

1 Phylogeny of the species-rich *Pilea* Lindl. (Urticaceae) supports its revised delimitation and
2 infrageneric classification, including the resurrection of *Achudemia* Blume

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18 Declarations of interest: none

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21 ABSTRACT

22 *Pilea* Lindl., with 933 published names is the largest genus within the Urticaceae. *Pilea* was
23 last monographed in 1869 and whilst the monophyly of the genus has been proposed by
24 previous authors, this has been based on incomplete taxon sampling and the failure to resolve
25 the position of key taxa. We aimed to generate a robust phylogeny for *Pilea* and allied genera
26 that could provide a framework for testing the monophyly of *Pilea*, revising its delimitation
27 and for answering broader scientific questions about this species-rich genus. To do so, we
28 sought to sample taxa representative of previous infrageneric classifications and with
29 anomalous inflorescences or flower configurations and to use the resulting phylogeny to
30 evaluate the delimitation of *Pilea* and to establish an infrageneric classification. In addition,
31 we included a representative of the Polynesian genus *Haroldiella* which, morphologically, is
32 very similar to *Pilea*. Using Sanger sequence data from two plastid and one nuclear regions
33 we constructed a phylogeny using Bayesian Inference, Maximum Likelihood and Maximum
34 Parsimony approaches. We used our phylogeny to evaluate the informativeness of 19
35 morphological traits and applied both to delimit a monophyletic genus and infrageneric
36 sections. Our results recovered *Pilea* as paraphyletic with respect to *Lecanthus*, a
37 consequence of the recovery of a monophyletic clade comprising sections *Achudemia* and
38 *Smithiella*, neither of which had been adequately sampled in previous studies. We also
39 recovered *Pilea* as polyphyletic with respect to *Haroldiella*. We identified isomery between
40 male and female flowers, flower part number and male sepal arrangement as being
41 phylogenetically informative traits that can be used to delimit two genera, *Achudemia*,
42 including section *Smithiella*, recovered as sister to *Lecanthus*, and *Pilea*, including
43 *Haroldiella*, recovered as sister to both. On the basis of our evaluation of both morphological
44 traits and phylogenetic relationships we propose a new infrageneric classification for the
45 genus comprising seven sections, five of which we describe for the first time, § *Trimeris*
46 Y.G.Wei & A.K.Monro, § *Lecanthoides* C.J.Chen, § *Angulata* L.F.Fu & Y.G.Wei, §
47 *Tetrameris* C.J.Chen, § *Verrucosa* L.F.Fu & Y.G.Wei, § *Plataniflora* L.F.Fu & Y.G.Wei and §
48 *Leiocarpa* L.F.Fu & Y.G.Wei. We also identify a trend of decreasing merism and fruit size,
49 and increasing species-richness as *Pilea* diverges. In addition, we recover strong geographical
50 structure within our phylogeny, sufficient to propose that *Pilea* originated in the IndoMalaya

51 biogeographic domain.

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53 *Key words: Lecanthus, Haroldiella, Aboriella, phylogenetics, taxonomy, merism*

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56 **1. Introduction**

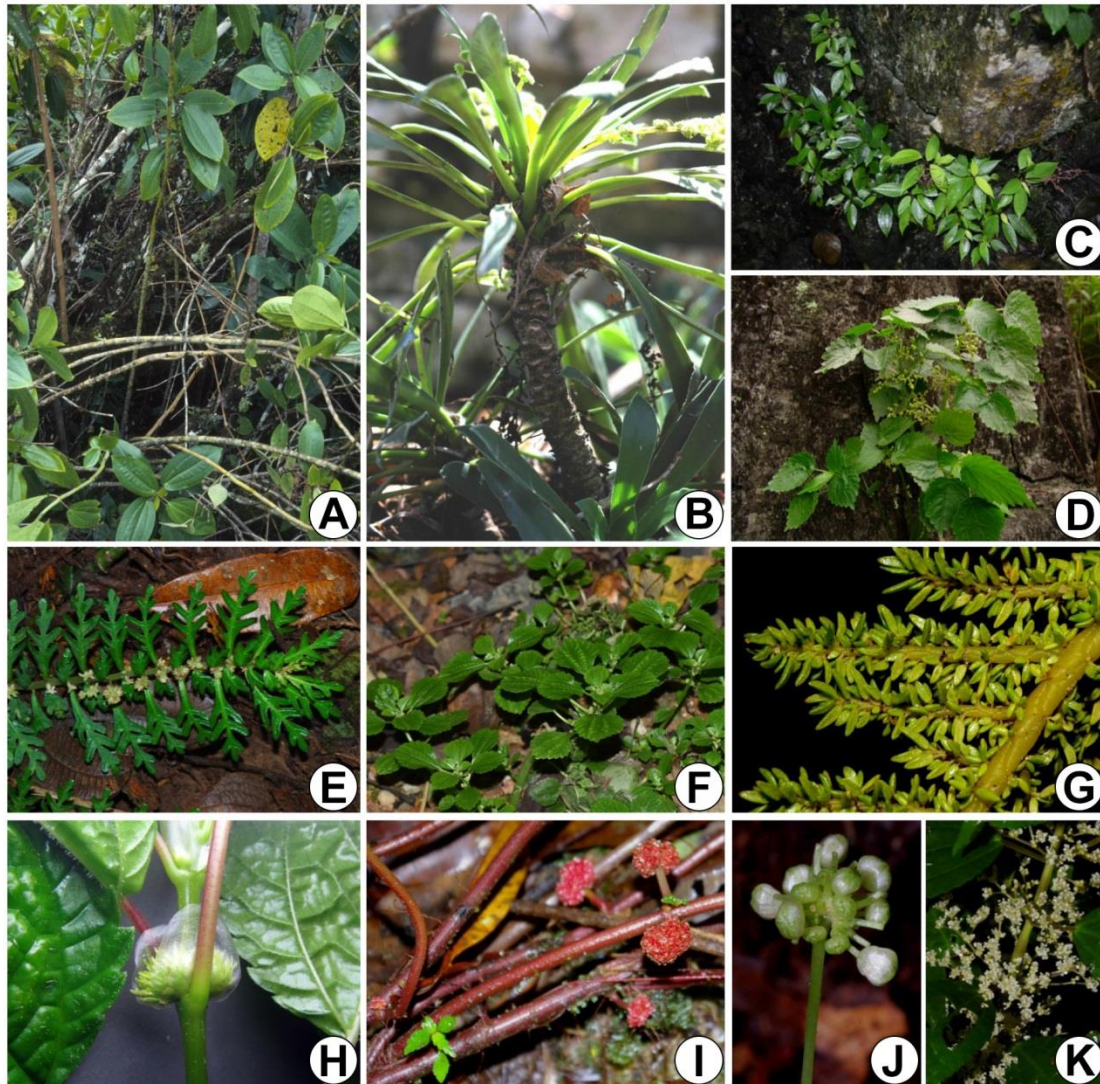
57 *Pilea* Lindl., with 933 published names (IPNI, 2020), 604 accepted names (WCVP, 2020)
58 and likely 715 species worldwide (Monro, 2004) is the largest genus within the Urticaceae
59 and has a pantropical and subtropical distribution. *Pilea* is characterized by succulent herbs,
60 shrubs and epiphytes whose flowers are wind pollinated, opening explosively, and seed which
61 is mechanically dispersed over short distances through the reflexing of the staminodes. It is
62 most species-rich in forested rocky habitats, especially on limestone or ultramafic rocks at
63 elevations between of 500 and 2,000 masl, in the Greater Antilles, Central America and the
64 Andes. Members of the genus may be distinguished from other genera in the family by the
65 combination of opposite (rarely alternate) leaves, intrapetiolar stipules, an absence of stinging
66 hairs, male inflorescences not fused to form a receptacle-like structure, and free female sepals
67 (Fig. 1 & Fig. 2). As is the case for many species-rich genera, *Pilea* has not been
68 monographed since the 19thC (Weddell, 1869) at which time the genus comprised ca 150 spp..
69 Instead, its taxonomy has been revised piecemeal through flora treatments (Monro, 2006).

70 *Pilea* belongs to the Elatostemeae tribe (Gaudichaud, 1830) which, including *Sarcopilea*
71 Urb., has been recovered as monophyletic and sister to *Lecanthus* Wedd. (Monro, 2006;
72 Jestrow et al., 2012; Wu et al., 2013; Tseng et al. 2019). The Elatostemeae comprises mainly
73 succulent, shade-loving, wind-pollinated species which, as is the case for each tribe in the
74 family, show a great variation in female inflorescence arrangement, ranging from open
75 panicles to spikes and fused receptacle-like structures. Despite several molecular studies,
76 doubts remain over the status of *Achudemia* Blume. Different accessions of *A. japonica*,
77 having been recovered within, or sister to, *Pilea* (Monro, 2006) and currently it is included
78 within *Pilea* (Friis, 1989; Chen and Monro, 2003). In addition, neither the Polynesian
79 endemic, *Haroldiella* J.Florence, whose morphological circumscription is congruent with
80 *Pilea*, or section *Smithiella*, characterised by strongly asymmetrical spicate inflorescences,
81 were sampled in previous studies, suggesting that the monophyly of the genus remains
82 untested.

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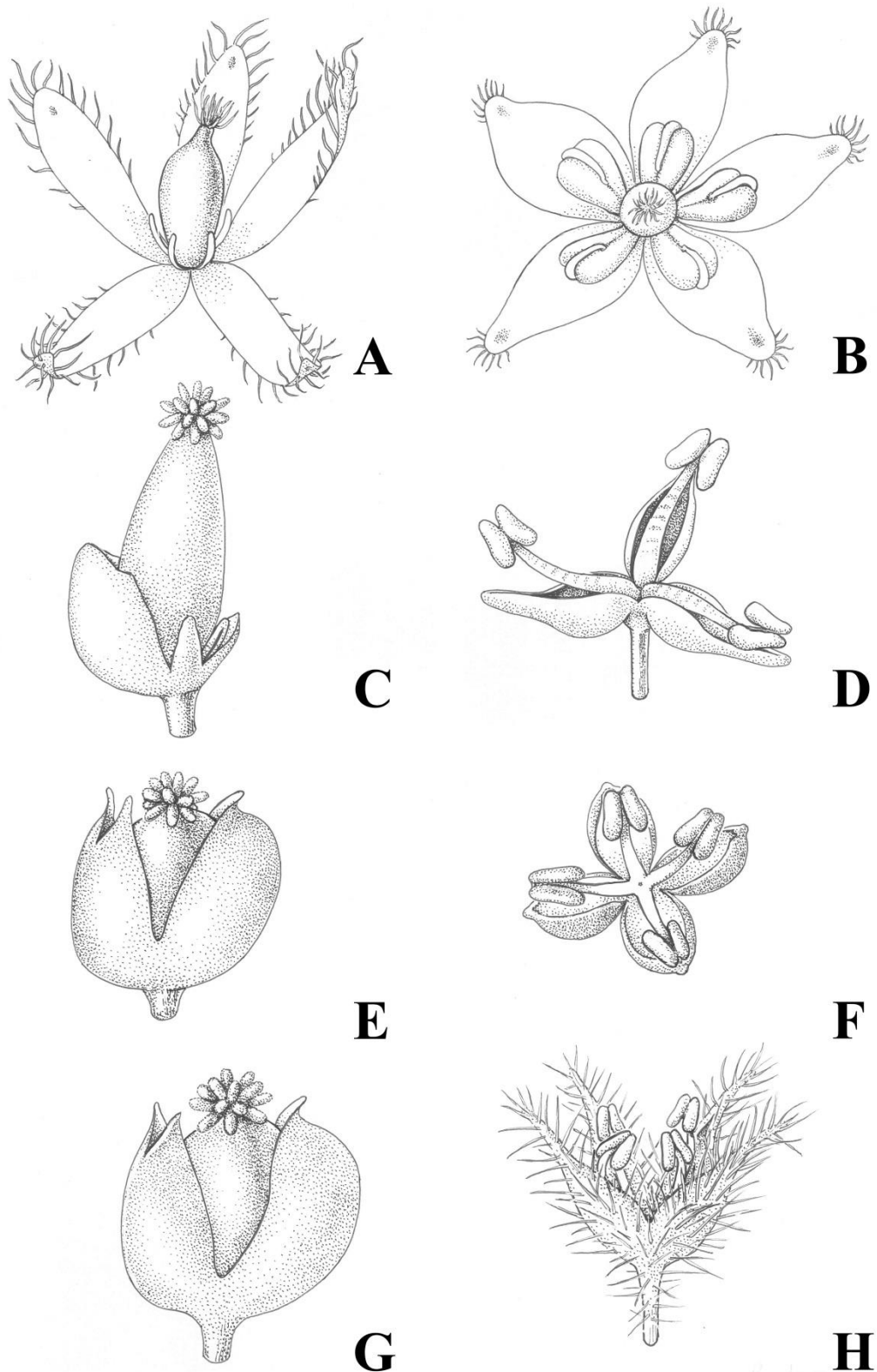
87 Fig. 1. Morphological diversity of *Pilea* and *Achudemia*. A, *P. longicaulis* (shrub); B, *P.*
88 *fairchildiana* (shrub, with alternate, spirally arranged leaves); C, *A. boniana* (herb, epipetric); D, *P.*
89 *paniculigera* (herb, epipetric); E, *P. matama* (unequal opposite leaves, epiphytic with capitate
90 female inflorescences); F, *P. peploides* (herb, clumped); G, *P. sp* aff. *microphylla*; H, *P. rivularis*
91 (female inflorescence enclosed by stipules); I, *P. aff. pittieri* (herb, male capitate inflorescence
92 arising from stolons); J, *P. angustifolia* (herb, male capitate inflorescence); *P. notata* (male cyme
93 inflorescence). A-B, E, G-J were photographed by Alexandre K. Monro; D was photographed by
94 Yi-Gang Wei; C-D, F, K were photographed by Long-Fei Fu.

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100 Fig. 2. Illustration of male and female flowers of *Pilea* and *Achudemia*. A, *A. javanica* (female
101 flower); B, *A. javanica* (male flower); C, *P. tripartite* (female flower); D, *P. tripartite* (male
102 flower); E, *P. plataniflora* (female flower); F, *P. plataniflora* (male flower); G, *P. microphylla*
103 (female flower); H, *P. microphylla* (male flower). Illustration by Margaret Tebbs.

104 *Achudemia*, currently treated as a section of *Pilea*, comprises four species of herb that
105 grow in deep forest shade, stream sides, gorges and caves in Indomalaya. *Achudemia* was
106 established by Blume (1856: 57) to account for a *Pilea*-like collection from Java (Indonesia)
107 which had bisexual (hermaphrodite) five-parted flowers. It appears that Blume described the
108 flowers as bisexual in error as neither the holotype, type illustration, or any other collections
109 have been observed to have bisexual flowers.

110 *Pilea* section *Smithiella*, comprises a single species of herb from Indomalaya, also
111 growing in deep shade (Chen, 1995; Chen and Monro, 2003). *Smithiella* was generated by
112 Dunn (1920) to account for *Pilea*-like material from the Eastern Himalayas characterised by
113 strongly asymmetrical spicate inflorescences of five-parted flowers. Dunn had been unaware
114 of an earlier homonym with priority, *Smithiella* H. Perag. & Perag. and in 1981, Bennet (1981)
115 created a replacement name, *Aboriella*.

116 *Haroldiella* comprises two species from Austral Polynesia growing on rocky outcrops or
117 in rain forest. *Haroldiella* was described by Florence (1997) based on plants with alternate,
118 spirally arranged pinnately nerved leaves. As with all *Pilea* from French Polynesia (Florence,
119 1997), they also share the trait of two-sepalate female flowers, a condition very rare
120 elsewhere in the genus. With the recovery of *Sarcopilea domingensis* Urb., a taxon with
121 spirally arranged, pinnately nerved alternate leaves, within a monophyletic *Pilea* (Monro,
122 2006, Jestrow et al., 2012), the characters used to delimit *Haroldiella* no longer support its
123 separation as a distinct genus.

124 Both *Achudemia* and *Aboriella* differ from other *Pilea* species in having male and female
125 flowers with five sepals (Fig. 2). Where free, the same number of perianth parts in male and
126 female flowers is uncommon within the Urticaceae and within *Pilea*, it is a condition
127 restricted to a basal, relatively species-poor clade (Monro, 2006) comprising Afrotropical,
128 Indomalayan and neotropical species.

129 Previous phylogenetic studies have consistently recovered *Lecanthus* as the genus most
130 closely related to *Pilea* (Monro, 2006; Wu et al., 2013, 2018), from which it be distinguished
131 by its male inflorescences being fused to form a concave receptacle-like structure reminiscent
132 of *Elatostema* J.R.Forst. & G.Forst. It also has an equal number of male and female perianth
133 parts, either four or five (Chen and Monro, 2003).

134 Given the above, and with the limited sampling of *Pilea* species with anomalous
135 inflorescence arrangements (spicate, receptacle-like) or flower-part number (five), together
136 with the ambiguous position of *Achudemia*, generic delimitation is potentially unstable.
137 Furthermore, the two main infrageneric classifications of *Pilea* (Weddell, 1856; Chen, 1982)
138 have been demonstrated to be largely para- or polyphyletic.

139 For the above reasons, we aimed to generate a robust phylogeny for *Pilea* and allied
140 genera that could provide a framework for revising the delimitation of the genus and the
141 answering of broader scientific questions about this species-rich and poorly studied genus. To
142 do so we sought to increase taxon sampling for the genus, encompassing all previous sections,
143 and the full range of morphological variation and geographical occurrence, using an
144 evaluation of the informativeness of morphological traits support the establishment of an
145 infrageneric classification.

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147 **2. Materials and methods**

148 *2.1. Taxon sampling*

149 We included 137 accessions representing 125 taxa (Table 1). These included 18 outgroup taxa
150 from the Cannabaceae, Moraceae and representatives of all Urticaceae tribes except for
151 Cecropieae (see Table 1) and 107 ingroup taxa (*Lecanthus* + *Pilea*). Within the Elatostemeae,
152 all genera were sampled except for the monotypic *Metapilea* which is likely extinct (Wu et al.,
153 2018), known only from the type and could not be sampled. This encompassed the following
154 taxa, *Elatostema* (3 spp), *Elatostematoides* (1 sp), *Lecanthus* (2 spp), *Pilea* (105 spp), *Procris*
155 (1 sp) and *Polychroa* (1 sp). We focussed on the ITS nuclear region and the *rbcL* and *trnL-F*
156 plastid regions. We combined sequences generated by previous studies (Monro, 2006; Jestrow
157 et al., 2012; Wu et al., 2013, 2018; Kim et al., 2015) 141 sequences of which were generated
158 by ourselves, excluding those accessions where we felt that the identifications were
159 ambiguous, or where sequence data for only a single region could be obtained. An exception
160 was made for the single sequence of *Haroldiella* that we were able to obtain. Our sampling of
161 *Pilea* included representatives of all infrageneric sections proposed by Chen (1982, Chen and
162 Monro, 2003). We did not structure our sampling to include representatives of Weddell's
163 sections as these were all demonstrated to be para- and polyphyletic by Monro (2006).

164 Sequence data were obtained for the four species of *P. sect. Achudemia*, the monotypic *P. sect.*
165 *Smithiella*, ten from *P. sect. Tetrameris* (approximately 2/3 of the species), 85 from *P. sect.*
166 *Urticella* (approximately 1/4 of the species), seven from *P. sect. Pilea* (approximately 2/3 of
167 the species), two from *P. sect. Dimeris* (1/2 of the species) and one from *P. sect. Lecanthoides*
168 (1/2 of the species). Four species of Moraceae (*Fatoua villosa* Nakai, *Morus alba* L., *Sorocea*
169 *affinis* Hemsl., *Trophis racemosa* (L.) Urb.) and two species of Cannabaceae (*Cannabis*
170 *sativa* L., *Humulus lupulus* L.) were chosen as outgroups based on the previous analyses
171 (Zhang et al., 2011; Kim et al., 2015). Species names, the accession numbers of sequences
172 downloaded from GenBank, and newly generated sequences used in this study are listed in
173 Supplementary Text 1.

174

175 Table 1. Statistics for the molecular datasets used in this study.

	Number of sequences (ingroup/outgroup)	Aligned length (bp)	Length variation (bp)	Variable characters (bp)	Parsimony-infor mative characters (bp)	Model selected (AIC)
ITS	119/18	716	472-588	490	398	GTR+I+G
<i>trnL-trnF</i>	117/18	1059	402-1059	532	356	GTR+I+G
<i>rbcL</i>	61/18	637	629-637	137	82	GTR+I+G
Combined plastid	119/18	1696	1038-1696	669	438	GTR+I+G
Combined all	119/18	2412	1572-2231	1159	836	GTR+I+G

176

177 2.2. DNA isolation, PCR amplification and sequencing

178 Genomic DNA was extracted from fresh or dried materials using a modified CTAB protocol
179 (Chen et al., 2014). The nrITS region was amplified using primers ITS 4 and ITS 5 (White et
180 al., 1990) and *rbcL* using primers 1F and 724R (Fay et al., 1997). The *trnL-F* spacer was
181 amplified using primers e and f (Taberlet et al., 1991) for most accessions while for few
182 problematic cases, we employed primers c and d to separate *trnL-F* into two overlap regions
183 then concatenated sequences (Taberlet et al., 1991). The PCR amplification were set at 94 °C
184 for 5 min, 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s and a final extension
185 at 72 °C for 10 min. The PCR products were checked on 1% agarose gels before being
186 purified using a QiaQuick gel extraction kit (Qiagen, Inc., Valencia, California, USA) and
187 directly sequenced in both directions using the amplification primers on an ABI 3730
188 automated sequencer (Applied Biosystems, Forster City, California, U.S.A.).

189 2.3. Phylogenetic analyses

190 Raw sequences were edited and assembled using the software Lasergene Navigator (DNAStar,
191 Madison, Wisconsin, USA) with subsequent manual adjustments. The output DNA sequences
192 were then aligned using MAFFT version 7.0 (Kato and Standley, 2013) with default settings,
193 followed by manual adjustment. The three datasets (nrITS, *rbcL*, and *trnL-F* spacer) were
194 aligned independently. Alignments were adjusted manually in MEGA 5.1 (Tamura et al.,
195 2011). Phylogenies were reconstructed based on the nrITS dataset, the combined plastid
196 datasets (*rbcL*, and *trnL-F* spacer), and all three datasets combined (nrITS, *rbcL*, and *trnL-F*
197 spacer), respectively. All of these reconstructions were analysed using Bayesian inference
198 (BI), maximum likelihood (ML), and maximum parsimony (MP) methods. A visual
199 comparison of the two best tree topologies generated by ML analyses of cpDNA and nrITS
200 datasets were performed to compare topological incongruence. A conflict in tree topologies of
201 each tree was considered significant when incongruent topologies both received bootstrap
202 values $\geq 80\%$ (Monro, 2006; Tseng et al., 2019).

203 Best-fit DNA substitution models were selected using the Akaike Information Criterion
204 (AIC) in Modeltest v 2.7 (Posada and Crandall, 1998) for each data partition. The substitution
205 model of the sequences was set to GTR+G+I for each single dataset based on Modeltest. BI
206 analyses were based on a Markov chain algorithm implemented in MRBAYES 3.2.6
207 (Huelsenbeck and Ronquist, 2001).

208 ML analyses with 1000 bootstrap resampling (MLBS) were conducted using the online
209 version of RAxML-HPC2 v8.2.9 (Stamatakis et al., 2008) available at the CIPRES Science
210 Gateway version 3.3 (<http://www.phylo.org/index.php/portal/>) (Miller et al., 2010) with the
211 gamma model of rate heterogeneity.

212 MP analyses were performed using PAUP* v4.0b10 (Swofford, 2002), in which all
213 characters were unordered and equally weighted, and gaps were treated as missing data.
214 Heuristic searches of MP were conducted with 100 random addition replicates with tree
215 tree-bisection–reconnection (TBR) branch swapping and MulTrees in effect. Branch supports
216 were assessed using 1000 bootstrap replicates (maximum parsimony bootstrap; MPBS) with
217 the sample settings the same as those for heuristic searches.

218 2.4. Estimates of support

219 In Bayesian analyses, posterior probabilities (PP) below 0.9 were considered as
220 providing no support, between 0.9 and 0.94 as providing weak support, between 0.95 and
221 0.99 as providing moderate support, and 1.0 as providing strong support (Tseng et al., 2019).

222 In bootstrap analyses of the ML (BSML) and MP (BSMP) analyses, values below 70%
223 were considered as providing no support, between 70-79% as providing weak support,
224 between 80-89% as providing moderate support, and 90-100% as providing strong support
225 (Tseng et al., 2019).

226 2.5. Morphological trait evolution

227 Based on existing phylogenetic studies we performed ancestral state reconstructions (ASR) in
228 order to evaluate the phylogenetic informativeness of selected morphological traits and so
229 apply these to the delimitation of *Pilea* and allied genera, and the establishment of an
230 infrageneric classification of *Pilea*. Our aim was to establish a classification that was both
231 phylogenetically congruent and morphologically diagnosable.

232 Nineteen morphological traits were coded for analysis (see Supplementary Text 2). Traits
233 were selected on the basis that they had been used in previous classifications and revisions of
234 *Pilea* (Weddell, 1856; Chen, 1982; Monro, 2006, 2015). Traits were scored based on the
235 examination of herbarium specimens and description in the literatures (Chen, 1982; Friis,
236 1989; Monro, 1999, 2015; Chen and Monro, 2003; Monro et al., 2012; Fu et al., 2017a; Yang
237 et al., 2018).

238 Likely transitions between trait states through evolution were reconstructed using ML
239 methods in Mesquite v.3.51 (Maddison and Maddison, 2015). We sampled the last 1000 trees
240 from the post burn-in set of the Bayesian analysis using combined dataset and an equal rate
241 model (Mk1) was selected for all traits. To account for phylogenetic uncertainty, we used
242 ‘Trace character over trees’. All reconstructions were integrated over the 1000 trees from the
243 post burn-in set and summarized on one of these trees that most matched our hypothesized
244 topology. The results were summarized as a percentage of changes of trait states using the
245 option of ‘Average frequencies across trees’.

246 2.6. Delimitation of infrageneric groupings

247 Given the number of species in *Pilea*, an infrageneric classification can be a practical way to
248 ease identification, as well as providing a framework for answering broader evolutionary

249 questions. With these aims our classification needed to reflect both phylogenetic relationships
250 and be morphologically diagnosable. We decided to base our classification of *Pilea* on
251 sections rather than subgenera as the distinction between the two is unclear (Brizicky, 1969)
252 and in this way we maintain the terminology adopted by Weddell (1856, denoted by the
253 symbol ‘§’) and Chen (1982). We have also aimed to establish sections in accordance with the
254 International Code of Nomenclature for algae, fungi and plants (Turland et al., 2018).

255

256 **3. Results**

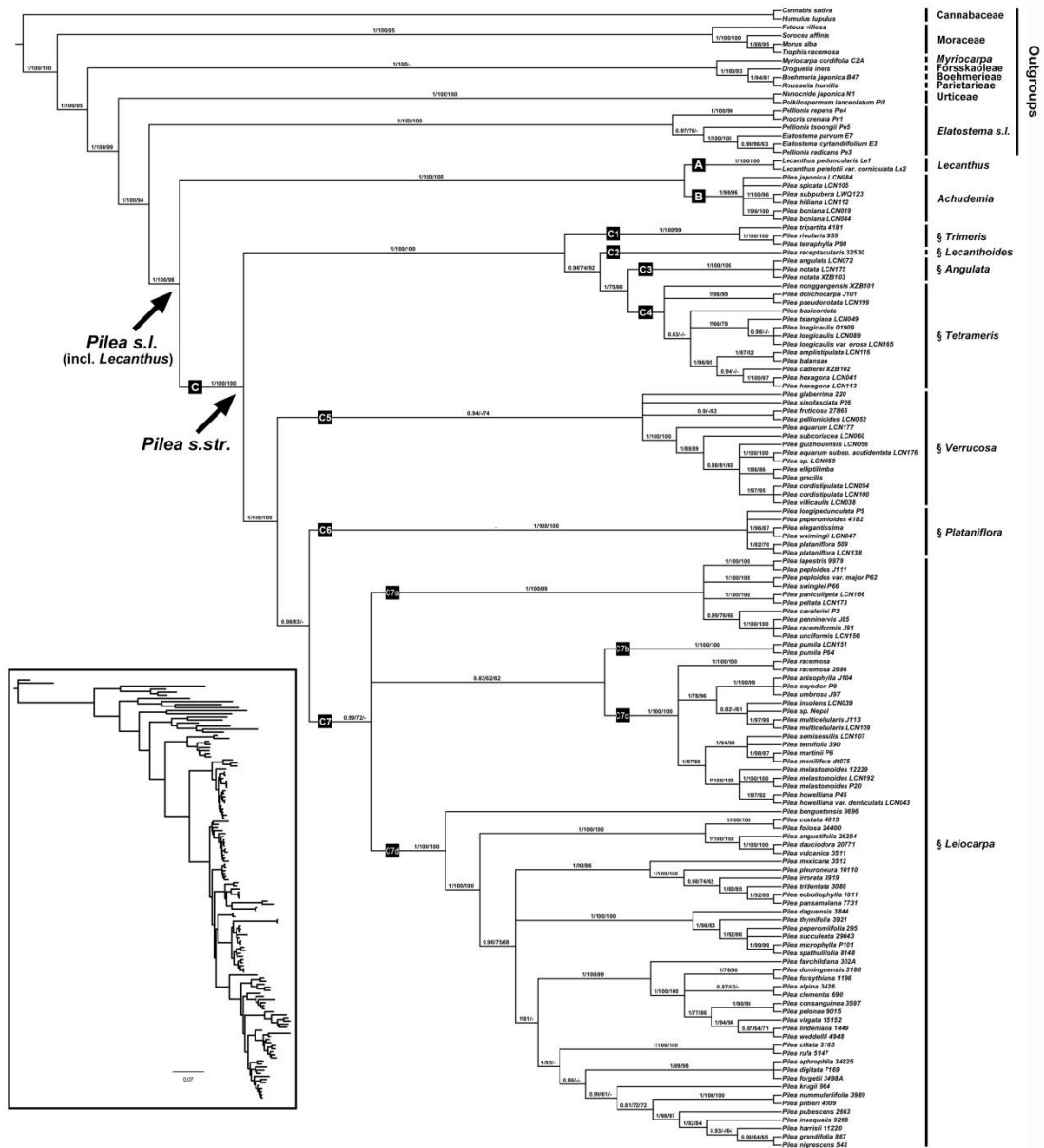
257 *3.1. Phylogenetic reconstruction*

258 Characteristics and statistics of the datasets used in this study are summarized in Table 1. The
259 comparison of trees for cpDNA (*trnL-F*, *rbcL*) and nrITS revealed an incongruence between
260 the outgroup taxa (Figs. S1 & S2). Because this incongruence did not affect the topology of
261 the ingroup taxa and the phylogeny of the combined dataset showed better resolved trees with
262 higher support values, than individual trees, we used the combined dataset for subsequent
263 analyses, including that of transitions between morphological trait states. The ingroup taxa
264 were recovered as monophyletic (Fig. 3) with strong support
265 (PP1.0/BSML100%/BSMP100%).

266 *3.2. Phylogenetic relationships of Pilea*

267 *Pilea*, including *Achudemia* and *Haroldiella* was recovered as paraphyletic with respect to
268 *Lecanthus* (Fig. 3 & Fig. S3). Two strongly supported clades attributable to *Pilea sensu lato*
269 were recovered. The first (Fig. 3, Clade B, labelled as *Achudemia*), was recovered sister to
270 *Lecanthus* (Clade A) and included all accessions from *P.* sect. *Achudemia* and *P.* sect.
271 *Smithiella* (*P. subpubera*, *P. boniana*, *P. hilliana*, *P. japonica*, *P. spicata*) with strong support
272 (PP1.0/BSML100%/BSMP100%). The second clade (Fig. 3, Clade C) comprised all other
273 accessions of *Pilea* with strong support (PP1.0/BSML100%/BSMP100%) and, in the analysis
274 of the ITS sequence data (Fig. S3), *Haroldiella*, with strong support (1/100/100). Within
275 Clade C, seven subclades were recovered with strong to weak support (C1 (1/100/99), C2
276 (0.96/74/92), C3 (1/100/100), C4 (-/-/-), C5 (0.94/-/74), C6 (1/100/100) and C7 (0.99/72/-))
277 (Fig. 3). *Haroldiella* was recovered within clade C7d (Fig. S3, 1/100/98). Clade C4 and C5
278 were no and weakly supported by all methods used to analyse the data, albeit they comprise

279 groups united by the morphological trait states of four-parted female flower and ornamented
 280 achenes, respectively. Clade C7 was recovered with strong to weak support but comprised
 281 four strongly supported subclades (C7a (1/100/99), C7b (1/100/100), C7c (1/100/100), C7d
 282 (1/100/100)).
 283



284
 285 Fig. 3. Phylogenetic tree of *Pilea* generated from Bayesian Inference (BI) of combined dataset
 286 (nrITS, *trnL-F* spacer and *rbcL*). Numbers on the branches indicate the posterior probability (≥ 0.8)
 287 of BI and bootstrap values ($\geq 60\%$) of the maximum likelihood (ML) and the maximum parsimony
 288 (MP) analyses.
 289

290 3.3. *Geographical structure*

291 Clade A (Figs. 4-5 & Fig. S23) comprises taxa with an Asia distribution, although *Lecanthus*
292 also includes species (not sampled) from Africa. Clade B (Figs. 4-5 & Fig. S23) comprises
293 taxa from East and Southeast Asia.

294 Clade C (Figs. 4-5 & Fig. S23) comprises species with a pantropical distribution except
295 for Australia and New Zealand. Within clade C, subclade C1 comprises taxa from Africa, Asia
296 and Latin America; subclades C2, C3, C4, C5 and C6 comprise taxa from East Asia; and
297 subclade C7 taxa from the pantropics. *Haroldiella*, restricted to Polynesia was recovered
298 within a polytomy within clade C7d meaning that it is more closely related to neotropical
299 species, than to African or Asian ones. Within clade C7, three subclades show a strong
300 geographical association. Subclade C7d, which harbours the greatest number of species, is
301 strongly associated with the neotropics, the Greater Antilles, Andes and Central America in
302 particular. Subclades C7b and C7c comprise predominantly palearctic taxa.

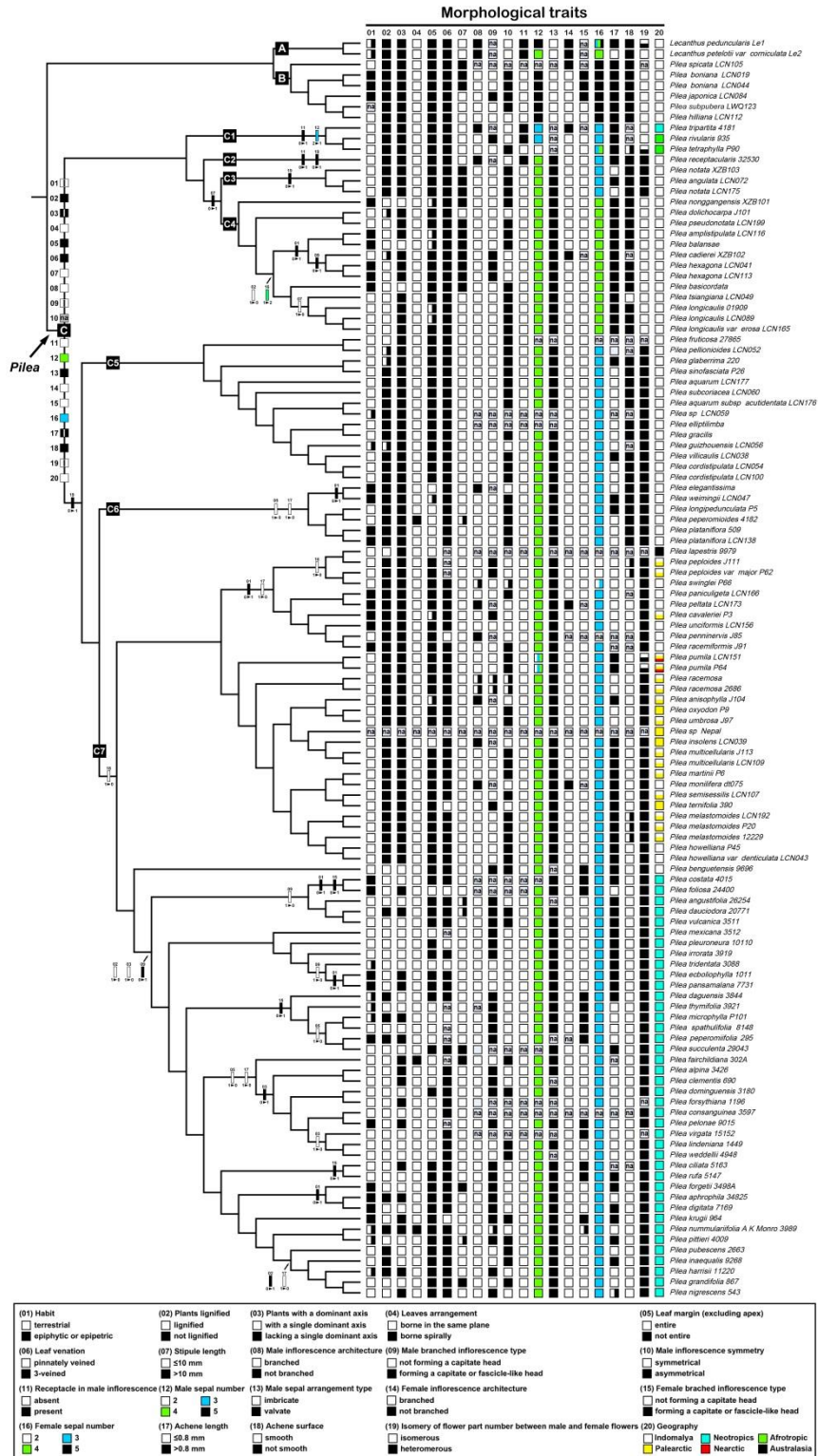
303 3.4. *Morphological trait evolution*

304 The 19 morphological traits were mapped onto the Bayesian Inference (BI) tree based on the
305 Maximum Likelihood analyses (Fig. 4 & Figs. S4-S22). This recovered five-sepalate male
306 and female flowers, and imbricate male flower sepals as synapomorphies for Clade A + B
307 (*Achudemia*), with a reversal in *L. petelotii* var. *corniculata* which has four-sepalate male and
308 female flowers. Achene bearing a crescent-shaped protuberance and unbranched male
309 inflorescences were recovered as autoapomorphies for Clade A (*Lecanthus*). Five-sepalate
310 female and male flowers were recovered as a plesiomorphy, and branched male inflorescences
311 as a synapomorphy, for Clade B (*Pilea* section *Achudemia* + *P.* section *Smithiella*). The
312 presence or absence of a crescent-shaped protuberance on the achene and branching, or not,
313 of the male inflorescence enable clades A and B to be readily distinguished from each other.

314 Four plesiomorphies were recovered for Clade C (all remaining *Pilea* species), stipules \leq
315 10 mm, male flowers 3-sepalate, achene $>$ 0.8 mm, achene ornamented. These were
316 manifested in the basal subclades, transforming to other states through the tree. All other traits
317 were recovered as homoplastic for the clade.

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321 Fig. 4. Reconstruction of the evolution of twenty morphological traits in *Pilea* based on our
 322 Bayesian Inference analysis of the combined dataset. The trait states at the *Pilea* node indicate the
 323 ancestral states of the genus. Transitions are indicated as filled boxes on the branches. Traits are
 324 shown above boxes and state transitions below. Descriptions of traits and their states are provided
 325 in the legend. The three clades (A–C) and seven subclades (C1–C7) correspond to those in Fig. 2.
 326 Reconstructions for each trait can be seen in Supplementary Figures S4 to S23.

327 Within subclades of Clade C, however, several plesiomorphic and synapomorphic trait
328 states were recovered. For clade C1, we recovered male flowers 3-sepalate (with a reduction
329 to two-sepalate for *Pilea tetraphylla*) as a synapomorphy and male sepals valvate as a
330 plesiomorphy. For C2, we recovered branches of the male inflorescence fused to form a
331 receptacle-like structure as a synapomorphy. For C3, we recovered stipules > 10 mm as a
332 synapomorphy. For C5 we recovered achene surface ornamented as a plesiomorphy. For C6
333 we recovered achene surface ornamented and leaf margin entire as plesiomorphies. For C7 we
334 recovered achene surface smooth as a plesiomorphy. For C4 we recovered no synapomorphies
335 or plesiomorphies. It could, however, be morphologically diagnosed based on unique
336 combinations of morphological traits (male and female flowers 4-sepalate), as could several
337 other monophyletic groupings within clade C.

338

339 **4. Discussion**

340 *4.1. Resurrection and expansion of Achudemia*

341 Previous studies (Monro, 2006; Jestrow et al., 2012; Wu et al., 2013, 2015, 2018) did not
342 attempt to establish formal infrageneric classifications because of limited morphological trait
343 and taxon sampling, especially of the more basal taxa. Monro (2006) did, however, suggest a
344 preliminary and informal classification of *Pilea* into six ‘units’ based on geography and
345 isomery of flower part number between male and female flowers, cystolith distribution on the
346 leaves and the presence of multicellular hairs. In this study we sought to propose a formal
347 classification based increased taxon, morphological and geographical sampling. Our approach
348 resolved the positions of all members of section *Achudemia*, including *Pilea subpubera*,
349 synonym of *Achudemia javanica*, genus type for *Achudemia*, and *Pilea (Achudemia) japonica*,
350 ambiguously recovered within and outside of *Pilea* by Monro (2006). In doing so recovered a
351 paraphyletic *Pilea*. The paraphyly can be resolved through the exclusion of 5-sepalate male
352 flowered taxa, formally assigned to Chen’s (Chen, 1982; Chen and Monro, 2003) sections
353 *Achudemia* and *Smithiella*, into a resurrected and expanded genus, *Achudemia*, which can be
354 distinguished from other *Pilea* species and *Lecanthus*, based on flower, male inflorescence
355 and achene morphology.

356 *Haroldiella* was recovered within *Pilea*. This result was not surprising given that the

357 justification for treating the taxa within a genus distinct to *Pilea* (Florence, 1997) was based
358 on the presence of alternate, spirally arranged leaves, a trait state expressed elsewhere within
359 *Pilea* (e.g. *P. peperomiodes*, *P. fairchildiana*).

360 Whilst almost all of the morphological trait states assessed were found to be homoplastic,
361 our expanded sampling did enable us to recover seven morphologically diagnosable clades
362 within *Pilea* and to use these to establish seven sections, partly congruent with those proposed
363 by Chen (1982) on morphology alone.

364 4.2. *Pilea* originated in IndoMalaya

365 Mapped onto our phylogeny, geographical occurrence suggests IndoMalaya as the centre of
366 origin for *Pilea* (Fig. 4 & Fig. S23), *Lecanthus*, *Achudemia* and the basal clades of *Pilea* (C2
367 to C6) predominantly comprising Indomalayan species. This confirms the findings of Monro
368 (2006) and Wu et al. (2018). Given the relationships between geographical areas suggested by
369 our results, the most plausible scenario for the dispersal of *Pilea* is of two independent events.
370 The first, early in the divergence of the genus (Fig. 4 & Fig. S23, clade C1) from IndoMalaya
371 to Africa and the northern Neotropics which has resulted in a lineage with a modest number
372 of species. The second, later in the divergence of the genus, involving dispersal to the
373 Palearctic (Fig. 4 & Fig. S23, clade C7) and later from the Palearctic to the Neotropics (clade
374 C7) resulting in species radiations in the Andes and Greater Antilles. According to the dated
375 Urticaceae phylogeny of Wu et al. (2018, Fig. 1), this first dispersal event would have
376 occurred ca 35 MYA (late Eocene) and the second ca 25 MYA (late Oligocene). A plausible
377 mechanism and route for second dispersal to the Neotropics would be the Bering land bridge
378 at some point between the late Cretaceous and late Neogene (Wen et al., 2016). Whilst long
379 distance dispersal, invoked by Wu et al. (2018) may have played a role in the first.

380 4.3. *Diversification accompanied by reductions in merism, achene size and ornamentation* 381 *and an increase in species number*

382 Chen (1982) proposed a reduction in female flower part number as an evolutionary trend in
383 *Pilea* and our results support this, five-parted flowers occurring amongst the basal clades of
384 our phylogeny (*Lecanthus*, *Achudemia*), followed by three or four-parted flowers (clades
385 C1-C4) and three or two-parted flowers (clades C5-C7). This includes a clade comprising
386 seven South Pacific species (sampled here as *Haroldiella*) characterised by two-parted female

387 flowers (Florence, 1997). Our analysis of morphological traits recovered three-parted female
388 flowers as plesiomorphy for the node comprising clades C5-C7 (Fig. S19). The trend in
389 reduced female part number was matched by a reduction in male flower part number from an
390 ancestral number of five to a derived condition of four, or three in the case of clade C1 (Fig.
391 S15) a transition from imbricate to valvate sepals (Fig. S16). The transition from imbricate to
392 valvate arrangement may suggest a transition from non-explosive to explosive anthesis
393 whereby imbricate sepals open in a controlled fashion whilst valvate sepals are torn apart by
394 the flexing filaments. The trend of reduced female and male flower part number parallels a
395 decrease in achene size and ornamentation, *Lecanthus*, *Achudemia* and clades C1-C4 having
396 achenes > 0.8 mm and ornamented as the ancestral state, whilst clades C5-C7 have achenes \leq
397 0.8 mm and clade C7 has smooth surfaces as their ancestral states. It also parallels a change
398 from imbricate to valvate sepals in the male flowers, suggesting a transition from gradual to
399 explosive flower opening (Pedersoli et al., 2019).

400 Whilst we did not seek to test hypotheses about key innovations, we are able to use the
401 results of our ASR analysis to propose hypotheses for future testing. Reduction in merism has
402 been associated with key innovations in floral evolution (De Craene, 2016; Kämpfers et al.,
403 2016; Simões et al., 2017). Our study supports this, a reduction in merism coinciding with an
404 evolutionary radiation, basal five-parted flowers of *Lecanthus* and *Achudemia* being relatively
405 species-poor (3 spp, and 5 spp respectively). *Pilea*, in contrast, which has four-, three-, or
406 two-parted flowers comprises ca 715 spp (Monro, 2004). According to De Craene (2016) a
407 reduction in merism may be driven by an advantage in reducing flower size. Whilst we have
408 not directly measured flower size, achene size is an effective surrogate for the size of the
409 female flower and fruit, both of which are dominated by the single ovary, and later achene. As
410 discussed above, there has been a decrease in achene size with the diversification of *Pilea*,
411 and specifically the species-rich C7 clade. The decrease in achene size coincides with a loss
412 of achene surface ornamentation. Based on an assumption that achene ornamentation
413 indicates animal dispersal, our results suggest a shift in both pollen and fruit dispersal, pollen
414 dispersal becoming explosive and more kinetic and fruit dispersal less reliant on animals.

415 Whilst the above discussion may provide the basis for future research into a reduction in
416 merism and achene surface ornamentation as key innovations, the increase in species number

417 with which they are associated may be unrelated. Increasing species number, focussed in the
418 Greater Antilles and Andes could also be the result of increased reproductive isolation and
419 subsequent speciation through random drift in steeply dissected shaded habitats devoid of
420 strong air-currents. It could, therefore be, an example of an intrinsic driver of speciation rather
421 than a response to the colonisation of novel habitats or development of novel pollination
422 syndromes (Tilston Smith et al., 2014).

423 4.4. Proposal for an infrageneric classification

424 To date, there have been only two main infrageneric classifications both of which were
425 based on morphological traits (Weddell, 1856, 1869; Chen, 1982; Chen and Monro, 2003).
426 Whilst these classifications facilitate the identification of taxa, they are not good indicators of
427 evolutionary relationships (Monro, 2006).

428 Chen (1982) proposed a classification for the Chinese taxa that focuses on the traits of
429 female flower merism, leaf nervation, male inflorescence arrangement and male sepal
430 arrangement. This was predated by Weddell's classifications (Weddell, 1856, 1869) based on
431 geographical distribution, leaf incision and heteromorphy. With the exceptions of male sepal
432 arrangement and male inflorescence becoming receptacle-like, the traits used in both
433 classifications were recovered as homoplastic, confirming the results of previous studies
434 (Monro, 2006; Wu et al., 2013, 2015).

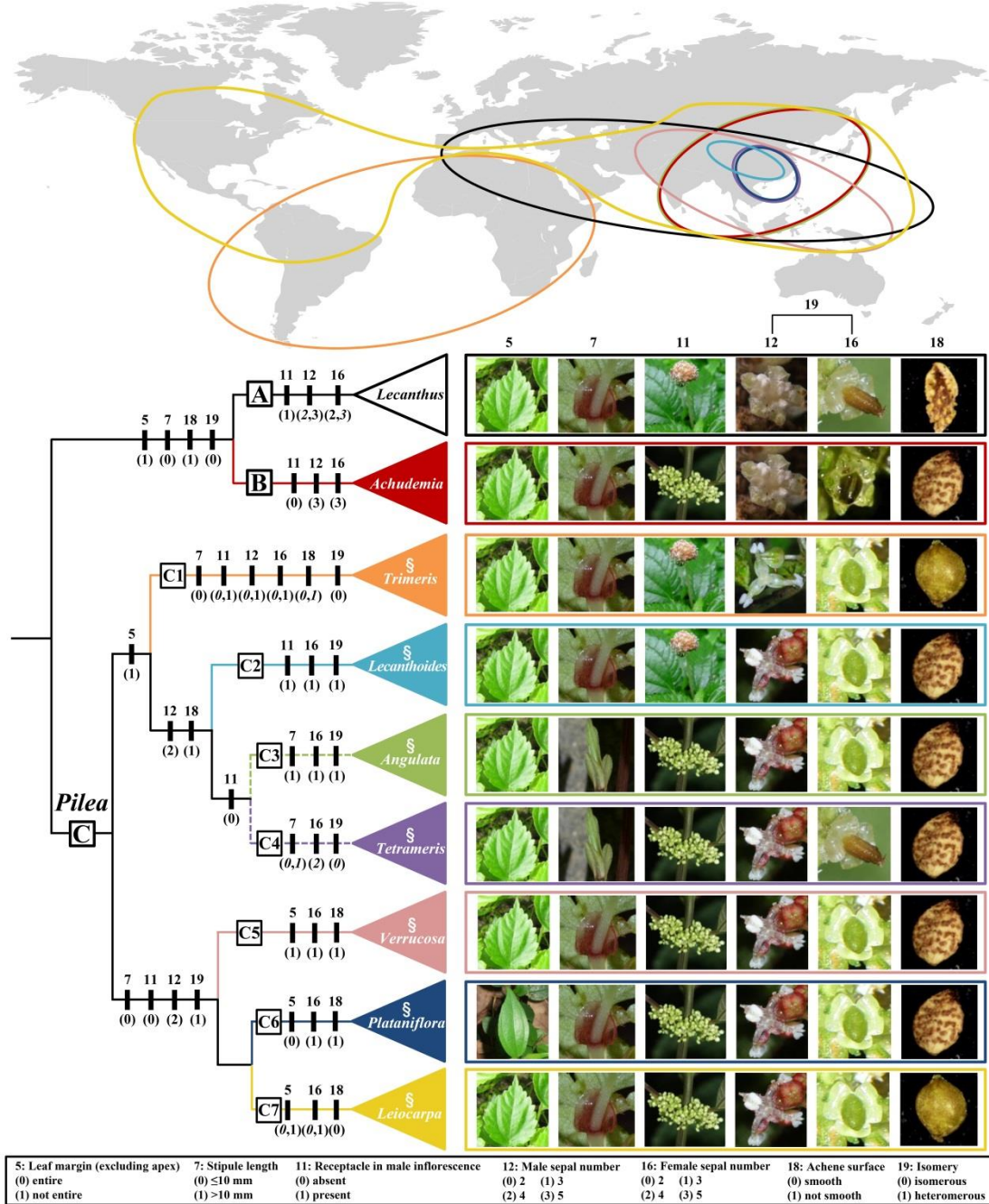
435 We did, however, recover achene morphology as a plesiomorphy useful in the distinction
436 of taxa in the species-rich clade C7 from clades C1-C6 (Fig. S21). Where traits were
437 recovered as homoplastic, they could still be used in combination, and or, with the addition of
438 geographical distribution to delimit monophyletic infrageneric groupings. For example, clade
439 C6, which we propose as section (§) *Plataniflora*, can be delimited by the combination of
440 entire leaf margins, four-parted valvate male flowers and ornamented achene surface. In this
441 way we were able to delimit seven infrageneric groupings.

442 The sections (§) that we propose for *Pilea* are, § *Trimeris*, § *Lecanthoides*, § *Angulata*, §
443 *Tetrameris*, § *Verrucosa*, § *Plataniflora* and § *Leiocarpa* (Fig. 3 & Fig. 5).

444

445

446



447

448 Fig. 5. Proposed infrageneric classification of *Pilea* and their geographical distribution. Filled
 449 boxes illustrate all states for each section. *Derived* states in italics, ancestral in regular font (based
 450 on ancestral state reconstructions summarised in Fig. 3). Images illustrate the ancestral states
 451 (replaced by common state when ancestral one is not available) for each infrageneric section.

452

453 5. Conclusions

454 We demonstrate that the species-rich genus *Pilea* is paraphyletic with respect to *Achudemia*
 455 and polyphyletic with respect to *Haroldiella*. We identify sepal number, flower isomery, male

456 sepal arrangement and achene surface ornamentation as phylogenetically informative traits
457 useful in both the delimitation of generic and infrageneric groupings and the generation of
458 hypotheses about the evolution of the genus and the richness of its neotropical clade.
459 Translating these findings into taxonomic actions resulted in the resurrection of *Achudemia*
460 and the revised delimitation and infrageneric classification of *Pilea*. Our findings provide a
461 stable framework for future research aimed at answering broader questions in evolutionary
462 biology, such as whether intrinsic factors can drive species radiations.

463

464 **6. Taxonomy**

465 *6.1. Revised delimitation of Achudemia and Pilea*

466 **Achudemia** Blume, Mus. Bot. 2: 57, 1856. Genus type: *A. javanica* Blume, Mus. Bot. 2: 57,
467 1856.

468 *Smithiella* Dunn. Bull. Misc. Inform. 1920: 210, 1920. Genus type: *Smithiella myriantha*
469 Dunn. nom. illeg., non *Smithiella* H. Perag. & Perag. = *Aboriella* Bennet, Indian Forester 107:
470 437, 1981. = *Dunniella* Rauschert, Taxon 31: 562, 1982.

471 Herbs, perennial or annual. Stems without stinging hairs, not releasing watery latex when
472 cut. Leaves opposite, frequently subequal at each node, the margins toothed; cystoliths
473 fusiform; stipules borne in axils of the leaves, persistent or caducous. Inflorescences
474 unisexual or bisexual, paniculate, capitate or racemose cymes; pedicels subtended by
475 inconspicuous bracteoles. Male flowers 5-merous; sepals imbricate in bud, equal, each
476 bearing a subapical appendage. Female flowers 5-merous; the sepals 5, equal or subequal, not
477 dimorphic. Achenes compressed ovoid, ornamented.

478 Five species, restricted to the Palearctic and IndoMalayan biogeographic regions.
479 Associated with forested rocky habitats. *Achudemia japonica* is used as a medicine to treat
480 fever and as a diuretic (Chen and Monro, 2003)

481 Note:— Blume (1856) in his description of the genus refers to polygamous, hermaphrodite
482 flowers. We believe this to have been an editorial error as neither, material collected by
483 Blume at L, or the illustration which serves as type, include polygamous or hermaphrodite
484 flowers.

485

486 *New combinations and typifications*

487 *Achudemia subpubera* (Miq.) Y.G.Wei & A.K.Monro. **comb. nov.** \equiv *Pilea subpubera* Miq.

488 Syst. Verz. Ind. Archip. 2: 102, 1854. Type: [Indonesia], Bandung Province, *H. Zollinger 870*

489 (holotype, U (U0226171*); isotypes P (P 02428341*), (P 02428342*))

490 *Achudemia javanica* Blume, Mus. Bot. 2: 57, 1856. TYPE: Mus. Bot. 2: 57, 1856, t. 20

491 (holotype). Epitype (selected here): [Indonesia] Java, *C.L. Blume s.n. L (10039782)**

492 We have selected an epitype as the type material comprises an illustration

493 (<https://www.biodiversitylibrary.org/item/200679#page/274/mode/1up>) which is not adequate

494 for making observations of anatomy of the leaf or stem. We selected material collected by

495 Blume in Java, which may have served as the subject of the type illustration.

496

497 *Achudemia boniana* (Gagnep.) L.F.Fu & Y.G.Wei. **comb. nov.** \equiv *Pilea boniana* Gagnap.,

498 Bull. Soc. Bot. France 75: 71. 1928. Type: Indochina [Vietnam], [Hà Nam Province]

499 Kien-khế Dong-ham rocks, *R.P. Bon 2522* (holotype P (P06817992)*). Epitype (selected

500 here): [Vietnam], Tonkin, Dong-Dang, on calcareous rocks, 12 Feb. 1886, *B. Balansa 581* (P

501 (P06817995)*).

502 *P. morseana* Hand.-Mazz., *Symb. Sin.* 7: 140. 1929. Type: China, Guangxi, Longzhou, *Morse*

503 495 (holotype K (K000708579)*)

504 *P. pentasepala* Hand.-Mazz., *Symb. Sin.* 7: 128. 1929. Type: China: Yunnan, mountains of

505 Mengzi, 1800 m, *Henry 9771* (holotype K (K000708578)*)

506 We have selected an epitype for *Achudemia boniana* as the holotype comprises leafless

507 material and leaves include several traits useful for species delimitation in *Achudemia*.

508

509 *Achudemia hilliana* (Hand.-Mazz.) L.F.Fu & Y.G.Wei. **comb. nov.** \equiv *Pilea hilliana*

510 Hand.-Mazz., *Symb. Sin.* 7: 129. 1929. Type: China, Yunnan, Mōngdse [Mengzi], *Henry*

511 10295 (lectotype (selected here) K (K000708583)*).

512

513 *Achudemia myriantha* (Dunn) L.F.Fu & Y.G.Wei. **comb. nov.** \equiv *Smithiella myriantha* Dunn,

514 Bull. Misc. Inform. Kew 1920: 211. 1920. Type: [India] Eastern Himalaya, Outer Abor Hills,

515 sunless side of the Dihong Gorge below Rotung, 300 m. Jan 3 1912, *Burkill 37636* (lectotype

516 (selected here) K (K000708616)*. *Pilea myriantha* (Dunn) C.J.Chen nom. illeg., non *P.*
517 *myriantha* Killip, *Bull. Bot. Res., Harbin* 2: 44. 1982. *P. spicata* C.J. Chen & A.K. Monro,
518 *Novon* 17: 26. 2007.

519

520 **Pilea** Lindl., nom. cons., *Coll. Bot. ad t. 4.* 1821. Genus type: *P. muscosa* Lindl. nom. illeg.
521 superfl. = *Parietaria microphylla* L. = *Pilea microphylla* (L.) Liebm.

522 *Adicea* Raf., nom. nud. *First Cat. Gard. Transylv. Univ.*: 13. 1824.

523 *Adicea* Raf. ex Britton & A. Br., *Ill. Fl. N. U.S.* 1: 533. 1896. nom. illeg. superfl.,

524 *Adike* Raf., *New Fl.* 1: 63. 1836. Genus type: *A. pumila* Raf.

525 *Chamaecnide* Nees & Mart. ex Miq., in C.F.P.von Martius & auct. suc. (eds.), *Fl. Bras.* 4:
526 203. 1853. Genus type: *C. microphylla* Nees ex Miq.

527 *Dubrueilia* Gaudich., *Voy. Uranie*: 495. 1830. Genus type: *D. peploides* Gaudich.

528 *Haroldiella* J.Florence, *Fl. Polynésie Franç* 1: 218. 1997. Genus type: *H. rapaensis*
529 J.Florence.

530 *Neopilea* Leandri, *Ann. Mus. Colon. Marseille, sér. 6, 7-8*: 46. 1950. Genus type: *N.*
531 *tsaratananensis* Leandri

532 *Sarcopilea* Urb., *Symb. Antill.* 7: 201. 1912. Genus type: *S. domingensis* Urb.

533 Herbs, rarely shrubs, occasionally epiphytic, perennial, rarely annual. Stems without
534 stinging hairs, not releasing watery latex when cut. Leaves opposite, frequently unequal at
535 each node, the margins toothed or entire; cystoliths fusiform; stipules borne in axils of the
536 leaves, persistent, rarely caducous. Inflorescences unisexual, rarely bisexual, paniculate,
537 capitate or rarely fused cymes; pedicels subtended by inconspicuous bracteoles. Male flowers
538 4- or rarely 2- or 3-merous; sepals valvate, equal, each bearing a subapical appendage. Female
539 flowers 3-, or rarely 2- or 4-merous, unequal, dimorphic, the adaxial sepal of the larger sepal
540 frequently bearing a dorsal thickening. Achenes weakly to strongly compressed ovoid to
541 sub-ellipsoid, smooth or ornamented. Approx. 710 spp. Cosmopolitan, except for Australia
542 and New Zealand. A number of species cultivated as ornamentals.

543

544 *New combinations*

545 ***Pilea australis*** L.F.Fu & A.K.Monro, *nom. nov.* Replaced name: *Haroldiella rapaensis*

546 J.Florence, *Fl. Polynésie Franç* 1: 220 (1997). TYPE: French Polynesia, Austral Islands,
547 Rapa, eastern flank of Mt. Perau, 610 m, 21 Jul. 1934, *H. St. John, FR. Fosberg & J. Maireau*
548 15643 (holotype BISH).

549 *Note*:— *Pilea australis* was created as a replacement name because a homonym, *Pilea*
550 *rapensis*, has been published by Forest Brown (Brown, 1935).

551

552 *Pilea sykesii* (J.Florence) L.F.Fu & A.K.Monro. **comb. nov.** \equiv *Haroldiella sykesii*

553 J.Florence, *Fl. Polynésie Franç* 1: 221 (1997). TYPE: French Polynesia, Austral Islands,

554 Raivavae, Anatonu, Falaise centrale, 140 m, 10 May 1992, *J. Florence & W.R. Sykes* 11336

555 (holotype P (P 00637067)*, isotype PAP)

556

557 6.2. Key to the sections of *Pilea*

558 1. Male and female flowers with the same merism, 3-parted or 4-parted **2**

559 1. Male and female flowers with different merisms, 2-, 3- or 4-parted **3**

560

561 2. Merism of 3, rarely 4 (*P. tetraphylla*), stipules \leq 10 mm in length, tropical Africa,

562 neotropics. **§ Trimeris**

563 2. Merism of 4, stipules $>$ 10 mm in length, Indomalaya. **§ Tetrameris**

564

565 3. Male inflorescence an unbranched and fused receptacle-like capitulum, involucrate.

566 **§ Lecanthoides**

567 3. Male inflorescence branched or unbranched, where unbranched capitulum globose or

568 subglobose, not involucrate **4**

569

570 4. Achenes ornamented **5**

571 4. Achenes not ornamented or rarely so, where ornamented Indomalayan and either *P.*

572 *melastomatoides*, or *P. peploides*. **§ Leiocarpa**

573

574 5. Stipules $>$ 10 mm in length. **§ Angulata**

575 5. Stipules \leq 10 mm in length **6**

576

577 6. Leaf margins incised. **§ Verrucosa**

578 6. Leaf margins entire. **§ Plataniflora**

579

580 *6.3. Infrageneric classification of Pilea*

581 *Pilea* § *Trimeris* Y.G. Wei & A.K. Monro, **sect. nov.** — Section type: *P. tripartita* A.K.

582 Monro.

583 Herbs. Stipules ≤ 10 mm in length. Leaf margin incised. Male inflorescence a capitate

584 cyme, involucrate; male and female flowers with the same merism, three- rarely four- parted;

585 the achene > 0.8 mm in length, not ornamented. Ca three spp. Tropical Africa, Neotropics.

586

587 *Pilea* § *Lecanthoides* C.J.Chen, Bull. Bot. Res. 2(3): 118. 1982. Section type: *P.*

588 *receptacularis* C.J.Chen.

589 Herbs. Stipules ≤ 10 mm in length. Leaf margin incised. Male inflorescence an

590 unbranched and fused capitulum, involucrate; male and female flowers with different merism,

591 male flowers four-parted, female flowers three-parted; the achene > 0.8 mm in length,

592 ornamented. Two spp. Indomalaya.

593

594 *Pilea* § *Angulata* L.F.Fu & Y.G.Wei, **sect. nov.** — Section type: *P. angulata* (Blume) Blume

595 Herbs. Stipules > 10 mm in length. Leaf margin incised. Male inflorescence a branched

596 cyme; male and female flowers with different merism, male flowers four-parted, female

597 flowers three-parted; achene $>$ or ≤ 0.8 mm in length, ornamented. Ca two spp. Indomalaya.

598

599 *Pilea* § *Tetrameris* C.J.Chen, Bull. Bot. Res. 2(3): 44. 1982. Section type: *P. basicordata*

600 W.T.Wang.

601 Herbs. Stipules > 10 or rarely ≤ 10 mm in length. Leaf margin incised. Male

602 inflorescence a branched cyme; male and female flowers with the same merism, flowers

603 four-parted; achene > 0.8 mm in length, ornamented. Ca 15 spp. Indomalaya.

604

605 *Pilea* § *Verrucosa* L.F.Fu & Y.G.Wei, **sect. nov.** Type: *P. gracilis* Hand.-Mazz.

606 Herbs. Stipules ≤ 10 mm in length. Leaf margin incised. Male inflorescence a branched
607 cyme; male and female flowers with different merism, male flowers four- or occasionally
608 two-parted, female flowers three-parted, rarely two-parted; achene $>$ or ≤ 0.8 mm in length,
609 ornamented. Ca 80 spp. Indomalaya.

610

611 *Pilea* § *Plataniflora* L.F.Fu & Y.G.Wei, sect. nov. Type: *P. plataniflora* C.H.Wright.

612 Herbs. Stipules ≤ 10 mm in length. Leaf margins entire. Male inflorescence a branched
613 cyme; male and female flowers with different merism, male flowers four-parted, female
614 flowers three-parted; achene $>$ or ≤ 0.8 mm in length, ornamented. Ca 34 spp. Indomalaya.

615

616 *Pilea* § *Leiocarpa* L.F.Fu & Y.G.Wei, sect. nov. Type: *P. micropylla* (L.) Liebm.

617 Herbs. Stipules $>$ or ≤ 10 mm in length. Leaf margins entire or incised. Male
618 inflorescence a branched or capitate cyme; male and female flowers with different merism,
619 male flowers four- or rarely two-parted, female flowers four- or rarely two-parted, achene $>$
620 or ≤ 0.8 mm in length, not ornamented. Ca 570 spp, Indomalaya, Neotropics, Australasia,
621 Palearctic, Nearctic.

622

623 6.4. Excluded names

624 *Metapilea* W.T.Wang, Bull. Bot. Res., Harbin 36: 164. 2016. Genus type: *M. jingxiensis*
625 W.T.Wang.

626 We have some doubt over the position of this taxon based on the poor quality of the
627 material upon which the description and illustration are based (a single sheet, very few
628 immature flowers). One individual of this taxon was collected once in a relatively common
629 habitat in Guangxi Province, China, in 1973 (Wang s.n.) and it has not been collected since. It
630 is possible that the original material may be an immature collection of *Pilea*, to which it is
631 vegetatively identical, or a distinct genus, which, based on the illustrations, could be allied to
632 *Elatostema* or *Procris*. Due to the sampling policy of the herbarium where the type collection
633 is stored it is not possible to sample this material for DNA and so its status and position
634 remains uncertain.

635

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645

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