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Testing Darwin's transoceanic dispersal hypothesis for the inland nettle family (Urticaceae)

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in the paper and to revisions.

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1 **Abstract**

2 Dispersal is a fundamental ecological process, yet demonstrating the occurrence and
3 importance of long-distance dispersal (LDD) remains difficult, having rarely been
4 examined for widespread, non-coastal plant groups. Therefore, we integrated
5 phylogenetic, molecular-dating, biogeographical, ecological, seed biology, and
6 oceanographic data for the cosmopolitan, primarily inland Urticaceae. We found that
7 Urticaceae originated in Eurasia ~69 Ma, followed by ≥ 92 LDD events between
8 landmasses. Under experimental conditions, seeds of many Urticaceae floated for >220
9 days, and remained viable after ten months in seawater, long enough for most detected
10 LDD events, according to oceanographic current modeling. Ecological traits analyses
11 indicated that preferences for disturbed habitats might facilitate LDD. Nearly half of all
12 LDD events involved dioecious taxa, so population establishment in dioecious
13 Urticaceae requires either multiple seeds, or occasional selfing. Our work shows that
14 seawater LDD might be a valid mode of transportation for inland plants, providing
15 empirical evidence for Darwin's transoceanic dispersal hypothesis.

16 **(a) Introduction**

17 Dispersal is a central biogeographical process shaping the present-day distributions of
18 organisms (Lomolino *et al.* 2010; Saastamoinen *et al.* 2018). For sessile organisms such
19 as plants, dispersal occurs via the movement of propagules or gametes (e.g. through
20 spores, seeds and pollen) away from parents (Clobert *et al.* 2001; Petit 2004); this, along
21 with environmental conditions and species interactions, determines when and where
22 species are found.

23 Darwin (1856) was an early advocate of the importance of dispersal, and he
24 demonstrated that many plant seeds could survive more than one month's immersion
25 in saltwater, thus potentially facilitating migration between landmasses, although he felt
26 that sinking might be an issue. For the next hundred years, long-distance dispersal
27 (LDD) was implicated as the cause of many present-day disjunctions across ocean
28 barriers, although successful LDD has been rarely witnessed (Nathan 2006). However,
29 during the 1960-70s, the validation of plate-tectonic theory and the spread of cladistic
30 thinking drastically reduced the perceived importance of oceanic LDD, providing
31 instead an attractive vicariant explanation for plant disjunctions (de Queiroz 2005;
32 Cowie & Holland 2006; Milne 2006). Consequently, for a time, LDD largely fell out of
33 favor, being regarded as unfalsifiable and even unscientific (Nelson 1978).

34 The advent of molecular dating, combining molecular phylogenetics with fossil
35 calibration, however, repeatedly revealed cases of transoceanic disjunctions that were
36 too young to result from tectonic vicariance (Givnish *et al.* 2004; Popp *et al.* 2011;
37 Bourguignon *et al.* 2018; Guo *et al.* 2018), forcing the scientific community to accept

38 other explanations for disjunctions, leading to increasing acceptance of the role of LDD
39 in shaping the present distributions of organisms (Raxworthy *et al.* 2002; Pyron 2014;
40 Rota *et al.* 2016; Carlton *et al.* 2017; Luebert *et al.* 2017). LDD events are now
41 universally accepted as a major factor shaping global biogeography, including large-
42 scale processes such as population spread, biodiversity evolution of oceanic islands,
43 and colonization of unoccupied habitats (Levin *et al.* 2003; Trakhtenbrot *et al.* 2005;
44 Cowie & Holland 2006; Gillespie *et al.* 2012).

45 Regarding mechanism, oceanic LDD has been reevaluated and is now
46 hypothesized as the main cause of disjunct distributions in many groups (Samonds *et*
47 *al.* 2012; Wee *et al.* 2014; Correia *et al.* 2018). Nonetheless, it remains difficult to
48 demonstrate experimentally or from observations how LDD occurred in any particular
49 case, largely because of the rarity and presumed unpredictability of such events
50 (Gillespie *et al.* 2012). Therefore, it is important that LDD hypotheses should be
51 testable using independent lines of evidence (Crisp *et al.* 2011).

52 Ocean currents have repeatedly been shown to be very important to LDD (Harwell
53 & Orth 2002; Thiel & Haye 2006; Geng *et al.* 2008; McMahon *et al.* 2014; Smith *et al.*
54 2018). However, most such studies concern relatively recent (post-Pliocene) LDD
55 events, focus on littoral species that often have specific adaptations to seawater
56 dispersal, concern groups with a relatively limited geological range, and/or rely either
57 on phylogeography or anecdotal observations alone (Table S1). Where widely
58 distributed inland groups have been examined, phylogenetic reconstruction has
59 normally been the focus, and LDD has only been hypothesized from this evidence

60 without direct investigation into possible mechanisms for it (Mao *et al.* 2010; Boer *et*
61 *al.* 2015; Cano *et al.* 2018; Yang *et al.* 2018).

62 LDD of plant propagules across oceans can occur by transportation through air
63 (wind or birds) or on ocean currents through immersion in seawater or on floating
64 vegetation islands (Nathan *et al.* 2008). To make a strong case for the oceanic transport
65 of propagules, for a given plant group, requires three lines of evidence. First, LDD
66 needs to be implicated as a potential explanation for the disjunct distribution of
67 populations within species or sister taxa on different landmasses, which in turn requires
68 precise molecular phylogenetic reconstruction. Second, ocean currents must be shown
69 to be the most likely mechanism of LDD. Third, the ability of seeds to make long
70 journeys in ocean currents must be demonstrated, using taxa descended from those
71 determined to have undergone LDD.

72 The nettle family (Urticaceae) constitutes an ideal group for examining LDD,
73 because it has a worldwide distribution, and a recent phylogeny revealed many
74 intercontinental disjunctions, indicating many probable LDD events (Wu *et al.* 2013).
75 Moreover, individual Urticaceous plants produce large numbers of seeds, and these
76 seeds are dispersed in tiny and hard achenes, mostly <10 mm and often <1 mm across,
77 that have no obvious adaptations to long dispersal by animals or wind. Most species
78 occur in wet understory environments along watercourses (Chen *et al.* 2003) where
79 exposure to wind is limited, making LDD by air, especially wind, highly unlikely,
80 whereas seeds could easily be carried downstream into the sea, making dispersal via
81 seawater seem a likely mechanism for LDD in this family.

82 For the current study, we first reconstructed the most complete generic-level
83 phylogeny of Urticaceae to date, and then used this as a framework to investigate the
84 biogeography and diversification of Urticaceae in space and time, identifying likely
85 LDD events across ocean barriers. Second, we selected four important ecological traits
86 that might be associated with particular modes of dispersal, and mapped these onto our
87 phylogeny in order to test whether each trait was more common in nodes where LDD
88 occurred than across the whole tree, which would imply that it promoted dispersal,
89 hence providing clues to likely mechanisms. Third, we conducted a rigorous
90 examination of seed biology to determine the buoyancy and viability of seeds after
91 long-term exposure to seawater, and combined this with oceanographic current
92 modeling to determine how far seed could be expected to travel in seawater. From this,
93 we examined the frequency, routes and mechanisms of LDD across Urticaceae.

94

95 **(b) Materials and Methods**

96 **Taxon sampling and phylogenetic analyses**

97 We sampled 298 accessions of 258 species from 52 genera of Urticaceae,
98 representing 94.5% of the recognized genera, and covering the entire geographical
99 range of the family. As outgroups, we used 26 species representing 14 genera from the
100 3 most related families (Moraceae, Cannabaceae, and Ulmaceae) in the Rosales. In total,
101 we sampled 325 accessions (Appendix S1, Table S2), and examined seven loci from
102 three genomes, comprising four chloroplast genes or intergenic regions (*trnL-trnF*,

103 *rpl4-rps8-infA-rpl36*, *matK* and *rbcL*), two nuclear regions (ITS and 18S), and one
104 mitochondrial gene (*matR*). The total aligned length was 11973 bp. DNA isolation, PCR
105 amplification, sequencing, and phylogenetic analysis all followed Wu *et al.* (2013)
106 (Table S3, Appendix S1).

107 **Divergence time estimation**

108 We estimated a time-calibrated phylogeny using the Bayesian program BEAST v
109 1.7.5 (Drummond *et al.* 2012), and the four most reliable Urticaceae fossils plus one
110 outgroup fossil were used as calibration points to determine absolute age of divergence
111 (Appendix S2).

112 **Ancestral area reconstruction and diversification analyses**

113 To determine the historical biogeography of Urticaceae, two distinct but widely
114 used methods of inferring biogeographic histories were employed: The dispersal-
115 extinction-cladogenesis (DEC) model was implemented in the software LAGRANGE
116 (Ree & Smith 2008), and S-DIVA (Yu *et al.* 2010) was implemented in RASP 3.2 (Yu
117 *et al.* 2015) (Table S4, Appendix S2).

118 To explore the tempo and mode of species diversification rates in Urticaceae over
119 time, we performed diversification analyses in the R package using the *ape* library
120 (Paradis *et al.* 2004) (Appendix S2).

121 **Evolutionary trends, correlations to LDD, correlated evolution and phylogenetic**
122 **signal in ecological traits**

123 **Evolutionary trends-** Dispersal tends to covary with a number of morphological,
124 ecological and behavioral traits (Stevens *et al.* 2014), hence such traits have a likely
125 impact on the feasibility of different modes of LDD. Hence we selected four important
126 ecological traits (sexual system, epiphytic life style, wet/dry habitat, and degree of
127 habitat disturbance) that might be associated with particular modes of dispersal, and
128 mapped them onto our phylogeny (Appendix S3). Ancestral states were reconstructed
129 for all such characters using the maximum likelihood (ML) method, following Wu *et*
130 *al.* (2015) (Table S5).

131 **Correlations to LDD-** For the definition of LDD in this study see Appendix S3.
132 For each trait, the likely state was determined for every node and terminal, giving an
133 average value across the tree; this was then compared to the mean value across only
134 those nodes associated with a detected LDD event.

135 **Correlated evolution** - To test the correlation among all four of these characters
136 for statistical validity, we employed Maximum likelihood (ML) and Markov chain
137 Monte Carlo (MCMC), using for both the discrete model conducted in BayesTraits v
138 1.0 (Appendix S3).

139 **Phylogenetic signal test** - a) To test whether presence/absence of LDD events are
140 distributed randomly across the phylogeny, we used the *phylo.d* function in the caper
141 package in R (Fritz & Purvis 2010). The value of D can be both smaller than 0 (highly
142 conserved) and greater than 1 (over-dispersed). b) We determined whether sexual

143 system, epiphytic life style, wet/dry habitat, and degree of habitat disturbance exhibited
144 phylogenetic signals. The first three were binary variables, and were also tested using
145 the function *phylo.d*. For habitat disturbance there were three possible states (Appendix
146 S3), so we firstly calculated the observed parsimony score (number of character
147 changes along the tree), then the null expectations were generated from the
148 randomizations of 999 times the habitat disturbance across the species. The
149 standardized effect size (SES) was calculated by subtracting the observed parsimony
150 score from the mean parsimony score from null randomizations and dividing by the
151 standard deviation of the randomized score (Maddison & Slatkin 1991).

152 **Saltwater immersion tolerance test for seeds**

153 To investigate whether seeds of Urticaceae can remain viable when immersed in
154 salt water, we carried out rigorous immersion experiments. Throughout this paper,
155 “seeds” refers to achenes, which is the form in which they are released in all Urticaceae.

156 Firstly, based on our biogeographic results, we identified 12 species (Table S6) that
157 had unequivocal intercontinental LDD events in their ancestry, representing all of the
158 four major subfamily-level clades (Clades I-IV) comprising the family (Fig. S1). Seeds
159 of each species were placed in plastic Petri dishes (90 mm diameter) with NaCl
160 solutions of concentrations below (0%, 1%), equal to (3.5%; Sverdrup *et al.* 1942) and
161 above (5%, 8%) that of seawater. Solutions were renewed every month. All dishes were
162 placed in a room with temperature at 18-25°C in the day and 10-15°C at night.

163 For each species, seeds were left in the NaCl solutions for each of one, three, five,

164 six, seven, eight, nine and ten months. After that, the seed germination of each species
165 from each combination of concentration and time period was determined, for details
166 see Appendix S3. An increase in salinity stress can induce physiological secondary
167 dormancy, delaying germination (Baskin & Baskin 1998); therefore, seeds that did not
168 germinate in our experiment might still be viable. Tetrazolium testing, a widely used
169 alternative means of testing for seed physiological quality (Lamarca & Barbedo 2014;
170 Oliveira *et al.* 2016), was therefore employed (Appendix S3).

171 From each replicate, viability percentage (VP) was calculated as total number of
172 seeds that germinated, plus estimated number alive by Tetrazolium testing, divided by
173 the total (excluding empty or infected seeds). A mean was then calculated for each
174 species, time and concentration.

175 The effect of immersion time and NaCl concentration on VP was evaluated using
176 Univariate General Linear Models performed using the SPSS 16.0 software package
177 (Chicago, IL, USA). The percentage data were arcsine transformed before analysis.
178 Differences obtained at a level of $p < 0.05$ were considered to be significant.

179 Additionally, to test whether 50% viability and maximum life span of the seeds
180 from these 12 species showed a significant phylogenetic signal, we calculated the
181 maximum likelihood value of Pagel's (1999) lambda (λ) using the functions 'fit discrete'
182 in the *Geiger* package with 50% viability and maximum life span as a discrete character.

183 **Seed buoyancy and LDD potential over water**

184 Successful LDD across water requires long-term seed buoyancy as well as survival, and

185 we assessed buoyancy for 21 species (those 12 tested for saltwater immersion tolerance,
186 plus nine others) (Table S7). A total of 270 seeds were taken from each species, then
187 following Lopez (2001), these were divided evenly among six glass bottles (250 mL),
188 of which three contained fresh water (0% NaCl) and three had 3.5% NaCl, mimicking
189 seawater. Conditions were otherwise the same as for the saltwater immersion tolerance
190 test. The number of floating seeds was counted at 5-day intervals.

191 For each species, mean seed mass (m) was determined by weighing 1000 seeds
192 using a precision electronic balance reading up to 0.0001 g (Table S8). Seed volume
193 could not reliably be determined using the liquid displacement method because of high
194 buoyancy. Therefore volume (v) per seed was calculated by measuring major (L_1),
195 intermediate (L_2) and minor (L_3) diameters from five randomly selected seeds of each
196 species, using a Vernier caliper (to 0.01 mm), and the equation $v = \pi (L_1 L_2 L_3) / 6$ (Miller
197 1987); Typical seed density (ρ) for each species was then calculated according to $\rho =$
198 m/v (Table S8).

199 Two separate mechanisms underlie all seed floatation, these are air trapped between
200 the cotyledons, and a spongy mesocarp or cork-like pericarp (van der Pijl 1972). We
201 used a Faxitron MX-20-DC12 X-ray image system (Faxitron X-ray Corporation,
202 Lincolnshire, IL, USA) to examine internal seed anatomy for these 21 species.

203 Finally, to observe the impact on the seed coat from prolonged salt water immersion,
204 the external morphology of achenes from those species subjected to eight months
205 immersion were compared to seeds from before immersion using a Sigma 300 scanning
206 electron microscope.

207 To predict the time required for seeds to disperse from the source landmass to the
208 destination landmass, ocean circulation models (Van Sebille *et al.* 2012; van Sebille
209 2014) were employed. Based on our biogeographic results, we selected eight species or
210 small clades within which unequivocal LDD events were detected (Table S9). In each
211 case, to set the starting point for dispersal, we first conflated the existing distributions
212 of all species within the source clade, and then selected the closest point within this
213 range to the destination landmass (Table S9). Next, in each case, results from ocean
214 modeling data were combined with seed survival and flotation data, to examine the
215 likelihood of seeds reaching the destination landmass via seawater.

216

217 **(c) Results**

218 **Relationships and molecular dating**

219 Our phylogenetic topology was consistent with Wu *et al.* (2013), strongly supporting
220 the monophyly of Urticaceae and that of all four main Clades (Fig. S1, Appendix S4).
221 Further, our analyses suggested that Urticaceae originated in Eurasia (56.2-) 68.7 (-87.1)
222 Ma (range indicates 95% highest probability densities; Fig. 1; Table S10).

223 **Ancestral area reconstruction and diversification**

224 Lagrange (Fig. 1) and S-DIVA (Fig. S2) analyses gave similar results for all nodes,
225 but the former gives more accurate reconstructions that are better aligned with
226 palaeogeographical evidence (Buerki *et al.* 2011), hence we describe the results here

227 entirely based on Lagrange.

228 Urticaceae arose and began to diversify within Eurasia, and a total of 92 LDD
229 events are indicated (Fig. 1; Table 1; Appendix S5). Detailed biogeographical
230 descriptions for all main clades of Urticaceae are provided in Appendix S4. A plot of
231 the cumulative number of lineages through time (LTT plot) for Urticaceae indicates
232 diversification perhaps accelerating around 45 Ma (Fig. S3, Appendix S4).

233 **Evolutionary trends of ecological traits and their correlations to LDD**

234 The ancestor of Urticaceae was dioecious, non-epiphytic and occupied dry habitats
235 (Fig S4). Within Urticaceae, 45.02% of nodes and terminals were monoecious, whereas
236 1.01% were epiphytic and 46.04% occupied wet habitats. The respective percentages
237 for these traits across only those nodes associated with LDD events were 51.65%,
238 3.30%, and 36.26%, but the differences were non-significant (see Appendix S4 for
239 details).

240 Regarding level of disturbance, 9.99% of nodes and terminals occupied
241 undisturbed habitats, 77.95% were semi-disturbed, and were 12.06% completely
242 disturbed. Scoring 0, 1 and 2 for these states respectively, the mean value across the
243 tree was 0.979 as opposed to 1.143 for nodes with LDD events ($t=3.740$; $p=0.00028$;
244 Appendix S4) indicating that the likelihood of LDD increases as the preferred habitat
245 becomes more disturbed.

246 Furthermore, we determined that LDD events were strongly phylogenetically
247 conserved ($D = -0.007$, $p < 0.001$), indicating that closely related species were likely to

248 be ecologically similar to one another (Appendix S4).

249 Using ML and MCMC analysis methods, very strong support was detected for
250 correlated evolution between habitat (wet/dry) and two other traits: sexual system ($p=$
251 0.00002; LBF = 24.85), and completely disturbed habitat ($p=$ 0.00023; LBF = 8.78)
252 (table S11).

253

254 **Effect of salt concentration and immersion time on seed survival**

255 Across all species, seed viability declined significantly as both immersion time and
256 salt concentration increased (Fig. 2, S5 & S6, Table S12 & S13). At salt water
257 concentration (3.5%), seeds of *Droguetia*, *Dendrocnide*, *Gonostegia*, *Maoutia* and
258 *Poikilospermum* survived until the seed supply ran out, lasting respectively 300, 240,
259 270, 240, and 240 days; however, seeds of *Urtica*, *Pilea*, *Parietaria* and *Debregeasia*,
260 survived only until 240, 210, 240, and 210 days, respectively. Similar results were
261 observed at 1% concentration. However, seeds of *Boehmeria* and *Elatostema* were all
262 dead after 180 days' immersion, at any concentration. Increased salt concentrations
263 caused a decline in viability for most species after 150 days (though not *Urtica*), and
264 only five genera showed any viability after 210 days at 8% (Table S13). There was no
265 phylogenetic signal detected for 50% viability and maximum life span (Table S14).

266 **Seed floatation**

267 Germinated seed will not survive a long journey in seawater, so these are treated

268 as having sunk or died. In distilled water, no germination was observed in 7 species
269 after 150 days' immersion, but 6 of the 21 species exhibited some germination from ten
270 days on (Fig. S7; Appendix S4). Regarding flotation, 12 species had at least 60% of
271 seeds still floating by the end, but five species had all seed sunk within 30 days (Fig.
272 S7).

273 In saltwater, no germination was observed for any species, and in general more
274 seeds remained buoyant than in fresh water (Fig. 3). After 220 days, twelve species had
275 at least 95% of seeds still floating, four had between 70% and 95% floating after 220
276 days, and four had <50% of their seeds floating (Fig. 3). Among most species, most
277 seeds that were buoyant after 20 days remained so after 220 days (Fig. 3, Appendix S4).

278 The mean seed density for all species was less than the density of both water (1.0
279 $\times 10^3$ kg/m³) and seawater (1.04×10^3 kg/m³), indicating that the seeds should be
280 buoyant in seawater. At least 12 of the 21 species examined using X-ray images were
281 found to possess small but obvious air-filled cavities (Fig. S8).

282 Examination of the external morphology of achenes demonstrated that long-term
283 salt water immersion produces little or no change to seed volume. This implies that the
284 seeds have excellent waterproof properties, and that neither imbibition of seawater nor
285 significant loss of water via osmosis has occurred. Damage was only observed to the
286 seed coat (Fig. S9) and parts not involved in seed protection, such as persistent perianth
287 lobes (e.g. *Boehmeria penduliflora* and *Debregeasia longifolia*, 1 and 8 in Fig. S9).

288 **Estimating the time required for LDD using ocean circulation modeling**

289 Regarding drift patterns and minimum transit times between continents (Fig. 4,

290 Table S9), three of the eight species analyzed could have reached the destination
291 landmass via seawater within six months, with *Debregeasia longifolia* needing the least
292 time, i.e. four months to reach the Philippines from Vietnam. *Droguetia inners* subsp.
293 *urticoides* would need 12 months to reach Tanzania from Java, whereas *Parietaria*
294 *micrantha* would need 22 months to float directly from China to Australia, but there are
295 ample islands in between, so the journey could have been made in stages (Fig. 4). It
296 would take the MRCA of *Neraudia*+sistergroups ca. 48 months to reach Hawaii from
297 Japan or thereabouts.

298 **(d) Discussion**

299 **Major disjunctions in Urticaceae and identifications of oceanic dispersal**

300 Urticaceae began to diversify in Eurasia around the Late Cretaceous (68.7 Ma),
301 with diversification perhaps accelerating around 45 Ma (Appendix S6). Remarkably, at
302 least 92 LDD events were indicated, mostly out of Eurasia and most often to the
303 adjacent regions of Africa and especially SE Asia. Incorporating paleogeographical
304 evidence, at least 76 must have crossed at least one ocean (Appendix S6).

305 **Potential for seed transoceanic LDD in Urticaceae**

306 Migratory birds can be a major mechanism for transoceanic LDD (van der Pijl 1972;
307 Heleno & Vargas 2015). Although most Urticaceae fruits are not edible, certain species'
308 achenes (e.g. *Debregeasia longifolia*) are ingested by some small birds (Zeng-Yuan Wu,
309 pers. obs.). Most birds do not retain seeds in their digestive tracts for long periods

310 (Proctor 1968) but small achenes could potentially make long journeys attached for
311 example to mud on birds' feet (Cleland 1952), (Fig. 5, Table S15). Furthermore,
312 occasional 'vagrant' birds blown off course by storms could aid dispersal in any
313 direction. However, many Urticaceae live in wet forests, and birds that live there might
314 be less likely to be blown off course than those frequenting open or coastal habitats.
315 Likewise, seed from such habitats seem unlikely to be carried long distances by wind,
316 as tree cover breaks up the flow of wind. Although some Urticaceae seeds have limited
317 adaptation for wind dispersal (e.g. membranous achene wings; Kravtsova 2009) (Fig.
318 5), these tend only to be associated with short dispersal distances, rather than LDD
319 (Higgins *et al.* 2003). Therefore, neither wind nor bird dispersal seems a likely
320 mechanism for Urticaceae, though neither can be eliminated entirely (Table S15).

321 Floating vegetation islands provide a plausible dispersal mechanism for trees or
322 epiphytes, and many account for some or all of three detected LDD events in *Procris*.
323 An untested possibility is whether floating trees might also transport terrestrial forest
324 species that are attached to soil around their roots (Fig. 5G). A robust test of whether
325 epiphytes are favoured for LDD events requires examination of a family or clade with
326 approximately equal numbers of epiphytic and terrestrial species.

327 Ocean current-mediated dispersal seems highly plausible within Urticaceae. Plant
328 propagules can be carried long distances first by inland watercourses (Boedeltje *et al.*
329 2003), then later by ocean currents (e.g. Fig. 5F). We found no proof that growing near
330 water increases the chance of LDD in Urticaceae, but seeds of species not growing close
331 to water might still blow into streams regularly. One caveat to this is that in around half

332 the species examined, most or all seeds will sink and/or germinate in fresh water, within
333 15 to 80 days depending on the species, and hence could not conduct or survive a
334 subsequent journey in seawater. Hence seawater dispersal will only work for those
335 species if preceded by little or no time in fresh water; hence LDD is more likely if these
336 grow relatively close to the coast. For others, notably *Maoutia puya* and *Oreocnide*
337 *integrifolia*, seeds can remain buoyant and not germinate during long periods in fresh
338 water, and likewise in seawater, suggesting that waterborne dispersal could work even
339 from a starting point far inland.

340 For LDD by seawater to succeed, seed must both float and remain viable for long
341 periods. Crucially, our data showed that all Urticaceae seeds examined are much less
342 dense than water, with air-filled cavities, hairs and wings all sometimes contributing to
343 buoyancy. Darwin (1856) observed that most kinds of seed sink, so Urticaceae might
344 be better equipped for LDD over water than many other families. Most species
345 examined exhibited little seed sinkage in salt water during 220 days, and what sinkage
346 there was occurred mostly in the first 20 days, implying that after this buoyancy
347 becomes stable, and could continue for a long time after 220 days. Only two of the 21
348 species tested, *Pellionia yunnanensis* and *Lecanthus peduncularis* looked as if seed
349 sinkage will provide a significant obstacle to LDD in seawater. Seed that only partially
350 sinks (i.e. is neutrally buoyant), can remain effectively suspended in the interior of the
351 water column, where like floating seed they can still be moved and influenced by
352 currents generated from tides, wind, waves, sea swell and hydrodynamics, as occurs for
353 some seagrass seeds (McMahon *et al.* 2014). Even seed that has sunk can be moved

354 along the sediment surface by currents or wave driven oscillatory flows (McMahon *et*
355 *al.* 2014), although this would not allow crossing of deep ocean.

356 Surface currents and winds provide passive transport for seeds drifting at sea
357 (Hawlitschek *et al.* 2017), and the mean speed of ocean currents is 0.1-0.3 m/s (Nathan
358 *et al.* 2008), Seeds of many Urticaceae remain viable following ten months' immersion
359 in seawater, making possible a dispersal distance of at least 2592 km via ocean currents,
360 close to the nearest distance between Africa and South America (~2800 km).
361 Oceanographic current modeling also indicated that many of the detected LDD events
362 could plausibly have occurred via ocean currents, before seed died or sank. Dispersal
363 across oceans might also be aided by archipelagos such as Hawaii (Harbaugh *et al.*
364 2009), serving as dispersal “stepping stones”, with our data suggesting at least one
365 possible LDD event out of Hawaii (Appendix S5).

366 Therefore, similar with the propagules of marine species (Harwell & Orth 2002;
367 Kennedy *et al.* 2016; Smith *et al.* 2018), viable seeds of Urticaceae could plausibly
368 travel long distances via seawater. A further significant obstacle, however, may be the
369 establishment stage.

370 **Implications of ecological traits for LDD mechanisms in Urticaceae**

371 For plants, LDD usually involves rare events driven by complex and highly
372 stochastic processes (Nathan 2006). Following long journeys in seawater, seeds could
373 be driven inland by extreme meteorological events such as stormy winds or floods,
374 although more frequent events allow arrival in coastal areas or tidal floodplains (Fig.

375 5). Extreme events could also aid the incoming seed by disturbing the recipient habitat,
376 reducing initial competition and favoring species adapted to disturbed environments.
377 Consistent with this, those Urticaceae nodes undergoing LDD tended to occupy
378 significantly more disturbed environments than did the family on average. Furthermore,
379 at least seven Urticaceae genera contain species that occur in periodically disturbed
380 littoral environments (Z.-Y. Wu, pers. obs.); such species could establish relatively
381 easily following ocean seed dispersal. LDD might also favour species of disturbed
382 habitats because these tend to be generalists, and hence more likely to establish in a
383 novel environment; crucially this third argument applies to LDD via any method,
384 whereas the first applies only to seawater dispersal, and the second to ocean dispersal
385 in general. Hence the observed trend fits seawater dispersal well, but does not exclude
386 other methods.

387 Seawater or wind dispersal both involve seeds travelling individually, introducing
388 a major obstacle at the establishment stage, i.e. going from one individual to a
389 population, unless the organism can self-fertilize. Conversely, birds or floating island
390 dispersal could bring seeds in groups (Fig. 5, Table S15). Hence monoecious and self-
391 compatible plants should be more likely to undergo LDD than dioecious or self-
392 incompatible (SI) species (Baker 1955). However, 48.35% of detected LDD events
393 involved dioecious taxa, compared to 54.98% of all nodes, so dioecy is not a major
394 obstacle to LDD in Urticaceae. This weak effect can be explained if most monoecious
395 Urticaceae are self-incompatible (data on SI in the family are lacking), meaning they
396 too should require two seeds for establishment. Potentially, a dioecious species might

397 occasionally self via an occasional female flower on a male plant, or *vice versa* (Vega-
398 Frutis *et al.* 2014; Varga & Kytöviita 2016). Moreover, monoecy arose at least 84 times
399 during Urticaceae evolution (Fig. S4), often associated with transitions of habit and
400 disturbance, suggesting an ability to switch breeding strategy when circumstances
401 select for it. Both might be triggered by founder effects, aiding establishment.
402 Otherwise, two seeds are needed. In a habitat that undergoes regular disturbance and
403 inundation involving seawater, including tidal inland riversides, a second seed might
404 arrive within the lifespan of the original plant. Alternately, multiple seeds might arrive
405 and germinate simultaneously following a catastrophic event (Fig. 5, N, Q). Otherwise,
406 scraps of plant washed into the sea (“vegetable rubbish”; Darwin, 1856) might float
407 across the ocean, with multiple viable seeds contained in fruiting bodies (Fig. 5, E).
408 Nonetheless, for Urticaceae, establishment represents a significant obstacle to
409 successful LDD, probably second only to sea crossing itself. Much more data are
410 needed, especially on SI, in order to determine the most likely way that this obstacle
411 was overcome.

412 Furthermore, we inferred that the LDD events and all four tested traits were
413 phylogenetically conserved, therefore, LDD events in Urticaceae were strongly
414 associated with the evolution of conserved traits. The traits we assessed might
415 adequately represent LDD-linked traits, but there are almost certainly other traits we
416 did not include in our analyses that could also be informative regarding LDD events.
417 Of course any traits associated with LDD would not have actually been selected to
418 optimize LDD, since there would not have been any population-level advantage locally,

419 but nonetheless their evolution influenced which lineages successfully underwent LDD.

420 To the best of our knowledge, this is the first study to use multiple lines of evidence
421 to examine seed transoceanic LDD in a widely distributed group that is not specialized
422 for coastal habitats. Within Urticaceae, we inferred at least 76 LDD events that must
423 have involved a journey over across oceanic waters, a result that emphasises the
424 frequency and hence profound global importance of LDD for generating modern plant
425 distributions. Seawater appears to provide the most feasible LDD medium, because
426 seeds of many Urticaceae can float for long periods, and they remain viable after ten
427 months in seawater. This permits very long distances to be covered by living and
428 floating seeds, via ocean currents, and many species' seeds might live well beyond our
429 10-month test period. Furthermore, a bias towards disturbed habitats among dispersing
430 lineages fits a hypothesis that seeds in seawater can come ashore onto viable ground
431 that is disturbed, either periodically by tides, or dramatically by extreme events.
432 Therefore, other than the ocean crossing itself, the biggest obstacle to LDD might be
433 the establishment of breeding populations, at least for dioecious or self-incompatible
434 taxa. Possible solutions to this include arrival of multiple seeds by various mechanisms
435 (Fig. 5), or occasional sex switching that allows selfing. Given the long time-scales
436 involved, the need for just one highly unlikely event is surmountable. Future work on
437 breeding systems is needed to investigate this issue further. Our work adds to the
438 growing body of evidence showing the importance and frequency of LDD, but
439 moreover contributes towards addressing that most intractable question of LDD: how
440 it happens.

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646

647 **Figure legends**

648 **Figure 1.** Global biogeographical patterns of Urticaceae inferred by Lagrange. Map
649 shows the eight biogeographical regions in colors as defined in this study. Clades are
650 marked by numbers in a black box. Circles with numbers indicate fossil calibration
651 points. Pie charts above nodes show the biogeographical ancestral reconstruction
652 results, with colors referring to the different regions as indicated in the map. Colored
653 boxes behind tips indicate the distributions of species, as indicated in the map. Inferred
654 dispersal events (D) are marked where appropriate on internal branches. (a)
655 relationships between main clades; (b) biogeographical patterns of Clade I; (c)
656 biogeographical patterns of Clade IV; (d) biogeographical patterns of Clade III; (e)
657 biogeographical patterns of Clade II.

658

659 **Figure 2.** Effect of salinity (1, 3.5, 5 and 8% NaCl) and immersion time on the seed
660 viability percentage (VP) of 12 species. a) *Debregeasia longifolia*, b) *Boehmeria*
661 *penduliflora*, c) *Droguetia iners* subsp. *urticoides*, d) *Parietaria micrantha*, e) *Pilea*
662 *pumila*, f) *Elatostema stewardii*, g) *Gonostegia hirta*, h) *Poikilospermum suaveolens*, i)
663 *Laportea bulbifera*, j) *Dendrocnide basirotunda*, k) *Maoutia puya*, l) *Urtica dioica*
664 subsp. *dioica*.

665

666 **Figure 3.** Percentage of floating seeds versus days in seawater for 21 selected species
667 in our study.

668

669 **Figure 4.** Simulation of dispersal process for a seed released from the closest extant
670 distribution point within the source clade, to a point within the range of the clade
671 derived from an LDD event, assuming passive drifting via ocean currents. (a)
672 *Debregeasia longifolia*, (b) *Laportea bulbifera*, (c) *Poikilospermum suaveolens*, (d)
673 *Musanga cecropioides*, (e) *Droguetia iners* subsp. *urticoides*, (f) *Parietaria micrantha*,
674 (g) *Pouzolzia elegans* var. *elegans*_Po11+*Pipturus* (Pip1+Pip7+Pip10), (h)
675 *Neraudia*+sistergroup.

676

677 **Figure 5.** Plausible mechanisms for long-distance dispersal across an ocean barrier in
678 Urticaceae. Colours indicate start (yellow), middle (orange) and end (red) of a journey
679 via water. **A-D** = means of seeds reaching the sea: **A**, seeds or scrap of plant enter sea
680 directly from land; **B**, same but begins by entering river then carried into sea by river;

681 C, growing on or around roots of large tree that falls into river and is carried to the sea,
682 becoming floating vegetation island; D, same as C but plant begins as epiphyte. E-H =
683 forms in which they cross the sea, in all cases propelled by ocean currents: E, seeds
684 contained within scrap of floating plant; F, seeds (achenes in Urticaceae) float on their
685 own; G and H, plants or roots or as epiphytes carried on floating tree, can be dead with
686 seeds on them, or still alive. I-J = transport by air: I, blown by wind; J, carried by bird,
687 mostly likely a vagrant blown off course. K-Q = methods of arrival on land from the
688 sea. K, scrap of plant washed ashore, after which seeds might blow inland; L, floating
689 seed washes ashore on beach or further inland if very high tide; M, carried ashore by
690 animals that move between sea and land; N, seeds carried inland by tsunami; O, carried
691 upriver (e.g. by tidal bore) and deposited on floodplain; P, floating vegetation island
692 comes ashore and seeds can then blow inland; Q, seeds carried inland by tornado,
693 waterspout or other extreme weather event. Where pairs of seeds are shown (E, G, H,
694 K, P) this indicates a mechanism with a high probability of transporting two seeds at
695 once, overcoming potential barriers to establishment from self-incompatibility or
696 dioecy.

697 **Figure S1.** Phylogenetic tree produced by Maximum Likelihood (ML) analysis based
698 on data matrix with plastid, mitochondrial, and nuclear datasets combined. Clades are
699 referred to by numbers in the box, numbers above branches (ML/MP/BI) are support
700 scores from Maximum Likelihood (bootstrap), Maximum parsimony (bootstrap) and
701 Bayesian posterior probability.

702

703 **Figure S2.** Global biogeographical patterns of Urticaceae inferred by S-DIVA. The map,
704 clade names, circles with numbers, pie chart, and colored boxes behind tips exactly
705 same as the scheme of Fig. 1. Inferred dispersal (D) is also marked where appropriate
706 on internal branches.

707

708 **Figure S3.** The lineage through time plot (LTT) for Urticaceae. The solid line is derived
709 from the maximum clade credibility tree, while the area shaded in grey represents the
710 95% confidence interval from the random 1000 trees of the BEAST analyses. The blue
711 interval represents a period when Urticaceae underwent relatively rapid diversification.

712

713 **Figure S4.** Ancestral state reconstruction of four selected characters in Urticaceae. The
714 clade names and plotted numbers exactly same as the scheme of Fig. 1. The character
715 states at the Urticaceae node indicate the ancestral states of the family. State changes
716 are indicated on the branches using same forms as nodes respectively.

717

718 **Figure S5. (A)** Seed germination of *Debregeasia longifolia* following immersion in 0%
719 NaCl solution (distilled water) for 10 days. **(B)-(D)** Seed germination of *Gonostegia*,
720 *Pilea*, and *Urtica* respectively, one week after being transferred to 1% water agarose
721 substrate following 30 days' immersion in 1% NaCl solution. **(E)-(H)** Seed germination
722 of *Droguetia* three weeks after being transferred to 1% water agarose substrate
723 following 90 days' immersion in 1% (E), 3.5% (F), 5% (G) and 8% (H) NaCl solution.
724 **(I)** Seed germination of *Gonostegia* two weeks after being transferred to 1% water

725 agarose substrate following 150 days' immersion in 3.5% NaCl solution. (J-K) Seed
726 germination of *Pilea* (J) and *Maoutia* (K) two weeks after being transferred to 1% water
727 agarose substrate following 150 days' immersion in 1% NaCl solution. (L) Seed
728 germination of *Parietaria* two weeks after being transferred to 1% water agarose
729 substrate following 150 days' immersion in 5% NaCl solution.

730

731 **Figure S6.** Photos of seeds of 12 species, following seed coat removal for Tetrazolium
732 tests. (A-B) living (red) and dead (white) seeds of *Dendrocnide basirotonda* (A) and
733 *Maoutia puya* (B). For all others, only live seed is shown: (C) *Elatostema stewardii*;
734 (D) *Debregeasia longifolia*; (E) *Laportea bulbifera*; (F) *Urtica dioica* subsp; *dioica*;
735 (G) *Parietaria micrantha*; (H) *Poikilospermum suaveolens*, (I) *Boehmeria penduliflora*;
736 (J) *Pilea pumila*; (K) *Droguetia iners* subsp *urticoides*; (L) *Gonostegia hirta*.

737

738 **Figure S7.** Percentage of floating and germinated seeds versus days in distilled water.
739 The X axis represents the soaking time (days), the Y-axis represents the
740 floating/germination percentage of the seeds.

741

742 **Figure S8.** Seed X-ray images. (a) *Boehmeria nivea*. (b) *Boehmeria penduliflora*. (c)
743 *Dendrocnide basirotonda*. (d) *Laportea bulbifera*. (e) *Pellionia yunnanensis*. (f)
744 *Poikilospermum suaveolens*. (g) *Droguetia iners* subsp. *urticoides*. (h) *Gonostegia hirta*.
745 (i) *Pilea pumila*. (j) *Maoutia puya*. (k) *Nanocnide lobata*. (l) *Girardinia diversifolia* subsp.
746 *diversifolia*.

747

748 **Figure S9.** Achene surface comparing no immersion (A) with eight months' immersion
749 into 3.5% NaCl solution (B), for the following species: **(1)** *Boehmeria penduliflora*, **(2)**
750 *Dendrocnide basirotunda*, **(3)** *Laportea bulbifera*, **(4)** *Parietaria micrantha*, **(5)** *Pilea*
751 *pumila*, **(6)** *Gonostegia hirta*, **(7)** *Droguetia iners* subsp. *urticoides*, **(8)** *Debregeasia*
752 *longifolia*, **(9)** *Maoutia puya*, **(10)** *Urtica dioica* subsp. *dioica*.

Table 1. Summary of all LDD events detected based on Lagrange analysis, incorporating ancestral states of four characters for correlative nodes.

Taxa/Clades	Geographic Disjunction (Lagrange)	Age of disjunction (Crown age) (Lagrange)	Sexual system	Epiphyticlife style	Habitat wetness	Habitat disturbance
3F	A→B	40.8 (30.9-57.1)-30.0 (20.3-41.4)	diecious	non-epiphytic	dry	semi-disturbed
<i>Discocnide mexicana_167A+Discocnide mexicana_Di6</i>	A→G	27.2 (14.7-40.9)-5.7(5.2-19.5)	diecious	non-epiphytic	dry	semi-disturbed
<i>Dendrocnode excelsa_D10</i>	A→E	4.5 (0.6-9.1)-present	diecious	non-epiphytic	wet	semi-disturbed
<i>Dendrocnode sinuata_D1</i>	A→D	0.2 (0-0.6)-present	diecious	non-epiphytic	dry	semi-disturbed
<i>Dendrocnode meyniana_D2</i>	A→D	0.2 (0-0.6)-present	diecious	non-epiphytic	wet	semi-disturbed
Clade III <i>Dendrocnode_sp_W1</i>	A→D	8.6 (3.3-14.5)-present	diecious	non-epiphytic	dry	semi-disturbed
<i>Urtica ferox_Q10</i>	A→E	15.8 (10.9-21.5)-present	diecious	non-epiphytic	dry	semi-disturbed
<i>Urtica pilulifera_Q20</i>	A→B	26.2 (18.7-33.8)-present	monecious	non-epiphytic	dry	completely disturbed
<i>U. sp_U18</i>	A→B	5.7 (2.3-9.5)-present	diecious	non-epiphytic	dry	semi-disturbed
<i>Urtica urens_U25+U. urens_U27+U. sp_U19</i>	A→E	5.5 (2.5-9.1)-2 (0.5-3.9)	diecious	non-epiphytic	dry	completely disturbed
<i>Urtica mollis_Q16+U. aquatica_Q6</i>	A→G	4.1 (1.8-6.8)-1.5 (0.3-3.1)	diecious	non-epiphytic	wet	semi-disturbed

<i>Urtica andicola_Q5+U. leptophylla_Q13+U. macbridei_Q14+U. magellanica_Q15+U. peruviana_Q19_U. magellanica_U33</i>	A→G	7.0 (6.5-13.6)-6.4 (4.3-9.1)	diecious	non-epiphytic	wet	semi-disturbed
<i>Urtica echinata_U34</i>	A→G	2.4 (0.9-3.8)--present	diecious	non-epiphytic	wet	semi-disturbed
<i>Urtica dioica subsp. dioica_J10+U21</i>	A→B&F	2.4 (0.9-3.8)-2.0 (0.7-3.3)	diecious	non-epiphytic	wet	completely disturbed
<i>Urtica sp_U21</i>	A→G	1.0 (0.2-2.0)-present	monecious	non-epiphytic	dry	semi-disturbed
<i>Urtica trichantha_Q24+Urtica echinata_Q9</i>	A→G	8.6 (5.7-12.0) -6.7 (4.3-9.1)	diecious	non-epiphytic	wet	semi-disturbed
<i>Hesperocnide tenella_331A</i>	A→F	7.0 (4.3-9.1)-present	monecious	non-epiphytic	wet	semi-disturbed
<i>Utica_angustifolia_U1</i>	A→D	3.2 (2.8-7.1)-present	diecious	non-epiphytic	wet	semi-disturbed
<i>Touchardia latifolia_T1+Urera (Ur1+Ur2+Ur18+B30)</i>	B→H	12.8 (9.8-24.0)-10. (5.1-15.6)	diecious	non-epiphytic	dry	semi-disturbed
<i>Laportea aestuans_L31+L30</i>	B→A& C& D& G	12.8-0.6 (0.03-1.46)	monecious	non-epiphytic	wet	semi-disturbed
<i>Laportea bulbifera_L5</i>	A→D	3.4 (0.7-7.0)-present	monecious	non-epiphytic	dry	completely disturbed
<i>Laportea mooreana_L12</i>	A→B	3.4 (0.7-7.0)-present	diecious	non-epiphytic	wet	semi-disturbed
<i>Laportea alatipes_L10</i>	A→B	10.0 (3.2-18.2)-present	diecious	non-epiphytic	dry	semi-disturbed
<i>Urera nitida_Ur26+Urera sp._Ur25+Urera baccifera_Ur21+Urera baccifera_C4A</i>	B→G	17.3 (11.1-25.6)-6.7 (3.0-10.9)	diecious	non-epiphytic	dry	semi-disturbed
<i>Poikilospermum suaveolens_Pi3+Poikilospermum lanceolatum_Pi1+Poikilospermum lanceolatum_Poi8</i>	B→A	17.3-4.1 (1.0-8.6)	diecious	non-epiphytic	dry	semi-disturbed
<i>Poikilospermum suaveolens_Pi3</i>	A→D	0.5 (0.04-1.2)-present	diecious	non-epiphytic	dry	semi-disturbed

Urera alceifolia_C11A+Urera

lianooides_313A+Poikilospermum tangaum_Poi5+Urera caracasana_21+Urera caracasana_23561 B→G 18.7 (11.1-27.6)-8.2 (3.5-13.4) dieocious non-epiphytic dry semi-disturbed

Poikilospermum tangaum_Poi5 G→D 4.7 (1.9-8.0)-present dieocious non-epiphytic dry semi-disturbed

Obetia pinnatifida_Ob1 B→C 2.7 (0.5-5.6)-present dieocious non-epiphytic dry semi-disturbed

Obetia radula_Ob2 B→C 4.6 (1.4-8.3)-present dieocious non-epiphytic dry semi-disturbed

Girardinia diversifolia_subsp_diversifolia_G9 A→B& C& D 14.0 (4.8-25.0)-present monocious non-epiphytic wet completely disturbed

Girardinia diversifolia_subsp_triloba_G19+Girardinia diversifolia_subsp_suborbiculate_G17 A→B& C& D 14.0 (4.8-25.0)-4.5 (1.0-9.0) monocious non-epiphytic wet completely disturbed

2A A→G 51.6-28.8 (16.7-42.8) dieocious non-epiphytic dry semi-disturbed

Pilea tetraphylla_P90 A→B&C 16.3 (6.7-27.0)-present dieocious non-epiphytic dry semi-disturbed

Pilea (P101+P100)+Sarcopilea domingensis_302A A→G 18.2 (11.1-25.8)-14.5 (7.8-21.2) dieocious non-epiphytic wet semi-disturbed

Pilea bemarivensis_P91+P92 A→C 18.2 (11.1-25.8)-1.6 (0.3-3.5) dieocious non-epiphytic dry semi-disturbed

Clade II

Pilea microphylla_P100+P101 G→A& B& C& D& E& F 14.5 (7.9-21.2)-5.9 (1.8-11.1) monocious non-epiphytic wet completely disturbed

Pilea peploides_var_major_P62 A→D 4.1 (1.0-8.4)-present monocious non-epiphytic wet semi-disturbed

Pilea melastomoides_P20 A→D 4.9 (2.3-7.8)-present monocious non-epiphytic wet semi-disturbed

Pilea pumila_P64 A→F 24.5 (17.3-32.5)-present monocious non-epiphytic dry semi-disturbed

	<i>Procris (Pr2+Pr9+Pr10)</i>	A→D	25.5 (13.7-37.5)-13.6 (5.7-22.6)	monecious	epiphytic	wet	semi-disturbed
	<i>Procris_pedunculata_var_ornata_Pr10</i>	D→E	8.9 (2.8-16.1)-present	monecious	epiphytic	wet	semi-disturbed
	<i>Procris_crenata_Pr2</i>	D→A&B	13.6 (5.7-22.6)-present	monecious	epiphytic	wet	semi-disturbed
	<i>Lecanthus_peduncularis_Le1</i>	A→B&D	9.8 (2.5-18.9)-present	monecious	non-epiphytic	wet	semi-disturbed
	<i>1E</i>	A→G	40.5 (31.4-50.6)-27.1 (14.7-39.7)	monecious	non-epiphytic	wet	semi-disturbed
	<i>Boehmeria</i> (<i>B34+B37+B73+B36+B20+B39+B53+B88+B9+B47+B46+B24+B72+B47+B26+B1+B33+B16+B21+B40</i>)	G→A	17.8 (12.6-23.6)-12.3 (8.3-16.6)	monecious	non-epiphytic	wet	semi-disturbed
	<i>Boehmeria sp._Cy35</i>	A→G	2.0 (0.3-4.5)-present	monecious	non-epiphytic	dry	semi-disturbed
	<i>Boehmeria_densiflora_B53</i>	A→D	6.5 (4.0-9.3)-present	monecious	non-epiphytic	wet	completely disturbed
Clade I	<i>Boehmeria_macrophylla_var_macrophylla_B24</i>	A→D	5.9 (1.6-10.8)-present	monecious	non-epiphytic	wet	semi-disturbed
	<i>Boehmeria_macrophylla_var_scabrella_B26</i>	A→D	5.2 (2.5-8.3)-present	monecious	non-epiphytic	wet	semi-disturbed
	<i>Boehmeria_zollingeriana_var_blinii_B1</i>	A→D	4.6 (1.2-8.2)-present	monecious	non-epiphytic	wet	semi-disturbed
	<i>Boehmeria_clidemioides_var_diffusa_B16</i>	A→D	2.6 (0.7-4.9)-present	diecious	non-epiphytic	dry	completely disturbed
	<i>Boehmeria_cylindrica_20</i>	G→F	6.8 (2.1-12.0)-present	diecious	non-epiphytic	dry	semi-disturbed
	<i>Boehmeria_glomerulifera_B5</i>	A→D	2.0 (0.3-4.5)-present	monecious	non-epiphytic	dry	semi-disturbed

<i>Boehmeria nivea</i> _var_ <i>nivea</i> _B6+ <i>Boehmeria nivea</i> _var_ <i>tenacissima</i> _B32	A→B& C&D& E	1.26 (0.36-2.35)-0.5 (0.04-1.1)	monecious	non-epiphytic	wet	completely disturbed
<i>Sarcochlamys pulcherrima</i> _S1	A→D	1.3 (0.4-2.4)-present	diecious	non-epiphytic	dry	semi-disturbed
<i>Cypholophus</i>	G→D	13.2 (8.6-18.5)-9.9 (5.6-14.5)	monecious	non-epiphytic	dry	semi-disturbed
<i>Astrothalamus reticulatus</i> (As2+23592)	A→D	11.1 (5.5-17.5)-4.5 (1.4-8.0)	monecious	non-epiphytic	wet	semi-disturbed
<i>Debregeasia saeneb</i> _De17+De25	A→B	4.5 (2.1-7.2)-1.7 (0.4-3.5)	diecious	non-epiphytic	wet	completely disturbed
<i>Debregeasia longifolia</i> _De10	A→D	1.7 (0.3-3.5)-present	diecious	non-epiphytic	wet	completely disturbed
<i>Debregeasia squamata</i> _De5	A→D	3.1 (1.2-5.5)-present	monecious	non-epiphytic	wet	completely disturbed
<i>Neraudia</i> +its sistergroups	A→H	14.8 (9.0-21.2)-12.9 (7.9-18.2)	diecious	non-epiphytic	wet	semi-disturbed
<i>Pouzolzia poeppigiana</i> _Po10	H→G	9.0 (4.3-14.1)-present	monecious	non-epiphytic	dry	semi-disturbed
<i>Pouzolzia sanguinea</i> _var_ <i>sanguinea</i> _Po6+Po2	A→D	8.9 (4.0-14.1)-5.1 (1.4-9.4)	diecious	non-epiphytic	dry	semi-disturbed
<i>Pouzolzia zeylanica</i> _var_ <i>zeylanica</i> _Po7	A→B& D& E& F& G	4.1 (1.3-7.6)-present	diecious	non-epiphytic	wet	completely disturbed
<i>Gonostegia parvifolia</i> _Go1	A→D	4.2 (1.1-8.2)-present	monecious	non-epiphytic	dry	semi-disturbed
<i>Gonostegia hirta</i> _Go3	A→D& E	4.2 (1.1-8.2)-present	monecious	non-epiphytic	dry	completely disturbed
<i>Pouzolzia elegans</i> _var_ <i>elegans</i> _Po11	D→A	1.0 (0.1-2.7)-present	monecious	non-epiphytic	dry	semi-disturbed
<i>Pipturus arborescens</i> _Pip1+Pip7	D→A	1.0 (0.05-2.66)-0.4 (0.02-1.12)	diecious	non-epiphytic	dry	semi-disturbed

<i>Pouzolzia elegans</i> _var_ <i>elegans</i> _Po11+ <i>Pipturus</i> (<i>Pip1</i> + <i>Pip7</i> + <i>Pip10</i>)	H→D	4.8-2.9 (1.0-5.2)	monecious	non-epiphytic	dry	semi-disturbed
<i>Hemistylus macrostachya</i> _23597+ <i>Rousselia</i> <i>humulis</i> _23596	B→G	10.6 (5.2-16.0)-2.5 (0.2-5.7)	monecious	non-epiphytic	dry	semi-disturbed
<i>Hemistylus macrostachya</i> _23597+ <i>Rousselia</i> <i>humulis</i> _23596+ <i>Neodistemon indicum</i> (279A+Ne6)+ <i>Pouzolzia maxta</i> _288A	A→B	15.3 (9.7-21.2)-12.7 (7.3-18.2)	monecious	non-epiphytic	dry	semi-disturbed
<i>Neodistemon indicum</i> (279A+Ne6)	B→D	10.6 (5.2-16.0)-1.7 (0-4.9)	monecious	non-epiphytic	wet	semi-disturbed
<i>Pouzolzia maxta</i> _288A	B→C	12.7 (7.3-18.2)-present	monecious	non-epiphytic	dry	semi-disturbed
<i>Pouzolzia guineensis</i> _282A	A→B	12.3 (6.7-18.2)-present	monecious	non-epiphytic	dry	semi-disturbed
<i>Oreocnide trinervis</i> _O33	A→D	2.3-present	diecious	non-epiphytic	dry	undisturbed
<i>Oreocnide frutescens</i> _subsp_ <i>frutescens</i> _O2	A→D	1.8 (0.2-3.9)-present	diecious	non-epiphytic	dry	undisturbed
<i>Oreocnide rubescens</i> _O15	A→D	10.6 (4.9-17.4)-present	diecious	non-epiphytic	dry	undisturbed
<i>Didymodoxa caffra</i> _23599+its sister groups	A→B	30.7 (20.6-41.1) -24.3 (14.5-33.9)	monecious	non-epiphytic	dry	semi-disturbed
<i>Parietaria judaica</i> _11077+J7	A→B	10.4 (4.5-17.1)-2.4 (0.4-5.1)	monecious	non-epiphytic	dry	completely disturbed
<i>Forsskaolea angustifolia</i> _6515	A→B	17.1 (6.6-27.3)-present	monecious	non-epiphytic	dry	semi-disturbed
<i>Forsskaolea tenacissima</i> _F5	A→B	17.1 (6.6-27.3)-present	monecious	non-epiphytic	dry	semi-disturbed

	<i>Parietaria micrantha_Pa1</i>	A→B& E& G	(5.4-24.6) 14.7-present	monecious	non-epiphytic	dry	completely disturbed
	<i>Parietaria debilis_Pa5</i>	A→E& G	(5.4-24.6) 14.7-present	monecious	non-epiphytic	dry	completely disturbed
	<i>Drouguetia iners_subsp_urticoides_Dr1</i>	B→A& D	9.0 (4.0-14.5)-present	monecious	non-epiphytic	dry	semi-disturbed
	Clade 4A	A→G	44.1 (27.3-60.1)-19.6 (11.0-29.7)	diecious	non-epiphytic	dry	semi-disturbed
	<i>Musanga</i>	G→B	7.1-1.4 (0-4.3)	diecious	non-epiphytic	dry	semi-disturbed
	<i>Myrianthus</i>	G→B	5.7-present	diecious	non-epiphytic	dry	semi-disturbed
Clade IV	<i>Gibbsia insignis_Gi1</i>	A→D	10.4 (3.4-19.2)-present	monecious	non-epiphytic	dry	semi-disturbed
	<i>Maoutia setosa_M4+M2</i>	A→D	4.0 (0.7-8.8)-0.7 (0-2.2)	monecious	non-epiphytic	dry	semi-disturbed
	<i>Leucosyke quadrinervia_Leu4</i>	A→D	28.3 (14.7-43.0)-present	diecious	non-epiphytic	dry	semi-disturbed

#Note: Dates for LDD events are presented in the form (W-) X - Y (-Z), where X and Y are the nodes before and after the branch on which the event occurred, and W and Z are the older and younger HPD limits for X and Y respectively. Certain nodes lack HPD values due to weak phylogenetic support; age ranges for LDD events bounded by such nodes are given in the form X – Y, and stated to be approximate.







