



Phylogenetic position of *Trocholejeunea* and a new infrageneric classification of *Acrolejeunea* (Lejeuneaceae, Marchantiophyta)

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Abstract

We address phylogenetic relationships of *Acrolejeunea* and *Trocholejeunea*, two putatively related members of Lejeuneaceae subfam. Ptychanthoideae, by using nuclear (ITS) and chloroplast (*rbcL*, *trnL-F*) DNA regions. Bayesian, likelihood and parsimony phylogenetic analyses of a broad sampling of the two genera resolved *Acrolejeunea* as monophyletic. The species of *Acrolejeunea* were resolved in five clades, which partly correspond to previously described sections. *Trocholejeunea* was nested in *Acrolejeunea* as a sixth, monophyletic lineage. Based on our molecular-phylogenetic results, the species of *Acrolejeunea* are arranged in six sections including two new ones, sect. *Recurvatae* sect. nov. for *A. recurvata* from subtropical Asia and sect. *Trocholejeunea* (Schiffn.) stat. nov. The five species of *Trocholejeunea* are transferred to *Acrolejeunea*, which becomes a pantropical genus of 21 species (including a fossil one). New lectotypes are designated for *A. infuscata* and *A. sandvicensis*.

Keywords: *Acrolejeunea* sect. *Recurvatae*; *Acrolejeunea* sect. *Trocholejeunea*; *Acrolejeunea* subg. *Acrolejeunea*; *Acrolejeunea* subg. *Isolejeunea*; *Frullanoides*; molecular phylogeny; Lejeuneaceae subfam. Ptychanthoideae; taxonomy

Introduction

Lejeuneaceae Cavers (1910: 291) with more than a thousand species in 69 genera are the largest family of leafy liverworts and notoriously difficult in terms of generic delimitation (e.g., Schuster 1963; Gradstein 1979, 2013). Several recent studies have analysed generic relationships in Lejeuneaceae using molecular evidence (e.g., Ahonen *et al.* 2003; Dong *et al.* 2013; Gradstein *et al.* 2006; Hartmann *et al.* 2006; Heinrichs *et al.* 2012a, b, 2013; Sukkharak *et al.* 2011; Wilson *et al.* 2007; Ye *et al.* 2013), the most comprehensive one being the study by Wilson *et al.* (2007). These studies showed that the number of genera is much lower than previously accepted and that several genera, including *Aphanolejeunea* A.Evans (1911: 272), *Chondriolejeunea* (Benedix 1953: 75) Kis & Pócs (2001: 239), *Cystolejeunea* A.Evans (1906: 16), *Dendrolejeunea* (Spruce 1884: 110) Lacouture (1908: 104), *Leucolejeunea* A.Evans (1907: 225), *Metzgeriopsis* K.I.Goebel (1888: 54), *Myriocolea* Spruce (1884: 305), *Oryzolejeunea* (R.M.Schuster 1970: 338) R.M.Schuster (1992: 249), *Pluvianthus* R.M.Schuster & A.Schäfer-Verwimp (1994: 213), *Sphaerolejeunea* Herzog (1938: 88) and *Taxilejeunea* (Spruce 1884: 212) Schiffner (1893: 125), should be reduced to subgeneric rank or synonymy. However, a large number genera of Lejeuneaceae have not been studied in detail based on molecular evidence.

This paper focuses on the relationships of *Acrolejeunea* (Spruce 1884: 115) Schiffner (1893: 128) and *Trocholejeunea* Schiffner in Dixon *et al.* (1932: 160) (Lejeuneaceae subfam. Ptychanthoideae). *Acrolejeunea* is a pantropical genus of 15 extant species, including two varieties and five subspecies (Gradstein 1975, Renner 2013). In addition, a fossil species has been described from Late Eocene amber from Ukraine (Mamontov *et al.* 2013). The centre of diversity is Southeast Asia with 10 species. *Acrolejeunea* is circumscribed to include species with predominantly *Lejeunea*-type branches, stems with hyalodermis, entire underleaves and entire margins of leaf lobe, cordate trigones, homogeneous oil bodies, epistatic male bracts with one antheridium, absence of innovations, 5–10-keeled perianths, and a non-articulate seta with 16 outer and four inner cell rows. The genus was monographed by Gradstein (1975) who

classified the species into two subgenera, subg. *Acrolejeunea* and subg. *Isolejeunea* Gradstein (1975: 83). Subgenus *Acrolejeunea* (5 spp.) was characterized by anisoplicate perianths and vegetative reproduction by caducous leaves, and was distributed in tropical America, Africa and continental Southeast Asia. The species of subg. *Isolejeunea* (10), in contrast, possessed isoplicate perianths (usually), lacked vegetative reproduction by caducous leaves, and were largely restricted to tropical Asia, Australasia and the Pacific region. Each subgenus was further subdivided in two sections based on characters of stem cross sections and leaves, but morphological separation of the sections was not sharp. One species of subg. *Acrolejeunea*, *A. pusilla* (Stephani 1911: 731) Grolle & Gradst. in Gradstein (1974: 332) from Japan, was separated as a monospecific section, sect. *Pusillae* Gradstein (1975: 59) based on very thin stems and non-squarrose leaves. The remaining species of this subgenus (*A. emergens* [Mitten 1879: 397] Stephani (1895a: 65), *A. heterophylla* [A.Evans 1918: 44] Grolle & Gradst. in Gradstein (1974: 332), *A. recurvata* Gradstein (1975: 79), *A. torulosa* [Lehm. & Lindenb. in Lehmann 1834: 41] Schiffner (1893: 128. [type]) constituted sect. *Acrolejeunea*. In subg. *Isolejeunea*, four species (*A. aulacophora* [Montagne 1843: 259] Stephani (1895b: 317) [type], *A. fertilis* [Reinwardt, Blume & Nees von Esenbeck 1824: 211] Schiffn. in Engler & Prantl (1893: 128), *A. securifolia* [Nees in Endlicher 1833: 5] Stephani (1895c: 59), *A. sikkimensis* [Mizut. in Hara 1966: 532] Gradstein [1975: 83]), were placed in sect. *Regulares* [Verdoorn 1934: 143] Gradstein (1975: 83) characterized by asymmetric epidermis and frequently squarrose leaves, while the remaining six species (*A. allisonii* Gradstein [1975: 103], *A. arcuata* [Nees 1830: 38] Grolle & Gradst. in Gradstein [1974: 332] *A. mollis* [Hook.f. & Taylor in Taylor 1846: 384] Schiffner [1894: 178], *A. parvula* [Mizutani in Hattori & Mizutani 1969: 97] Gradstein [1975: 115], *A. pycnoclada* [Taylor 1846: 385] Schiffner in Engler & Prantl [1893: 128], *A. tjobodensis* [Verdoorn 1933: 227] Grolle & Gradst. in Gradstein [1974: 333]) constituted sect. *Isolejeunea* Gradstein (1975: 83) characterized by symmetric epidermis and non-squarrose leaves. The latter name proved illegitimate and is replaced by *A. sect. Minores* (Verd.) L.Söderstr. & Hagborg.

Trocholejeunea was established by Schiffner (in Dixon *et al.* 1932) based on *Brachiolejeunea levieri* Schiffn. ex Stephani (1912: 134) (= *Trocholejeunea infuscata* [Mitten 1861: 111] Verdoorn [1934: 190]). The genus is restricted to eastern Asia and currently contains five species: the type *T. infuscata* (Mitt.) Verd. known from China, Himalayas, India, Sri Lanka, Myanmar and Thailand (Gradstein 1991; Mizutani 1989), *T. sandvicensis* (Gottsche 1857: 344) Mizutani (1962: 169) widespread in Indochina, Japan and the Pacific (Gradstein 1991; Zhu & So 2001), *T. crassicaulis* (Stephani 1914: 550) Mizutani (1989: 280) from Thailand, Sabah, and Papua New Guinea (Mizutani 1989; present study), *T. meghalayensis* A.P.Singh & V.Nath (2008: 2) from India (Singh & Nath 2008), and *T. sinensis* J.Wang bis, R.L.Zhu & Gradst. in Wang *et al.* (2014: 296) from China (Wang *et al.* 2014). The genus shares many characters with *Acrolejeunea* such as stem with a hyalodermis, entire-margined leaves and underleaves, cordate trigones, homogeneous oil bodies, epistatic male bracts with one antheridium, pluriplicate perianth and a non-articulate seta. It differs mainly by the predominance of *Frullania*-type branching, presence of *Frullania*-type innovations, a seta with 4–16 rows of inner cells and 16–32 outer rows, and possibly more numerous elaters per capsule in *Trocholejeunea* (Thiers 1985; Van Slageren 1985; Wang *et al.* 2014; Weis 2001; Zhu & So 2001).

Cladistic analyses of Lejeuneaceae using morphological characters (Gradstein 1994; Gradstein *et al.* 2003) have recovered *Acrolejeunea* and *Trocholejeunea* as members of a clade together with *Frullanoides* Raddi (1822: 13), a mostly neotropical genus of six species (van Slageren 1985), and Schuster (1980, 1992) even combined *Trocholejeunea* and *Frullanoides* into a single genus. However, the latter genus is readily separated from *Acrolejeunea* and *Trocholejeunea* by blackish pigmentation, hypostatic male bracts with two antheridia, *Radula*-type innovations and an articulate seta. The putative close relationship between *Acrolejeunea* and *Frullanoides* was not supported by recent molecular-phylogenetic evidence, which resolved these two genera in distant clades (Wilson *et al.* 2007). The relationships of *Trocholejeunea* have not yet been analysed by molecular methods.

The goals of the present study are to (1) evaluate the relationships of *Acrolejeunea* and *Trocholejeunea* using evidence from three molecular markers (*rbcL*, *trnL-F*, nrITS) and morphology, (2) test the monophyly of the subgenera and sections of *Acrolejeunea*, and (3) evaluate the significance of morphological characters for species circumscriptions and intrageneric classification in the light of the molecular results.

Materials and methods

Sampling, DNA extraction and sequencing.

We analysed 48 species (54 samples) of 15 genera of Ptychanthoideae, including 14 (of 15) species of *Acrolejeunea* (17 samples), representing all described sections of the genus, and 4 (of 5) of *Trocholejeunea* (6 samples) (Table 1).

These samples were the ingroup in this study. We were unable to sequence the rare *Acrolejeunea allisonii* from New Zealand and *Trocholejeunea meghalayensis* from India due to the lack of sufficiently recent specimens for DNA studies. Members of five genera of subfam. Lejeuneoideae tribe Brachiolejeuneae were employed as outgroups, including *Acanthocoleus aberrans* (Lindenb. & Gottsche in Gottsche *et al.* 1847: 751) Kruijt (1988: 62), *Blepharolejeunea incongrua* (Lindenb. & Gottsche in Gottsche *et al.* 1847: 750) van Slageren & Kruijt (1985: 133), *Brachiolejeunea laxifolia* (Taylor 1847: 341) Schiffner (1893: 128), *Dicranolejeunea axillaris* (Nees & Montagne 1836: 59) Schiffner (1893: 128) and *Lindigianthus cipaconeus* (Gottsche 1864: 150) Kruijt & Gradstein (1985: 166). Thirty *trnL-F*, 30 *rbcL* and 29 nrITS sequences from Wilson *et al.* (2007) were downloaded from Genbank, all other sequences were newly generated for this study. Voucher information and GenBank accession numbers used for phylogenetic analyses are given in Table 1.

TABLE 1. Information on voucher specimens and GenBank accession numbers in the present study. An asterisk indicates sequences generated by the present study; a dash indicates a missing sequence.

Taxa	Voucher	Gen bank Accession numbers		
		<i>rbcL</i>	<i>trnL</i>	nrITS
<i>Acanthocoleus aberrans</i> (Lindenb. & Gottsche) Kruijt	Spain, Canary Islands, Schäfer-Verwimp 4213 (GOET)	DQ983647	DQ987461	DQ987365
<i>A. javanicus</i> (Steph.) Kruijt	Indonesia, Schäfer-Verwimp 20817 (GOET)	DQ983648	DQ987398	DQ987291
<i>Acrolejeunea arcuata</i> (Nees) Grolle & Gradst. I	Indonesia, Gradstein 12053 (HSNU)	KP182103*	KP182080*	KP182126*
<i>A. arcuata</i> II	Malaysia, Gradstein 10418 (HSNU)	KP182104*	KP182081*	KP182127*
<i>A. aulacophora</i> (Mont.) Steph.	Reunion, Schäfer-Verwimp 19800 (GOET)	KP182105*	KP182082*	KP182148*
<i>A. emergens</i> (Mitt.) Steph.	Thailand, Schäfer-Verwimp 23710/B (GOET)	KP182106*	KP182083*	KP182128*
<i>A. fertilis</i> (Reinw. <i>et al.</i>) Schiffn.	Malaysia, Gradstein <i>et al.</i> 10358 (HSNU)	KP182107*	KP182084*	KP182129*
<i>A. heterophylla</i> (Evans) Grolle & Gradst.	U.S.A., Lassiter 2258 (HSNU)	KP182108*	KP182085*	KP182130*
<i>A. mollis</i> (Hook & Taylor) Schiffn.	New Zealand, Beveridge NL-1 (HSNU)	KP182109*	KP182086*	KP182131*
<i>A. parvula</i> (Mizut.) Gradst.	Thailand, Kornochalert 1424 (HSNU)	KP182110*	KP182087*	KP182132*
<i>A. pusilla</i> (Steph.) Grolle & Gradst.	China, Schäfer-Verwimp 18449 (GOET)	KP182111*	KP182088*	KP182133*
<i>A. pycnoclada</i> (Taylor) Schiffn.	Thailand, Schäfer-Verwimp 16261/B (GOET)	KP182112*	KP182089*	KP182134*
<i>A. recurvata</i> Gradst. I	Thailand, Schäfer-Verwimp 23935 (GOET)	KP182113*	KP182090*	KP192135*
<i>A. recurvata</i> II	Thailand, Zhu 20111218-2 (HSNU)	KP182114*	KP182091*	KP182136*
<i>A. securifolia</i> (Nees) Steph. ssp. <i>securifolia</i>	Australia, Pöcs 1078/O (HSNU)	KP182115*	KP182092*	KP182137*
<i>A. sikkimensis</i> (Mizut.) Gradst.	China, Wang 20120424-8 (HSNU)	KP182116*	KP182093*	KP182138*
<i>A. tjibodensis</i> (Verd.) Grolle & Gradst. I	Indonesia, Schäfer-Verwimp 25108 (GOET)	KP182117*	KP182094*	KP182139*
<i>A. tjibodensis</i> II	Malaysia, Gradstein 10397 (HSNU)	KP182118*	KP182095*	KP182140*
<i>A. torulosa</i> (Lehm. & Lindenb.) Schiffn.	Colombia, Gradstein 9019 (HSNU)	KP182119*	KP182096*	KP182141*
<i>Archilejeunea fuscescens</i> (Hampe ex Lehm.) Fulf.	Costa Rica, Bernecker 97-53 (GOET)	DQ983655	DQ987384	DQ987267
<i>A. parviflora</i> (Nees) Schiffn.	Costa Rica, Gradstein & Dauphin 9370 (GOET)	DQ983657	DQ987456	DQ987356
<i>A. planiuscula</i> (Mitt.) Steph.	Thailand, Schäfer-Verwimp 16237 (GOET)	AY548081	DQ987395	DQ987285
<i>Blepharolejeunea incongrua</i> (Lindenb. & Gottsche) van Slageren & Kruijt	Brazil, Schäfer-Verwimp 12800 (GOET)	DQ983659	DQ987397	DQ987290
<i>Brachiolejeunea laxifolia</i> (Tayl.) Schiffn.	Ecuador, Wilson <i>et al.</i> 04-03 (GOET)	DQ983660	DQ987434	DQ987330
<i>Bryopteris diffusa</i> (Sw.) Nees	Bolivia, Acebey & Villavicencio 855 (GOET)	AY548085	AM237147	AM237095
<i>B. filicina</i> (Sw.) Nees	Bolivia, Drehwald 4751 (GOET)	AY548087	AM237158	AM237106
<i>Caudalejeunea reniloba</i> (Gottsche) Steph.	Malaysia, Gradstein <i>et al.</i> 10378 (GOET)	DQ983661	DQ987419	DQ987315
<i>Dicranolejeunea axillaris</i> (Nees & Mont.) Schiffn.	Bolivia, Drehwald 4829 (GOET)	DQ983674	DQ987377	DQ987255
<i>Frullanoides corticalis</i> (Lehm. & Lindenb.) van Slageren	French Guiana, Hartmann 04-080 (GOET)	DQ983681	AM237196	AM237143
<i>F. mexicana</i> van Slageren	Mexico, Burghardt 4421a (GOET)	DQ983682	DQ987464	DQ987366
<i>Fulfordianthus evansii</i> (Fulford) Gradst.	Costa Rica, Dauphin s.n. (GOET)	DQ983683	AM237197	AM237144
<i>F. pterobryoides</i> (Spruce) Gradst.	Costa Rica, Dauphin 2518 (GOET)	DQ983684	AM237198	AM237145

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TABLE 1. (Continued)

Taxa	Voucher	Gen bank Accession numbers		
		<i>rbcL</i>	<i>trnL</i>	nrITS
<i>Lindigianthus cipaconeus</i> (Gottsche) Kruijt & Gradst.	Ecuador, Kottke & Preußing MPEO2390 (GOET)	DQ983702	DQ987445	DQ987343
<i>Lopholejeunea eulopha</i> (Tayl.) Schiffn.	Australia, Pócs & Streimann 9987/H1 (GOET)	AY548067	DQ987381	DQ987262
<i>L. subfusca</i> (Nees) Schiffn.	Malaysia, Gradstein <i>et al.</i> 10368 (GOET)	DQ983705	DQ987430	DQ987326
<i>Marchesinia brachiata</i> (Sw.) Schiffn.	Ecuador, Gradstein <i>et al.</i> 10052 (GOET)	AY548069	DQ987390	DQ987278
<i>M. robusta</i> (Mitt.) Schiffn.	Ecuador, Wilson <i>et al.</i> 04-05 (GOET)	DQ983710	DQ987436	DQ987332
<i>Mastigolejeunea auriculata</i> (Wilson & W.J.Hooker) Schiffn.	Bolivia, Churchill 21275 (GOET)	AY548070	DQ987385	DQ987268
<i>M. ligulata</i> (Lehm. & Lindenb.) Schiffn.	Thailand, Schäfer-Verwimp 16246 (GOET)	DQ983711	DQ987465	-
<i>Phaeolejeunea amicornum</i> (Hürlimann) Pócs	Fiji, Pócs 03304L (GOET)	DQ983718	DQ987412	DQ987308
<i>Ptychanthus striatus</i> (Lehm. & Lindenb.) Nees I	Japan, Deguchi 36758 (GOET)	DQ983722	DQ987415	DQ987311
<i>P. striatus</i> II	Indonesia, Gradstein 10215 (GOET)	DQ983721	DQ987406	DQ987300
<i>Schiffneriolejeunea nymannii</i> (Steph.) Gradst. & Terken	Malaysia, Gradstein <i>et al.</i> 10321 (GOET)	DQ983725	DQ987424	DQ987320
<i>S. tumida</i> (Nees & Mont.) Gradst. var. <i>haskarlana</i> (Gottsche) Gradst. & Terken	Indonesia, Schäfer-Verwimp 20952 (GOET)	AY548077	DQ987396	DQ987286
<i>Spruceanthus theobromae</i> (Spruce) Gradst.	Ecuador, Andersson & Kautz AK 10 (GOET)	DQ983727	DQ987407	DQ987301
<i>S. thozetianus</i> (Gottsche & F.v.Müll) B.Thiers	Australia, Pócs 01107/M (GOET)	AM384877	DQ987460	DQ987362
<i>Thysananthus comosus</i> Lindenb. ex Lehm.	Malaysia, Gradstein <i>et al.</i> 10366 (GOET)	DQ983736	DQ987425	DQ987321
<i>T. convolutus</i> Lindenb.	Malaysia, Gradstein <i>et al.</i> 10324 (GOET)	DQ983738	DQ987431	DQ987327
<i>Trocholejeunea crassicaulis</i> (Steph.) Mizut.	Thailand, Schäfer-Verwimp 24029 (GOET)	KP182120*	KP182097*	KP182142*
<i>T. infuscata</i> (Mitt.) Verd. I	China, Yu 20100920-6 (HSNU)	KP182121*	KP182098*	KP182143*
<i>T. infuscata</i> II	China, Yu 20100918-5 (HSNU)	KP182122*	KP182099*	KP182144*
<i>T. sandvicensis</i> (Gottsche) Mizut.	China, Wei 20110214-43 (HSNU)	KP182123*	KP182100*	KP182145*
<i>T. sinensis</i> J.Wang bis <i>et al.</i> I	China, Wang 20121122-28 (HSNU)	KP182124*	KP182101*	KP182146*
<i>T. sinensis</i> II	China, Zhu & Wei 20110420-37A (HSNU)	KP182125*	KP182102*	KP182147*

The youngest parts of the shoots were isolated from fresh specimens or up to seven years old herbarium specimens. Total genomic DNA was extracted using a Qiagen DNeasy Plant® Mini Kit. The *trnL-F*, *rbcL* and nrITS regions were amplified using previously published protocols: *trnL-F* region from Taberlet *et al.* (1991), *rbcL* from Wilson *et al.* (2004) and Gradstein *et al.* (2006), and nrITS region from Hartmann *et al.* (2006). The PCR program was as follows: 3 min initial denaturation at 94°C, followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 50°C, 1 min elongation at 72°C and a final extension step of 10 min at 72°C before being held at 4°C.

Sequence alignment and phylogenetic analysis.

Sequences were manually aligned using PhyDE v.0.997 (Müller *et al.* 2008). Regions of ambiguous alignment were determined and excluded from phylogenetic analysis.

With the command files of the parsimony ratchet (Nixon 1999) generated from the program PRAP2 (Müller 2007), Maximum parsimony (MP) analyses were conducted using PAUP v.4.0b10 (Swofford 2002). The heuristic search mode was run with 10 000 random-addition-sequence replicates and TBR branch swapping. All characters were treated as equally weighted and unordered. Maximum likelihood (ML) analyses were performed using PhyML3.0 (Guindon & Gascuel 2003). jModeltest 2.1.3 (Posada 2008) was used to infer the best-fit model of evolution of the combined dataset based on hierarchical likelihood ratio test (hLRT) and Akaike Information Criterion (AIC). The model of GTR+I+G was chosen. Number of random starting tree is 5 and bootstrap values for ML analyses were obtained via 100 bootstrap replicates in PhyML. Bayesian inference analyses (BI) were performed using MrBayes v.3.2 (Ronquist *et al.* 2012). The optimum model for evolution was based on criteria for the hierarchical likelihood ratio test (hLRT) and Akaike Information Criterion (AIC) using MrModeltest v.2.3 (Nylander 2004). GTR+I+G is best-fit model

for nrITS and *rbcL* and GTR+G for *trnL-F*. Nst = 6, rates = invgamma were employed for nrITS and *rbcL* and rates = gamma for *trnL-F*. Four simultaneous Markov chain Monte Carlo (MCMC) were run for two million generations and sampled every 1000th generation. Tracer v1.5 (Rambaut & Drummond 2009) was used to determine the burn-in point and examine the log likelihoods for each run. Bayesian posterior probability (PP) were generated from the remaining trees after discarding the first 1000 trees of each run.

All three markers were first explored separately to check for possible incongruence among them. The incongruence length difference (ILD) test (Farris *et al.* 1995) was used to detect potential conflicts between different DNA regions, using the partition homogeneity test implemented in PAUP 4.0b10 (Swofford 2002), with 1000 replicates and the heuristic search option, with 100 random taxon addition and TBR branch swapping. As the ILD test does not actually reveal the amount of incongruence and can be insensitive to small but significant topological differences generated by different datasets (Siddall 1997), the topology and support values of each dataset were compared visually to evaluate the likelihood of incongruence. The strict consensus trees from three separate analyses were compared to identify conflicting nodes supported by at least 70% (Mason-Gamer & Kellogg 1996). As there was no significant incongruence among topologies, the three datasets were combined into a single matrix as partitions. The phylogenetic analyses were reconstructed based on combined dataset. The topology and support values from different analyses were compiled in Treegraph2 (Stöver & Müller 2010).

Results

The alignment of the combined dataset included 1086 *rbcL* sites (913 constant, 77 variable, 96 informative), 344 *trnL-F* sites (237 constant, 36 variable, 71 informative) and 962 nrITS sites (305 constant, 91 variable, 566 informative). Of a total of 2392 investigated sequence characters, 1455 were constant, 733 were parsimony informative and 204 were autapomorphic. MP analysis resulted in four maximally parsimonious trees (MPTs) with a length of 3893 steps, and with a consistency index (CI) of 0.413 and a retention index (RI) of 0.640. All ML searches yielded consistent topologies. Since the ML ($\ln = -21618.5831$), MP and Bayes analyses reveal similar topologies except for a few nodes with low bootstrap support, only the majority rule consensus tree derived from BI is shown (Fig. 1). Subfamily Ptychanthoideae, the core group of the subfamily (= "clade A4" of Wilson *et al.* 2007) and *Acrolejeunea* formed well-supported clades (MLBS = 100, PP = 1; MPBS = 100 for Ptychanthoideae and *Acrolejeunea*, MPBS = 99 for the core Ptychanthoideae). The four species of *Trocholejeunea* were nested in *Acrolejeunea* and formed a strongly supported monophyletic group (MLBS = 100, PP = 1, MPBS = 100) sister to *A. aulacophora* and *A. securifolia* (Fig. 1). *Frullanoides* was resolved at the base of Ptychanthoideae, far removed from *Acrolejeunea* and *Trocholejeunea*, in an unsupported clade together with *Marchesinia*.

Six well-supported lineages were detected in *Acrolejeunea*, labeled A, B, C, D, E and F (Fig. 1). All six lineages received maximum support except for lineage A (MLBS = 97, PP = 1, MPBS = 82). Lineage A consisted of three species of sect. *Acrolejeunea* including the type of this group (*A. emergens*, *A. heterophylla*, *A. torulosa*). A further member of this section, *A. recurvata*, was recovered as a separate lineage (E). Two other members of sect. *Regulares* (*A. aulacophora* [type], *A. securifolia*) and one species of sect. *Minores* (*A. mollis*) formed lineage B and the four species analysed of *Trocholejeunea* constituted lineage C. Lineage D contained the type of sect. *Pusillae*, *A. pusilla*, as well as *A. sikkimensis* of sect. *Regulares*. Lineage F, finally, contained one species of sect. *Regulares* (*A. fertilis*) and four of sect. *Minores* including the type of this section (*A. arcuata*, *A. parvula*, *A. pycnoclada* [type], *A. tjibodensis*).

Discussion

The molecular phylogenetic reconstructions obtained in this study (Fig. 1) corresponds well with those of Wilson *et al.* (2007), which were based on the same molecular markers and similar sets of genera but with a much more limited sampling of *Acrolejeunea* and without *Trocholejeunea*. Our results confirm that *Acrolejeunea* is monophyletic and indicate that *Trocholejeunea* is nested in it. They also show that *Frullanoides* is quite unrelated to *Acrolejeunea* and *Trocholejeunea*. As opposed to morphological treatments identifying *Trocholejeunea* as a separate genus (Mizutani 1989; Zhu & So, 2001; Singh & Nath, 2008; Wang *et al.*, 2014), our results indicate that *Trocholejeunea* needs to be synonymized with *Acrolejeunea*, adding a further example to the growing number of Lejeuneaceae genera being sunk into synonymy based on molecular data (these results are summarized in Gradstein 2013). The results are consistent

with the overall morphological similarity of the two genera, both possessing stems with a hyalodermis, entire-margined leaves, underleaves, bracts and bracteoles, leaf cells with cordate trigones and homogeneous oil bodies, androecia with epistatic bracts containing one antheridium, and pluriplicate perianths. The main features separating *Trocholejeunea* from *Acrolejeunea* are the presence of gynoecial innovations of the *Frullania*-type and a more massive seta built of 4–16 inner and 16–32 outer rows of cells (Verdoorn 1934; Mizutani 1961; Wang *et al.* 2014). In addition, vegetative branches of the *Frullania*-type are more common in *Trocholejeunea* than *Lejeunea*-type branches (Mizutani 1989). In *Acrolejeunea*, gynoecial innovations are lacking, the seta is built of only 4 rows of inner cells, and *Lejeunea*-type vegetative branches are more common than *Frullania*-type ones (Gradstein 1975). However, the differences between the two genera do not seem to be entire stable. For example, Mizutani (1989) observed the occasional absence of innovations in *T. sandvicensis* and Thiers (1985) found only *Lejeunea*-type branches in a Hawaiian population of this species, no *Frullania*-type ones. Furthermore, a seta built of 4–8 rows of inner cells occurs in *A. pusilla* (Mizutani 1961). This overlap in the differential features of the two taxa lends further support to the synonymy of *Trocholejeunea* under *Acrolejeunea*.

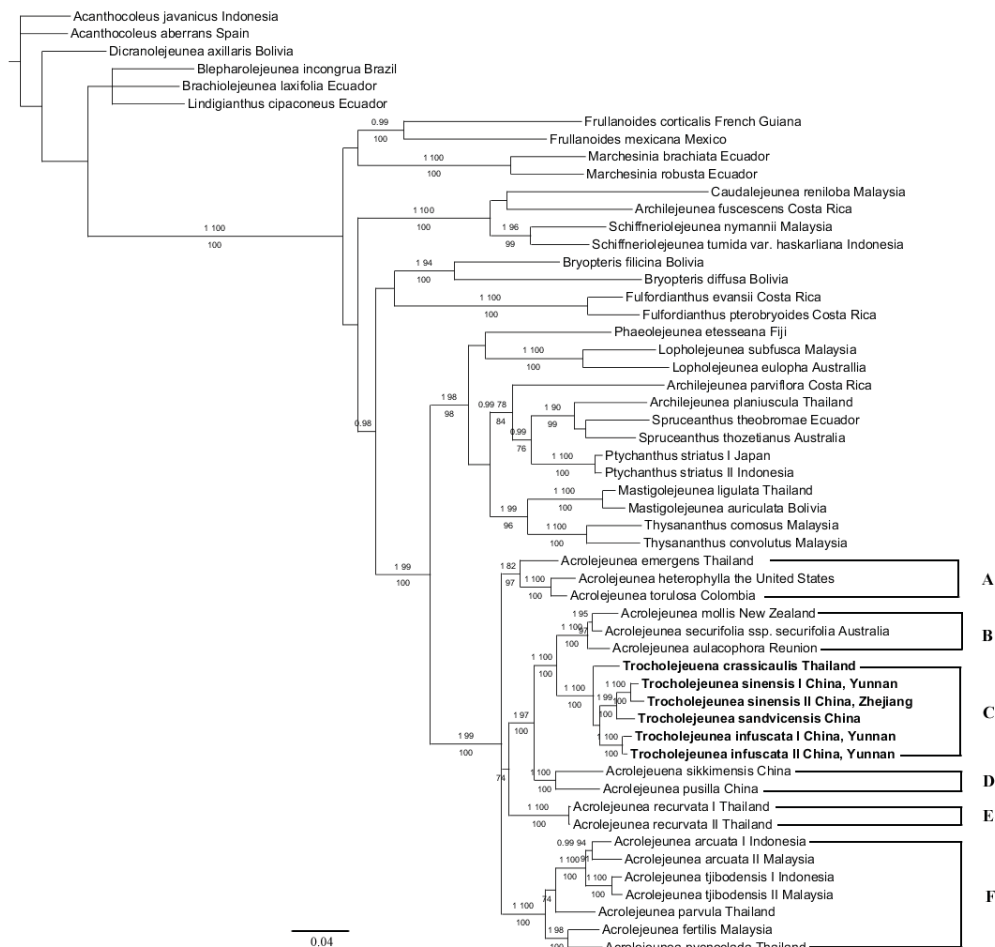


FIGURE 1. Majority consensus trees from the Bayesian analysis based on the combined dataset of *rbcL*, *trnL-trnF*, and *nrITS*. Bootstrap support > 70% and Bayesian PP > 0.95 are indicated at branches. Bootstrap values above the branches indicate Bayesian posterior probability (left) and MP bootstrap support (right), those below the branches ML bootstrap support.

The species of *Acrolejeunea* represented by multiple accessions in the molecular analysis proved to be monophyletic. A classification of the species into two subgenera, subg. *Acrolejeunea* and subg. *Isolejeunea* (Gradstein 1975), was not suggested by our study. The four sections of the genus proposed by Gradstein were partially supported. Deviating positions were detected for *A. fertilis*, which was resolved in sect. *Minores* instead of sect. *Regulares*, *A. mollis* (sect. *Regulares* instead of sect. *Minores*), *A. sikkimensis* (sect. *Pusillae* instead of sect. *Regulares*) and *A. recurvata*, which constituted a separate lineage (instead of being a member of sect. *Acrolejeunea*). Disagreement of molecular phylogenies with intrageneric classifications based on morphology is not uncommon in liverworts and has

recently been demonstrated in, e.g., *Porella* (Hentschel *et al.* 2007), *Radula* (Devos *et al.* 2010), *Diplasiolejeunea* (Dong *et al.* 2012), *Lejeunea* (Heinrichs *et al.* 2013) and *Odontoschisma* (Aranda *et al.* 2014).

The separate position of *Acrolejeunea recurvata*, outside sect. *Acrolejeunea*, is supported by unusually broad ventral merophytes, to 10 cells wide, and flexuose perianth keels. *Acrolejeunea sikkimensis* was only tentatively placed in sect. *Regulares* by Gradstein (1975) because the available material (type only) was poor. Several collections of this species have recently been made in Anhui, Yunnan and Zhejiang, China, and the morphology of the species has become better known (Fig. 2). It appears that the close molecular relationship of this species to *A. pusilla* (sect. *Pusillae*) correlates well with the similar gynoeceum in these two species, which stands out in the short lobule of the inner female bract, which is hardly extended beyond the keel, the flat, ligulate inner female bracteole, and the perianth with only 4–5 keels. Other species of *Acrolejeunea* have usually more than five perianth keels. Specimens with a 5-keeled perianth and short female bract lobule identified by Gradstein (1975) as *A. fertilis* (e.g. Vietnam, leg. Eberhardt, Gradstein 1975: Plate XIII-3) need to be checked; may be they belong to *A. sikkimensis*.

The sections *Minores* and *Regulares* of *Acrolejeunea* are morphologically very similar and can hardly be separated (see below). The positions of *A. fertilis* in sect. *Minores* instead of sect. *Regulares*, and of *A. mollis* in sect. *Minores* instead of sect. *Regulares*, clearly support this. The four species of *Trocholejeunea* analysed in this study, finally, constitute a well-defined monophyletic group in *Acrolejeunea* and may be treated as a further section of this genus.

Taxonomic conclusions: infrageneric classification of *Acrolejeunea*

Based on the molecular results *Acrolejeunea* is subdivided into six sections, corresponding to lineages A–F (Fig. 1). By describing the lineages uniformly as sections, only few taxonomic changes are necessary. For each section a brief morphological description, type, distribution and included species are given. Lineage names are cited following Devos *et al.* (2011). Two rare species, *A. allisonii* from New Zealand and *A. meghalayensis* from India (each marked by an asterisk), could not be sequenced and are tentatively assigned to a section based on morphology.

Acrolejeunea (Spruce) Schiffn., in Engler & Prantl, Nat. Pflanzenfam. 1, 3: 128. 1893. ≡ *Lejeunea* subg. *Acrolejeunea* Spruce, Trans. & Proc. Bot. Soc. Edinburgh 15: 115. 1884. Lectotype (designated by Gradstein 1974): *Acrolejeunea torulosa* (Lehm. & Lindenb.) Schiffn. (≡ *Jungermannia torulosa* Lehm. & Lindenb.).

Plants glossy green when alive. Branching *Lejeunea*-type or *Frullania*-type. Stems rather fragile, with hyalodermis; 20–50 medullary cells; dorsal epidermis cells usually larger than ventral epidermis cells; ventral merophyte (2–)4(–10) epidermis cells wide. Leaves imbricate (often strongly so), when dry convolute-suberect, when moist widely spreading and convex to squarrose; leaf apex rounded; margins of leaves, underleaves, bracts and bracteoles entire. Lobules with 1–9 teeth. Leaf cells with cordate trigones. Oil bodies homogeneous. Male bracts epistatic, with one antheridium. Innovations absent or *Frullania*-type (no *Radula*-type innovations). Perianth with (4–)5–10 smooth keels. Elongated seta non-articulate, with (14–)16(–17)–32 outer cell rows and 4–16 inner cell rows. Elaters ca. 36 per capsule (more in sect. *Trocholejeunea*?). Vegetative reproduction in some species by small, caducous leaves produced on upright flagelliform shoots.

Pantropical; contains 21 species (including one fossil).

Key to the sections of *Acrolejeunea*

1. Branching predominantly *Frullania*-type. Innovations (*Frullania*-type) present. Perianth keels flexuose. (SE Asia, Hawaii).....sect. *Trocholejeunea*
1. Branching predominantly *Lejeunea*-type. Innovations absent. Perianth keels straight (flexuose only in *A. recurvata*)2
2. Perianth 4–5-keeled, anisoplicate. Female bract lobule much shorter than lobe, scarcely elongated beyond the keel. (Japan, China, Sikkim) sect. *Pusillae*
2. Perianth (5–)6–10-keeled, isoplicate or anisoplicate. Female bract lobule shorter than to almost as long as lobe, mostly elongated beyond the keel.....3
3. Vegetative reproduction by small, caducous leaves produced on upright flagelliform shoots. Perianth anisoplicate. Dorsal epidermis cells in zig-zag longitudinal rows4
3. Vegetative reproduction absent. Perianth isoplicate. Dorsal epidermis cells in straight longitudinal rows5
4. Ventral merophyte 4–10 cells wide. Perianth keels flexuose. (India, Thailand, Laos) sect. *Recurvatae*
4. Ventral merophyte 4 cells wide. Perianth keels straight. (Tropical America, Africa, Sri Lanka, India, Thailand) sect. *Acrolejeunea*

5. Leaf lobules $1/2$ – $2/3$ × lobe length. Stem with 10–20(–25) medullary cells. (Africa, tropical Asia, Australasia, Pacific region).....
 sect. *Minores*
5. Leaf lobules $2/5$ – $1/2$ × lobe length. Stem with 20–30 medullary cells. (E Africa, E Malesia, Australasia, Pacific region).....
 sect. *Regulares*

Lin. A: *Acrolejeunea* sect. *Acrolejeunea*.

Branching predominantly *Lejeunea*-type. Stem with 20–40 medullary cells, dorsal epidermis cells in zig-zag longitudinal rows, ventral merophyte 4 epidermis cells wide. Leaf lobules with 2–9 teeth. Underleaf apex plane. Autoicous or dioicous. Lobule of female bract slightly shorter than lobe. Perianth anisplicate, with 5–10 rounded or sharp, straight keels. Vegetative reproduction by small, caducous leaves produced on upright flagelliform shoots.

TYPE: *A. torulosa* (Lehm. & Lindenb.) Schiffn.

DISTRIBUTION: Tropical America, Africa, Sri Lanka, S India, Thailand; on bark in rather dry lowland and lower montane forests, scrub, isolated trees, gardens and plantations, usually in rather open locations; from sea level to 1500 m.

INCLUDED SPECIES: *A. emergens*, *A. heterophylla*, *A. torulosa*.

Lin. B: *Acrolejeunea* sect. *Regulares* (Verd.) Gradst., Bryophyt. Biblioth. 4: 83. 1975 ≡ *Ptychocoleus* Trevis. sect. *Regulares* Verd., Ann. Bryol. suppl. 4: 143. 1934.

Branching predominantly *Lejeunea*-type. Stem with 20–30 medullary cells, dorsal epidermis cells in straight longitudinal rows, ventral merophyte 4 epidermis cells wide. Leaf lobule $2/5$ – $1/2$ × lobe length, with 1–5 teeth. Underleaf apex plane or recurved. Paroicous, autoicous or dioicous. Lobule of female bract shorter than to almost as long as lobe. Perianth isoplicate, with 7–10 rounded, straight keels. Vegetative reproduction lacking.

TYPE: *A. aulacophora* (Mont.) Steph.

DISTRIBUTION: E Africa, E Malesia, Australasia, Pacific region; in lowland and lower montane rainforests, semi-deciduous forests, scrub, isolated trees, gardens and plantations, usually in rather open locations; from sea level to ca. 1500 m.

INCLUDED SPECIES: **A. allisonii*, *A. aulacophora*, *A. mollis*, *A. securifolia*.

Lin. C: *Acrolejeunea* sect. *Trocholejeunea* (Schiffn.) J.Wang bis & Gradst., *stat. nov.* ≡ *Trocholejeunea* Schiffn., Ann. Bryol. 5: 160. 1932.

Branching predominantly *Frullania*-type. Stem with 20–55 medullary cells, dorsal epidermis cells in straight longitudinal rows, ventral merophyte 4–7 epidermis cells wide. Leaf lobules with 1–6 teeth. Underleaf apex plane or recurved. Autoicous or dioicous. Lobule of female bract absent or present, when present shorter than lobe. Perianth with (6–)10 rounded or sharp, flexuose keels. Vegetative reproduction lacking.

TYPE: *Trocholejeunea levieri* Steph. ex Schiffn. [= *Trocholejeunea infuscata* (Mitt.) Verd.].

DISTRIBUTION: Tropical and subtropical SE Asia, Hawaii; on bark in lowland and lower montane forests, scrub, isolated trees, gardens and plantations; also found on rock; from sea level to 3000 m.

INCLUDED SPECIES: *A. crassicaulis*, *A. infuscata*, **A. meghalayensis*, *A. sandvicensis*, *A. sinensis*.

NEW COMBINATIONS:

Acrolejeunea crassicaulis* (Steph.) J.Wang bis & Gradst., *comb. nov.

≡ *Hygrolejeunea crassicaulis* Steph., Sp. Hepat. 5: 550. 1914 ≡ *Trocholejeunea crassicaulis* (Steph.) Mizut., J. Hattori Bot. Lab. 66: 280. 1989. Type: Malaysia. Sabah: Labuk River, unknown collector, *hb. Levier* 4655 (holotype, G-14058, n.v.).

Acrolejeunea infuscata* (Mitt.) J.Wang bis & Gradst., *comb. nov.

≡ *Lejeunea infuscata* Mitt., J. Proc. Linn. Soc. London 5: 111. 1861 ≡ *Trocholejeunea infuscata* (Mitt.) Verd., Ann. Bryol., Suppl. 4: 190. 1934. Type: India. Assam: Mt. Khasia, 4000–6000 ft, *J.D.Hooker*. & *Thomson* 1515 (lectotype, NY-1049438!, here designated).

Acrolejeunea meghalayensis (Singh & Nath) J.Wang bis & Gradst., comb. nov. \equiv *Trocholejeunea meghalayensis* Singh & Nath, J. Jap. Bot. 83: 2. 2008. Type: India. Meghalaya: West Khasi Hills, Nongstoin, Mawkadiang, 17 July 2000, A. P. Singh 208658-A (LWG, n.v.).

Acrolejeunea sandvicensis (Gottsche) J.Wang bis & Gradst., **comb. nov.**

\equiv *Phragmicoma sandvicensis* Gottsche, Ann. Sci. Nat., sér. 4 (Bot.), 8: 344. 1857 \equiv *Lejeunea sandvicensis* (Gottsche) A.Evans, Trans. Connecticut Acad. Arts 8: 253. 1892, *nom. illeg.* (non Steph. 1890) \equiv *Mastigolejeunea sandvicensis* (Gottsche) Steph., Bull. Herb. Boissier 5: 842. 1897 \equiv *Brachiolejeunea sandvicensis* (Gottsche) A.Evans, Trans. Connecticut Acad. Arts 10: 419. 1900 \equiv *Trocholejeunea sandvicensis* (Gottsche) Mizut., Misc. Bryol. Lichenol. 2: 169. 1962 “1961”. Type: Hawaii, “Insul. Sandwich, *Phragmicoma sandvicensis* G. (Gottsche scripsit), Herb. Mus. Paris” (lectotype, PC-703330, here designated; isolectotypes (?), PC-Montagne nrs. MC2935 [PC-703331] & MB2837 [PC-703332]).

The type specimen of *Phragmicoma sandvicensis* Gottsche was originally cited as follows: “Habitat in insulis Sanvicensibus, ubi planta fructifera a cel. Gaudichaud mensibus septembre et octobre anni 1836, in itinere navis la Bonite circa terram collecta est. (Herb. Mus. Paris., fol. no 120 et fol. 180.)” (Ann. Sci. Nat., sér. 4 (Bot.), 8: 344. 1857). Although material with the full original label information lacks in PC, we have chosen the specimen PC-703330 from Hawaii as the lectotype because it contains the names of the basionym and the locality in Gottsche’s handwriting, and the sheet on which the specimen is attached is labelled «Herb. Mus. Paris». Two probable duplicates labelled «Ins. Sandwich, Bonite, *Phragmicoma bicolor* Nees” are kept in the Montagne herbarium. Since the Montagne herbarium was in private hands and not in PC at the time of the publication of the basionym, these specimen may be considered isolectotypes (B. Denetiere, pers. com.).

Acrolejeunea sinensis (J.Wang bis *et al.*) J.Wang bis & Gradst., **comb. nov.** \equiv *Trocholejeunea sinensis* J.Wang, R.L.Zhu & Gradst., Phytotaxa, 174: 296. 2014.

Type: China. Guangxi: Jinxiu Co., Dayaoshan National Nature Reserve, *Cathaya argyrophylla* Protection Station, on rotten logs, 900–1000 m, 19 July 2009, Wen Ye & Yu-Mei Wei 20090719-47A (holotype, HSNU!).

Lin. D: *Acrolejeunea* sect. *Pusillae* Gradst., Bryophyt. Biblioth. 4: 59. 1975.

Branching predominantly *Lejeunea*-type. Stem with 7–25 medullary cells, dorsal epidermis cells in straight longitudinal rows, ventral merophyte 4 epidermis cells wide. Leaf lobule with 1–5 teeth. Underleaf apex plane. Autoicous or dioicous. Lobule of female bract much shorter than lobe. Perianth anisoplicate, with 4–5 sharp, straight keels. Vegetative reproduction present or lacking, when present by small, caducous leaves produced on upright flagelliform shoots.

TYPE: *A. pusilla* (S.Hatt.) Grolle & Gradst.

DISTRIBUTION: Japan, China, Sikkim; on bark in broad-leaved evergreen forests from sea level to 150(–900?) m.

INCLUDED SPECIES: *A. pusilla*, *A. sikkimensis* (Fig. 2).

Lin. E: *Acrolejeunea* sect. *Recurvatae* J.Wang bis & Gradst., **sect. nov.**

Branching predominantly *Lejeunea*-type. Stem with 25–35 medullary cells, dorsal epidermis cells in zig-zag longitudinal rows, ventral merophyte 4–10 epidermis cells wide. Leaf lobule with 3–6 teeth. Underleaf apex recurved. Dioicous. Lobule of female bract shorter than lobe. Perianth isoplicate, with 5–8 sharp, flexuose keels. Vegetative reproduction by small, caducous leaves produced on upright flagelliform shoots.

TYPE: *A. recurvata* Gradst.

DISTRIBUTION: N India, N Thailand, Laos; on bark in rather dry lower montane forests and on roadside trees; also found on rock; 350–1500 m.

INCLUDED SPECIES: *A. recurvata*.

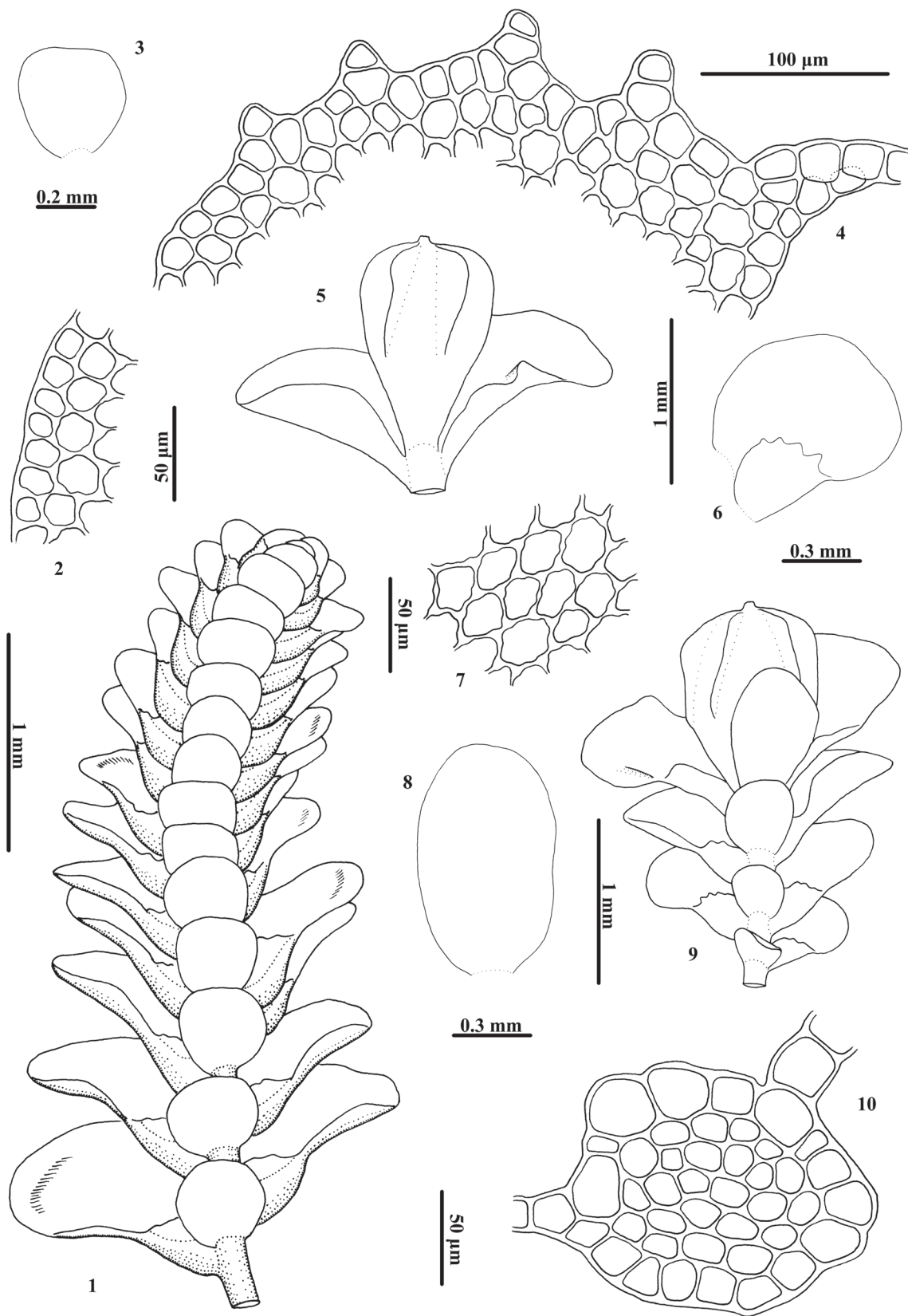


FIGURE 2. *Acrolejeunea sikkimensis*. 1. Portion of male shoot, ventral view. 2. Cells of lobe margin. 3. Underleaf. 4. Part of leaf lobule, showing free margin with four teeth. 5. Female bracts and perianth. 6. Leaf. 7. Median lobe cells. 8. Female bracteole. 9. Portion of female shoot. 10. Stem section. All from *Deng-Ke Li 00815*.

Lin. F: *Acrolejeunea* sect. *Minores* (Verd.) L.Söderstr. & A.Hagborg, **comb. nov.** \equiv *Ptychocoleus* Trevis. sect. *Minores* Verd., Ann. Bryol. suppl. 4: 132. 1934 \equiv *Acrolejeunea* sect. *Isolejeunea* Gradst., Bryophyt. Biblioth. 4: 103. 1975, *nom. illeg.*

Branching predominantly *Lejeunea*-type. Stem with 10–20(–25) medullary cells, dorsal epidermis cells in straight longitudinal rows, ventral merophyte (2–)4 epidermis cells wide. Leaf lobule 1/2–2/3 \times lobe length, with 1–9 teeth. Underleaf apex plane or recurved. Autoicous or dioicous. Lobule of female bract slightly shorter than lobe. Perianth isoplicate, with 5–10 rounded, straight keels. Vegetative reproduction lacking.

TYPE: *A. pycnoclada* (Taylor) Schiffn.

DISTRIBUTION: Tropical Africa, tropical Asia, Australasia, Pacific region; on bark in lowland and lower montane forests, scrub, isolated trees, gardens and plantations, usually in rather open locations; from sea level to 3500 m.

INCLUDED SPECIES: *A. arcuata*, *A. fertilis*, *A. parvula*, *A. pycnoclada*, *A. tjibodensis*.

NOTE: This section is morphologically very similar to the previous one. Further study focusing on hitherto neglected features (e.g., sporophytes, secondary metabolites), might reveal better characters separating the two groups.

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