

NEW AND NEGLECTED MORPHOLOGICAL FEATURES IN THE TAXONOMY OF ASIAN *LEJEUNEA* (MARCHANTIOPHYTA)

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Abstract. The infrageneric classification of the large genus *Lejeunea* Lib. is poorly understood due to the lack of stable morphological characters characterizing supraspecific groups. Phenetic analysis of 26 morphological features of 31 Asian *Lejeunea* species separated two main species clusters based on the number of superior central cells at underleaf bases. The number of superior central cells had not previously been utilized in the classification of *Lejeunea* and appears to be new and stable morphological feature within this genus. The presence of surface wax was confirmed in *L. flava* (Sw.) Nees and was newly recorded in *L. mimula* Hürtl. and *L. tuberculosa* Steph. We suggest that wax ornamentation may be a useful taxonomic feature at species level in *Lejeunea*.

Key words: *Lejeunea*, Malaysia, phenetic analysis, superior central cells, taxonomy, wax ornamentation

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INTRODUCTION

Lejeunea Lib. is the second-largest genus of Lejeuneaceae (Marchantiophyta), after *Cololejeunea* (Spruce) Schiffn., with ca 300 currently accepted species. Based on its current circumscription the genus is characterized by vegetative branches of *Lejeunea*-type; epidermal cells thin-walled and larger than the medullary cells; leaf lobules occasionally reduced and with one tooth (very rarely two); hyaline papilla positioned at the proximal side of the apex of the lobule (very rarely distal); leaf cells thin-walled or with rather small trigones; oil bodies granular or homogeneous, smooth to finely segmented; ocelli absent; underleaves usually bifid (rarely entire) and with upright lobes; gynoecea innovations with lejeuneoid leaf sequence (Lee 2013; Czumay *et al.* 2013).

A comprehensive, modern infrageneric classification of *Lejeunea* does not exist. Attempts to classify the species of the genus into subgenera or sections have been based mainly on morphology and on species of the New World (Schuster

1980; Reiner-Drehwald 1999; Gradstein *et al.* 2001). The first global phylogeny of *Lejeunea* based on molecular analysis of 91 species separated two main subgenera, subg. *Lejeunea* and subg. *Crossotolejeunea* (Heinrichs *et al.* 2013). Morphological analysis and hierarchical classification of the species into subgenera and sections were not attempted, however, and the infrageneric classification of *Lejeunea* remains poorly understood due to the lack of stable morphological characters characterizing the infrageneric groups.

The purpose of the present work was to assess the taxonomic relevance of selected morphological features within the genus *Lejeunea*, in particular among the Asian species. The study is based on the results of a taxonomic revision of the genus *Lejeunea* in Malaysia (Lee 2013). The results may contribute to the infrageneric classification of *Lejeunea* and should be tested on a worldwide scale using morphological and molecular evidence.

MATERIALS AND METHODS

A total of 26 morphological characters in 31 species of *Lejeunea* were scored for phenetic analysis (Tables 1 & 2). Characters and character states were derived from the results of a comprehensive morphological-anatomical study of the Malaysian species of *Lejeunea* (Lee 2013). The presence of wax on leaf surfaces was examined by scanning electron microscopy, following washing of leafy shoots with chloroform (1–3 min) prior to coating (Heinrichs & Reiner-Drehwald 2012). Phenetic analysis of the data matrix was carried out using five different methods: unweighted pair group

method with arithmetic mean (UPGMA; e.g., Zander 2006), weighted pair group method with arithmetic mean (WPGMA), farthest neighbor, median and centroid.

RESULTS AND DISCUSSION

All four analyses yielded two main species clusters; moreover, the species composition of the two clusters was the same in all four analyses. Therefore only one dendrogram (resulting from UPGMA) is shown here (Fig. 1). Visual inspection of the database showed that the two main clusters are

Table 1. Morphological characters and character states in Asian *Lejeunea* species.

1. Plant width	0 = <1.0 mm, 1 = >1.0 mm
2. Sexuality	0 = autoicous, 1 = dioicous
3. Superior central cells	0 = two, 1 = four
4. Number of medullary cells across stem cross section	0 = >10, 1 = <10
5. Leaf apex when dry	0 = plane to slightly recurved, 1 = strongly recurved
6. Shape of leaf apex	0 = rounded to obtuse, 1 = acute, 2 = apiculate
7. Rhizoids on margin of leaf lobe	0 = lacking, 1 = present
8. Angle between ventral leaf margin and keel (when leaf lobe flattened)	0 = narrow angle, 70°–100°; 1 = wide angle, 110°–180°
9. Margin of leaf lobe	0 = entire, 1 = weakly crenulate, 2 = strongly crenulate with projecting cells
10. Trigones of leaf cells	0 = absent or very small, 1 = well-developed
11. Intermediate thickenings of leaf cells	0 = absent, 1 = present
12. Two intermediate thickenings per adjacent trigones	0 = absent, 1 = present
13. Cuticle	0 = smooth, 1 = rough by minute papillae, 2 = rough by small warts coarser than papillae (wax)
14. Leaf lobule shape	0 = ovate-triangular, 1 = ovate-oblong to rectangular
15. Free margin of leaf lobule	0 = partially recurved, to 1/2–3/4 its length, 1 = fully recurved, 2 = flat
16. Rectangular disc cell of leaf lobule	0 = large and clearly distinguishable from adjacent cells, 1 = small and indistinguishable from adjacent cells
17. Underleaf apex	0 = emarginate to undivided, 1 = bifid
18. Underleaf base	0 = straight to shallowly curved, 1 = cordate
19. Underleaf shape	0 = longer than wide, 1 = wider than long, 2 = as long as wide
20. Width of base of underleaf lobe	0 = 2–9 cells, 1 = 10–20 cells
21. Underleaf arrangement	0 = distant, 1 = contiguous, 2 = imbricate
22. Secondary rhizoid disc	0 = absent, 1 = present
23. Male bracts	0 = smooth, 1 = crenulate, not winged, 2 = crenulate, usually winged
24. Perianth beak	0 = 2–4 cells long, 1 = 5–12 cells long, 2 = beak lacking
25. Perianth ornamentation	0 = smooth, 1 = mammillose (on keels), 2 = winged or ciliate
26. Perianth keels	0 = 5 keels, 1 = 2–4 keels, 2 = no keels

Table 2. Data matrix of morphological character states in Asian *Lejeunea* species.

Taxon	Character and character state																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26		
<i>L. alata</i> Gottsche	1	0	1	0	0	0	0	1	0	1	0	0	0	0	2	1	1	0	1	1	0	0	1	0	1	0	1	0
<i>L. albescens</i> (Steph.) Mizut.	1	1	1	0	0	0	0	0	2	1	1	0	1	0	0	1	1	0	1	1	1	1	0	0	2	0	3	0
<i>L. anisophylla</i> Mont.	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	0	1	0	1	0
<i>L. apiculata</i> Sande Lac.	0	0	0	1	0	2	0	1	0	1	1	0	1	0	2	1	1	0	0	0	0	0	0	1	0	2	0	0
<i>L. cocoes</i> Mitt.	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	1	0
<i>L. compacta</i> (Steph.) Steph.	1	1	1	0	1	0	0	0	1	1	0	1	1	0	1	1	2	1	1	2	1	1	0	?	0	0	0	0
<i>L. contracta</i> Mizut.	1	1	1	0	1	0	0	1	0	0	0	1	0	0	1	1	2	1	1	2	1	1	0	0	0	0	0	0
<i>L. dimorpha</i> Kodama	1	0	1	0	0	0	0	1	0	1	1	1	0	0	0	1	1	0	2	0	0	1	1	0	0	0	0	0
<i>L. dipterota</i> (Eifrig) G. E. Lee	1	1	1	0	0	1	0	0	1	1	0	1	0	1	0	1	0	1	1	1	1	0	1	0	1	0	2	0
<i>L. discreta</i> Lindenb.	1	1	1	1	0	1	0	1	0	1	1	0	1	1	1	0	1	0	2	0	1	1	0	0	0	0	0	0
<i>L. eifrigii</i> Mizut.	1	0	1	0	0	2	0	1	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	1	0	1
<i>L. exilis</i> var. <i>exilis</i> (Reinw. et al.) Grolle	0	1	0	1	0	1	1	1	1	0	0	0	0	0	0	1	1	0	2	0	0	0	2	0	0	3	0	3
<i>L. exilis</i> var. <i>abnormis</i> (Herzog) G. E. Lee	0	1	0	1	0	1	1	1	1	0	0	0	0	0	0	1	1	0	2	0	0	0	2	0	0	0	0	0
<i>L. flava</i> (Sw.) Nees	1	0	1	0	0	1	0	0	0	1	1	0	1	0	0	1	1	1	2	1	1	0	0	0	1	0	1	0
<i>L. fleischeri</i> (Steph.) Mizut.	1	1	1	0	1	0	0	1	1	1	1	0	1	1	1	1	0	1	0	1	1	0	0	1	0	1	0	1
<i>L. gradsteinii</i> G. E. Lee et al.	1	1	1	0	0	0	0	1	1	1	1	0	1	0	2	1	1	0	2	0	0	0	0	0	0	1	0	0
<i>L. kinabalensis</i> Mizut.	1	1	1	0	0	0	0	2	1	1	0	1	0	1	0	2	1	1	2	1	1	0	0	0	0	1	0	0
<i>L. lumbricoides</i> (Nees) Nees	1	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	2	1	0
<i>L. nicholitzii</i> Mizut.	1	1	1	0	0	0	0	1	0	1	1	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0
<i>L. microloba</i> Taylor	1	1	1	0	0	2	0	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	2	0	3	0
<i>L. mimula</i> Hürl.	1	1	1	0	0	0	0	1	0	1	1	0	2	0	1	1	0	0	1	0	1	0	0	1	1	0	1	0
<i>L. mizutanii</i> Grolle	1	1	1	0	1	0	0	0	1	1	1	0	0	1	2	0	1	1	0	1	1	0	?	1	0	0	0	0
<i>L. papilionacea</i> Steph.	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0	1	1	0	2	1	0	1	2	0	2	1	0	0
<i>L. patersonii</i> (Steph.) Steph.	0	1	1	0	0	0	0	2	1	0	0	0	0	0	2	1	1	0	1	0	0	0	1	0	2	0	0	0
<i>L. patriciae</i> Schäf.-Verw.	0	1	0	1	0	1	1	1	1	1	1	1	0	0	2	1	1	0	2	0	0	1	2	0	1	0	1	0
<i>L. pectinella</i> Mizut.	1	1	1	0	1	0	0	0	1	1	0	1	0	1	1	1	0	1	0	1	1	0	0	1	0	1	0	1
<i>L. sordida</i> (Nees) Nees	1	1	1	0	0	0	0	0	0	1	1	0	1	0	0	1	1	0	1	1	0	1	0	0	0	1	0	1
<i>L. stephaniana</i> Mizut.	1	1	1	0	1	0	0	0	0	1	0	0	0	1	0	1	1	0	2	1	0	0	?	0	0	0	0	0
<i>L. tamasposcii</i> G. E. Lee	0	0	0	1	0	1	0	0	1	1	1	1	1	0	2	1	1	0	1	0	1	0	0	1	0	2	0	0
<i>L. tuberculosa</i> Steph.	0	1	1	0	0	0	0	0	0	1	0	0	2	0	0	1	1	0	2	0	0	1	0	0	1	0	1	0
<i>L. umbilicata</i> (Nees) Nees	1	1	1	0	0	0	0	1	1	1	1	0	0	0	1	1	1	0	1	1	0	1	1	0	1	2	0	3
<i>L. utriculata</i> (Steph.) Mizut.	1	1	1	0	0	0	0	1	0	1	1	0	1	1	1	1	0	1	0	1	1	0	?	1	0	1	0	1

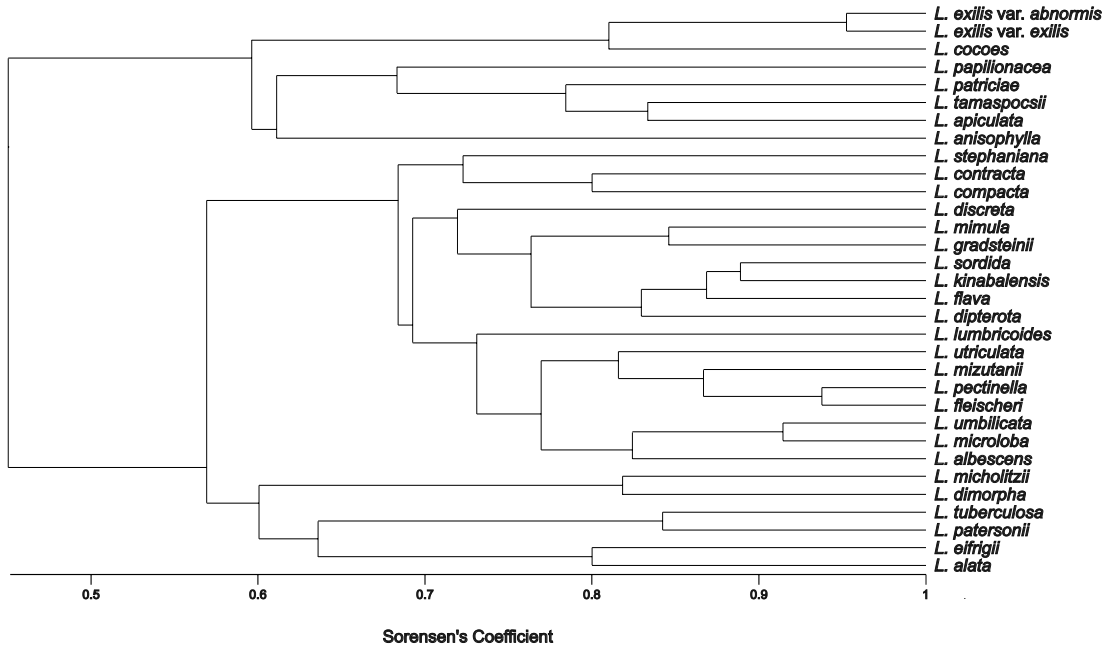


Fig. 1. Dendrogram based on UPGMA analysis of the morphological data of Asian *Lejeunea* species.

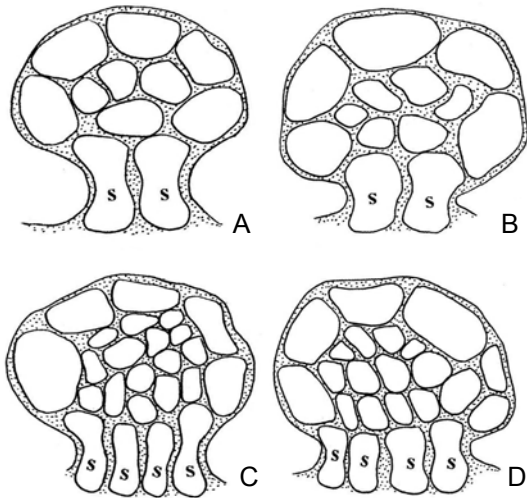


Fig. 2. Transverse sections of underleaf attachment to stem in Malaysian *Lejeunea*. A – *Lejeunea patriciae* Schäf.-Verw., B – *L. anisophylla* Mont., showing 2 superior central cells (s). C – *L. dimorpha* Kodama, D – *L. micholitzii* Mizut., showing 4 superior central cells (s). A from Fraser's Hill, Pahang, *G.E. Lee* 1099 (UKMB). B from Perlis State Park, Perlis, *G.E. Lee* 2240 (UKMB). C from Gunung Chamah, Dakota trail, Kelantan, *A. Damanhuri* 11-370 (UKMB). D from Gunung Brinchang, Pahang, *G.E. Lee* 2299 (UKMB).

characterized by one single morphological character, the number of superior central cells at the base of underleaves (Fig. 2). The morphological characterization and species composition of the two clusters are as follows:

CLUSTER I. Underleaves attached to the stem by two superior central cells: *Lejeunea anisophylla*, *L. apiculata*, *L. cocoes*, *L. exilis*, *L. papilionacea*, *L. patriciae*, *L. tamaspocsii*.

CLUSTER II. Underleaves attached to the stem by four superior central cells: *Lejeunea alata*, *L. eifrigii*, *L. albescens*, *L. microloba*, *L. umbilicata*, *L. compacta*, *L. lumbricoides*, *L. mizutanii*, *L. stephaniana*, *L. contracta*, *L. flava*, *L. kinabalensis*, *L. dipterota*, *L. discreta*, *L. fleischeri*, *L. pectinella*, *L. utriculata*, *L. sordida*, *L. mimula*, *L. gradsteinii*, *L. dimorpha*, *L. micholitzii*, *L. patersonii*, *L. tuberculosa*.

Studies on underleaf base anatomy in *Lejeuneaceae* have suggested that members of *Lejeuneaceae* subfam. *Ptychanthoideae* possess four or more superior central cells while those of

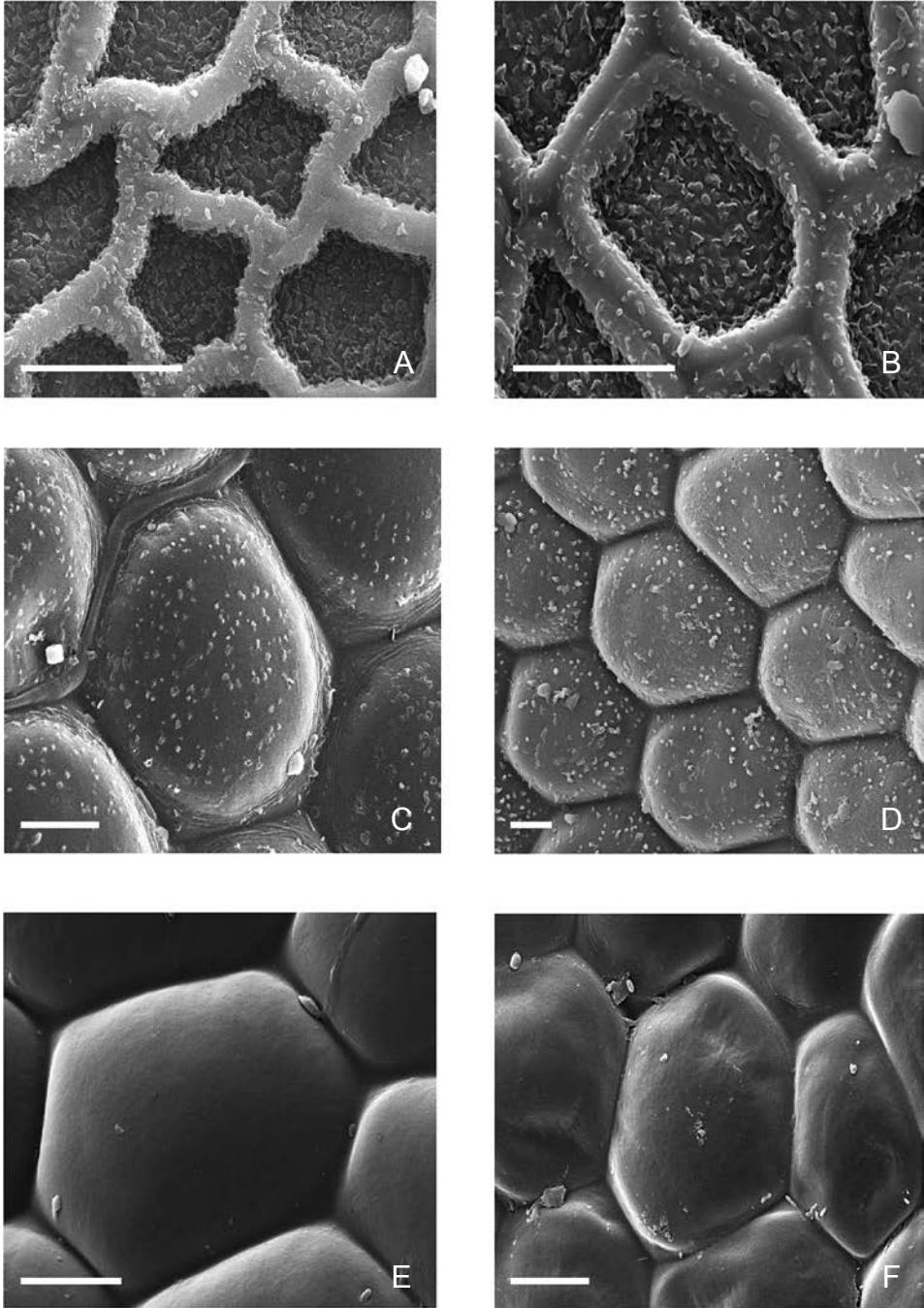


Fig. 3. Surface ornamentation of leaf cells in Malaysian *Lejeunea*. A – *Lejeunea flava* (Sw.) Nees, B – *L. tuberculosa* Steph., showing rough surface with small warts (wax). C – *L. microloba* Taylor, D – *L. kinabalensis* Mizut., showing rough surface with numerous minute papillae. E – *L. micholitzii* Mizut., F – *L. alata* Gottsche, showing smooth surface. A from *G.E. Lee 1191* (UKMB). B from *G.E. Lee 1164* (UKMB). C from *T. Kodama 40783* (NICH). D from *G.E. Lee 2138* (UKMB). E from *G.E. Lee 1833* (UKMB). F from *G.E. Lee 1199* (UKMB). Scale bars: 10 μ m.

subfam. Lejeuneoideae usually have only two (Bischler 1969; Winkler 1970; Gradstein 1975, 1979). However, *Lepidolejeunea* and *Pycnolejeunea* of subfam. Lejeuneoideae have 4–8(–10) superior central cells (Piippo 1986; He 1999), indicating that the number of superior central cells may vary within the two subfamilies and does not serve to sharply separate the two groups. In the present study, however, the number of superior central cells was constant at the species level and subdivided the 31 Malaysian *Lejeunea* species into two main groups. The number of superior central cells, which can be ascertained with ease from a small portion of the stem, had not previously been utilized in the classification of *Lejeunea* and appears to be a new and taxonomically informative feature in this genus.

The surface of leaf cells in Malaysian *Lejeunea* is usually smooth or finely roughened by minute papillae (Lee 2013). In *L. flava*, *L. mimula* and *L. tuberculosa*, however, small warts coarser than papillae are present (Fig. 3). Heinrichs & Reiner-Drehwald (2012) demonstrated that the warts of *L. flava* are wax projections and provided the first evidence for the occurrence of surface wax in Lejeuneaceae. Our SEM analysis of chloroform-treated shoots of *L. flava*, *L. mimula* and *L. tuberculosa* showed that the small warts found in all three species are cuticular wax projections. Our observations are the first records of wax ornamentation in *L. mimula* and *L. tuberculosa* and suggest that wax ornamentation may be a useful taxonomic feature at species level in *Lejeunea*.

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