadleaf forest with dominant *Nothofagus truncata* and *N. solandri* Hook.f. has moderate to strong insolation with evidence of maturity and windfall. *Cheilolejeunea rodneyi* there was in scattered patches on the trunk of *N. truncata* with other bryophytes including *Drepanolejeunea aucklandica* Steph., *Frullania aterrima* (Hook.f. & Taylor) Gottsche, Lindenb. & Nees, *F. pentapleura* Taylor, *Radula* sp. and *Hypnum chrysogaster* Müll.Hal.

Recognition: Although within the New Zealand *Cheilolejeunea* flora, there are a few species that can be identified with confidence on vegetative features alone, for most, gynoecial material is required with innovations, and ideally with perianths, to determine whether gynoecial innovations are lejeuneoid or pycnolejeuneoid and facilitate identification. Examination of the key below will show that the pycnolejeuneoid condition is shared by 7 species, *C. albovirens*, *C. ceylanica*, *C. intertexta*, *C. campbelliensis*, *C. novaezealandiae*, *C. morganii*, and with this paper, *C. rodneyi*. Some of these species are readily distinguished from *C. rodneyi* and *C. morganii* by their vegetative features. *Cheilolejeunea albovirens* and *C. ceylanica* differ by being small with narrow shoots, less than 500 μm wide and with long, rectangular lobules at least 0.5 lobe length. *Cheilolejeunea campbelliensis* and *C. novaezealandiae* are distinguished by their having papillose leaf cells. The remaining pycnolejeuneoid species was formerly known as *Pycnolejeunea glauca* and recognised in New Zealand at present as *C. intertexta*, is distinguished from *C. morganii* and *C. rodneyi* by its short rostrum and underleaves that are large, up to four times stem width, orbicular with a narrow sinus and with underleaf lobe apices commonly crossing over each other.

Cheilolejeunea rodneyi is most similar to *C. morganii* but is able to be distinguished by a number of features. The perianths of *C. rodneyi* have a rounded apex and a low-profile dorsal carina ridge in contrast to the truncate or retuse perianth apex and sharply carinate dorsal carina in *C. morganii*. Underleaves are usually obovate to suborbicular and distant in *C. rodneyi* while typically obovate and contiguous in *C. morganii*. The distinctive oil-body features in *C. rodneyi* of linear sequences of mainly single large homogenous segments up to 4 µm wide x 20 µm long contrast with the spherical segments, 2.5 µm diameter, in *C. morganii*. The lobule features of *C. rodneyi* include a single or two-celled second tooth and a large papilla oriented over the lobule external surface. The papilla is erect on the lobule margin in *C. morganii* and the second tooth 4–5-celled. Cladia, sometimes conspicuous, are present only in *C. rodneyi*.

A summary of these and other features by which *Cheilolejeunea rodneyi* can be distinguished from *C. morganii* may be found in Table 2.

Table 2. Features for distinguishing between *Cheilolejeunea rodneyi* and *C. morganii*.

Character	<i>Cheilolejeunea rodneyi</i>	<i>C. morganii</i>
Leaf lobe apices	rounded	rounded to obtuse-angled point
Oil-bodies	homogeneous, 2–4, 3–5 µm wide 7.5–20 µm long, segments 2.5–8 µm long in linear sequences	botryoidal, 2–3, 3–4 µm wide, 10–12.5 µm long, segments spherical, ca 2.5 µm in diam., moniliform or double rows
Underleaves (typically)	distant x length of an underleaf	contiguous
Underleaf shape	obovate to suborbicular	obovate
Lobule second tooth	1–2 cells	4–5 cells
Lobule papilla	large marginal extending onto lobule external surface	small marginal erect
Rostrum shape	cylindrical	truncate-conical
Rostrum cell tiers	5–7 (75–100 µm long)	5–7 (ca 65 µm long)
Rostrum cells	quadrate–rectangular, length to width to x2	isodiametric to short rounded
Perianth dorsal carina	low profile ridge	sharply carinate
Cladia	commonly present	not seen

Conservation Status: *Cheilolejeunea rodneyi* is known at present from only three locations in the Wellington area and was not previously represented in the herbaria at CHR and WELT. The species was unknown at the last published threat classification (de Lange *et al.* 2014). Its classification in the meantime, according to the New Zealand Threat Classification System (Townsend *et al.* 2008) should be ‘Data Deficient’.

Etymology: The name honours the late Rodney Lewington (1935–2018), a long-time member of the Wellington Botanical Society, supporter of the Otari-Wilton’s Bush Trust, and student of the New Zealand liverwort flora, generous in sharing his knowledge and enthusiasm, and a frequent companion in the field.

Key to species of *Cheilolejeunea* in New Zealand

The following key to the New Zealand species of *Cheilolejeunea* is modified from that for Australia in Thiers (1997), with additional modifications after Zhu and Lai (2005).

1. Leaves ovate; lobule rectangular, 2.0–3.5 times longer than wide, at least 0.5 lobe length; innovation leaf sequences pycnolejeuneoid 2
1. Leaves ovate, ovate-acute or orbicular; lobule ovoid, 1–2 times longer than wide, 0.25–0.5 lobe length; innovation leaf sequence lejeuneoid or pycnolejeuneoid 3
2. Shoots less than 500 µm wide; stem cortex of less than 5 cells in cross-section; leaves erect, innovations usually absent *C. albovirens*
2. Shoots at least 500 µm wide; stem cortex of at least 7 cells in cross-section; leaves widely spreading; innovations usually present *C. ceylanica*
- 3 Underleaf insertion straight or moderately arched, bases cuneate to rounded 4
- 3 Underleaf insertion strongly arched (∩-shaped), bases cordate *C. trifaria*
- 4 Innovation leaf sequence lejeuneoid 5
- 4 Innovation leaf sequence pycnolejeuneoid 7

- 5 Perianths sharply 5-keeled 6
- 5 Perianth keels completely lacking *C. comitans*
- 6 Leaf apex acute; lobule apex attenuate, lobule teeth subequal, composed of single cells, isodiametric *C. hamlinii*
- 6 Leaf apex obtuse; lobule apex not attenuate, lobule teeth not equal in size and shape, second tooth pointed towards leaf apex *C. mimosa*
- 7 Rostrum (intact) >4 cell tiers tall 8
- 7 Rostrum (intact) <4 cell tiers tall 9
- 8 Second tooth composed of 1 or 2 cells, if 2 then uniseriate *C. rodneyi*
- 8 Second tooth composed of 4–5 cells, uniseriate or not *C. morganii*
- 9 Dorsal leaf cell surfaces smooth *C. intertexta*
- 9 Dorsal leaf cells each bearing a single dome-shaped papilla 10
- 10 Leaf apices rounded-acute to obtuse, underleaves remote, papillae on lobule carina, lobe margins, but not on underleaves, dioicous *C. campbelliensis*
- 10 Leaf apices acute, narrowly triangular, underleaves contiguous or slightly imbricate, papillae on dorsal lobe surface and margins and underleaf disc, autoicous *C. novaezelandiae*

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References

- Allan HH (1961) Flora of New Zealand. Vol. I. Government Printer, Wellington
- Braggins JE, Renner MAM, de Lange P (2014) Additions to the liverwort flora of the Kermadec Islands, New Zealand Botanical Region. *Telopea* 17: 183–194. <https://doi.org/10.7751/telopea20147709>
- Beveridge P, Glenny D, Shepherd L (2019) *Cheilolejeunea morganii* Bever. & Glenny (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>
- Beveridge P, Shepherd L (2019) Molecular and morphological evidence support the reinstatement of *Cheilolejeunea comitans* from synonymy of *C. krakakammae*. *Telopea* 22: 89–98. <https://doi.org/10.7751/telopea13390>
- 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>
- Engel JJ, Glenny D (2008) A liverwort and hornwort flora of New Zealand. Vol. 1. *Monographs in Systematic Botany*. St Louis: Missouri Botanical Garden.
- Engel JJ, Glenny D (2019a) A liverwort and hornwort flora of New Zealand. Vol. 2. *Monographs in Systematic Botany*. St Louis: Missouri Botanical Garden.
- Engel JJ, Glenny D (2019b) A liverwort and hornwort flora of New Zealand. Vol. 3. *Monographs in Systematic Botany*. St Louis: Missouri Botanical Garden.
- Glenny D (1996) *Nephelolejeunea papillosa*, a new liverwort species from New Zealand, with notes on the distribution of *Kymatolejeunea bartlettii* Grolle. *New Zealand Journal of Botany*. 34(2): 195–198. <https://doi.org/10.1080/0028825X.1996.10410683>
- Grolle R (1973) *Nephelolejeunea*—eine neue Gattung der Tuyamaelloideae. *Journal of the Hattori Botanical Laboratory*. 37: 251–261.
- Grolle R. (1982) Übersicht der Lejeuneaceae in Tasmanien. *Wissenschaftliche Zeitschrift der Friedrich-Schiller- Universität 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>
- Lewington RJ, Beveridge P, Renner MAM (2013) *Lejeunea hodgsoniana*, a newly described, long-recognised *Lejeunea* (Jungermanniopsida, Lejeuneaceae) from lowland coastal forest habitats in New Zealand. *PhytoKeys*. 29: 1–15. <https://doi.org/10.3897/phytokeys.29.5376>
- Mizutani M (1982) Notes on the Lejeuneaceae 6. Japanese species of the genus *Cheilolejeunea*. *Journal of the Hattori Botanical Laboratory*. 51: 151–173.
- McCarthy PM (2003) Catalogue of Australian liverworts and hornworts. *Flora of Australia Supplementary Series*. 21: 1–137.
- Pacak A, Szweykowska-Kulinska Z (2000) Molecular data concerning allopolyploid 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/>.
- Renner MAM (2010) Another new species of *Austrolejeunea* (Lejeuneaceae) from New Zealand's subalpine environs. *The Bryologist*. 113: 781–787. <https://doi.org/10.1639/0007-2745-113.4.781>
- Renner MAM (2012) Further insight into lobule teeth homology in Lejeuneaceae subf. Lejeuneoideae from *Cheilolejeunea oscilla*, a new species from Australia. *The Bryologist*. 115: 536–556. <https://doi.org/10.1639/0007-2745-115.4.536>
- Renner, MAM (2013) *Lejeunea subelobata* and *Lejeunea drummondii* (Jungermanniopsida) in Australasia. *Polish Botanical Journal* 58: 193–203. <https://doi.org/10.2478/pbj-2013-0018>
- Renner MAM, Brown EA, Wardle GM (2009) Evidence for species recognition on the basis of a single specimen: *Nephelolejeunea carcharias* sp. nov. Lejeuneaceae: Jungermanniopsida). *Systematic Botany*. 34: 615–624. <https://doi.org/10.1600/036364409790139754>
- Renner MAM, Brown EA, Wardle GM (2010) The *Lejeunea tumida* species group (Lejeuneaceae: Jungermanniopsida) in New Zealand. *Australian Systematic Botany* 23: 443–462. <https://doi.org/10.1071/SB10037>
- Renner MAM, de Lange PJ (2011) Additions to the Lejeuneaceae flora of New Zealand: new species from the Kermadec Islands and range extensions of New Zealand species into the South Pacific. *New Zealand Journal of Botany* 49: 421–433. <https://doi.org/10.1080/0028825X.2011.580765>
- Renner MAM, Pócs T (2011) *Cololejeunea grossepapillosa* (Lejeuneaceae: Jungermanniopsida), an inconspicuous species new for New Zealand. *New Zealand Journal of Botany*. 49: 295–303. <https://doi.org/10.1080/0028825X.2010.548071>
- Schuster RM (1980) *The Hepaticae and Anthocerotae of North America. Vol. IV*. New York: Columbia University Press.
- Schuster RM (1985) Austral Hepaticae, XIX. Some taxa new to New Zealand and New Caledonia. *Phytologia* 56: 449–464.
- 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>
- Söderström L, Hagborg A, von Konrat MJ, Bartholomew-Began S, Bell N, Briscoe L, Brown EA, Cargill DC, da Costa DP, Crandall-Stotler BJ, Cooper ED, Dauphin G, Engel JJ, Feldberg K, Glenny DS, Gradstein SR, He XL, Heinrichs J, Hentschel J, Ilkiu-Borges AL, Katagiri T, Konstantinova NA, Larraín J, Long DG, Nebel M, Pócs T, Puche F, Reiner-Drehwald E, Renner MAM, Sass-Gyarmati A, Schäfer-Verwimp A, Moragues JGS, Stotler RE, Sukkharak P, Thiers BM, Uribe J, Váña J, Villarreal JC, Wigginton M, Zhang L, Zhu RL. (2016) World checklist of hornworts and liverworts. *PhytoKeys*. 59: 1–828. <https://doi.org/10.3897/phytokeys.59.6261>

- 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>
- 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.
- Townsend AJ, de Lange PJ, Norton DA, Molloy J, Miskelly C, Duffy C. (2008) *The New Zealand threat classification system manual*. Wellington: Department of Conservation.
- von Konrat M, Hagborg A, Söderström L, Mutke J, Renner M, Gradstein SR, Engel JJ, Zhu R-L, Pickering J (2008) Early land plants today: global patterns of liverwort diversity, distribution, and floristic knowledge. Pp 425–438 in: Mohamed H, Baki BB, Nasrulhaq-Boyce A, Lee PKY (eds) *Bryology in the New Millennium*. Kuala Lumpur: University of Malaya,
- Ye W, Gradstein SR, Shaw J, Shaw, B, Hoe, 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>
- Ye W, Zhu R-L (2018) *Gaolejeunea*, a new genus from China and new member of subtribe Echinolejeuneinae (Lejeuneaceae, Marchantiophyta). *The Bryologist*. 121: 41–48. <https://doi.org/10.1639/0007-2745-121.1.041>
- 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.
- 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 (2001) Epiphyllous liverworts of China. *Nova Hedwigia Beiheft*. 121: 1–418.
- Zhu R-L, Shu L, Mustapeng AM, Suleiman M (2017) *Thiersianthus* (Marchantiophyta: Lejeuneaceae), a new genus from lowland rainforests in Borneo. *The Bryologist*. 120: 511–520. <https://doi.org/10.1639/0007-2745-120.4.xxx>
- Zhu R-L, Shu L, He Q, Wei Y-M (2018) *Soella* (Marchantiophyta: Lejeuneaceae), a new genus from China and Japan. *The Bryologist*. 121: 324–339. <https://doi.org/10.1639/0007-2745-121.3.324>

