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

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Abstract

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

(a) Introduction

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

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

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

other explanations for disjunctions, leading to increasing acceptance of the role of LDD

in shaping the present distributions of organisms (Raxworthy *et al.* 2002; Pyron 2014;

Rota *et al.* 2016; Carlton *et al.* 2017; Luebert *et al.* 2017). LDD events are now

universally accepted as a major factor shaping global biogeography, including large-

scale processes such as population spread, biodiversity evolution of oceanic islands,

and colonization of unoccupied habitats (Levin *et al.* 2003; Trakhtenbrot *et al.* 2005;

Cowie & Holland 2006; Gillespie *et al.* 2012).

Regarding mechanism, oceanic LDD has been reevaluated and is now

hypothesized as the main cause of disjunct distributions in many groups (Samonds *et al.* 2012; Wee *et al.* 2014; Correia *et al.* 2018). Nonetheless, it remains difficult to

demonstrate experimentally or from observations how LDD occurred in any particular

case, largely because of the rarity and presumed unpredictability of such events

(Gillespie et al. 2012). Therefore, it is important that LDD hypotheses should be

testable using independent lines of evidence (Crisp et al. 2011).

Ocean currents have repeatedly been shown to be very important to LDD (Harwell

& Orth 2002; Thiel & Haye 2006; Geng *et al.* 2008; McMahon *et al.* 2014; Smith *et al.*

2018). However, most such studies concern relatively recent (post-Pliocene) LDD

events, focus on littoral species that often have specific adaptations to seawater

dispersal, concern groups with a relatively limited geological range, and/or rely either

on phylogeography or anecdotal observations alone (Table S1). Where widely

distributed inland groups have been examined, phylogenetic reconstruction has

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 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   *rpll4-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).

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

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

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

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

Phylogenetic signal test - a) To test whether presence/absence of LDD events are distributed randomly across the phylogeny, we used the *phylo.d* function in the caper package in R (Fritz & Purvis 2010). The value of D can be both smaller than 0 (highly 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).

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

16

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

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

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

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

Estimating the time required for LDD using ocean circulation modeling

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

17

Table S9), three of the eight species analyzed could have reached the destination

landmass via seawater within six months, with *Debregeasia longifolia* needing the least

time, i.e. four months to reach the Philippines from Vietnam. *Droguetia inners* subsp.

urticoides would need 12 months to reach Tanzania from Java, whereas *Parietaria*

micrantha would need 22 months to float directly from China to Australia, but there are

ample islands in between, so the journey could have been made in stages (Fig. 4). It

would take the MRCA of *Neraudia*+sistergroups ca. 48 months to reach Hawaii from

Japan or thereabouts.

(d) Discussion

Major disjunctions in Urticaceae and identifications of oceanic dispersal

Urticaceae began to diversify in Eurasia around the Late Cretaceous (68.7 Ma),

with diversification perhaps accelerating around 45 Ma (Appendix S6). Remarkably, at

least 92 LDD events were indicated, mostly out of Eurasia and most often to the

adjacent regions of Africa and especially SE Asia. Incorporating paleogeographical

evidence, at least 76 must have crossed at least one ocean (Appendix S6).

Potential for seed transoceanic LDD in Urticaceae

Migratory birds can be a major mechanism for transoceanic LDD (van der Pijl 1972;

Heleno & Vargas 2015). Although most Urticaceae fruits are not edible, certain species'

achenes (e.g. *Debregeasia longifolia*) are ingested by some small birds (Zeng-Yuan Wu,

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 that water, with air-filled cavities, hairs and wings all sometimes contributing to

343 bouyancy. 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 bouyancy

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

22

occasionally self via an occasional female flower on a male plant, or *vice versa* (Vega-

Frutis *et al.* 2014; Varga & Kytöviita 2016). Moreover, monoecy arose at least 84 times

during Urticaceae evolution (Fig. S4), often associated with transitions of habit and

disturbance, suggesting an ability to switch breeding strategy when circumstances

select for it. Both might be triggered by founder effects, aiding establishment.

Otherwise, two seeds are needed. In a habitat that undergoes regular disturbance and

inundation involving seawater, including tidal inland riversides, a second seed might

arrive within the lifespan of the original plant. Alternately, multiple seeds might arrive

and germinate simultaneously following a catastrophic event (Fig. 5, N, Q). Otherwise,

scraps of plant washed into the sea ("vegetable rubbish"; Darwin, 1856) might float

across the ocean, with multiple viable seeds contained in fruiting bodies (Fig. 5, E).

Nonetheless, for Urticaceae, establishment represents a significant obstacle to

successful LDD, probably second only to sea crossing itself. Much more data are

needed, especially on SI, in order to determine the most likely way that this obstacle

was overcome.

Furthermore, we inferred that the LDD events and all four tested traits were

phylogenetically conserved, therefore, LDD events in Urticaceae were strongly

associated with the evolution of conserved traits. The traits we assessed might

adequately represent LDD-linked traits, but there are almost certainly other traits we

did not include in our analyses that could also be informative regarding LDD events.

Of course any traits associated with LDD would not have actually been selected to

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.

24

(e) Acknowledgements

(f) References

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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

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

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

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

Figure 5. Plausible mechanisms for long-distance dispersal across an ocean barrier in Urticaceae. Colours indicate start (yellow), middle (orange) and end (red) of a journey via water. **A-D** = means of seeds reaching the sea: **A,** seeds or scrap of plant enter sea 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

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

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

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

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

agarose substrate following 150 days' immersion in 3.5% NaCl solution. **(J-K)** Seed

germination of *Pilea* (J) and *Maoutia* (K) two weeks after being transferred to 1% water

agarose substrate following 150 days' immersion in 1% NaCl solution. **(L)** Seed

germination of *Parietaria* two weeks after being transferred to 1% water agarose

substrate following 150 days' immersion in 5% NaCl solution.

Figure S6. Photos of seeds of 12 species, following seed coat removal for Tetrazolium

tests. **(A-B)** living (red) and dead (white) seeds of *Dendrocnide basirotunda* (A) and

Maoutia puya (B). For all others, only live seed is shown: **(C)** *Elatostema stewardii*;

(D) *Debregeasia longifolia*; **(E)** *Laportea bulbifera*; **(F)** *Urtica dioica* subsp; *dioica*;

(G) *Parietaria micrantha*; **(H)** *Poikilospermum suaveolens,* **(I)** *Boehmeria penduliflora*;

(J) *Pilea pumila*; **(K)** *Droguetia iners* subsp *urticoides*; **(L)** *Gonostegia hirta*.

Figure S7. Percentage of floating and germinated seeds versus days in distilled water.

The X axis represents the soaking time (days), the Y-axis represents the

floating/germination percentage of the seeds.

Figure S8. Seed X-ray images. **(a)** *Boehmeria nivea*. **(b)** *Boehmeria penduliflora*. **(c)**

Dendrocnide basirotunda. **(d)** *Laportea bulbifera.* **(e)** *Pellionia yunnanensis.* **(f)**

Poikilospermum suaveolens. **(g)** *Droguetia iners* subsp. *urticoides*. **(h)** *Gonostegia hirta.*

(i) *Pilea pumila*. **(j)** *Maoutia puya*. **(k)** *Nanocnide lobata*. **(l)** *Girardinia diversifolia* subsp.

diversifolia.

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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
	Discocnide mexicana_167A+Discocnide mexicana_Di6	A→G	27.2 (14.7-40.9)-5.7(5.2-19.5)	diecious	non-epiphytic	dry	semi-disturbed
	Dendrocnide excelsa_D10	A→E	4.5 (0.6-9.1)-present	diecious	non-epiphytic	wet	semi-disturbed
	Dendrocnide_sinuata_D1	A→D	0.2 (0-0.6)-present	diecious	non-epiphytic	dry	semi-disturbed
	Dendrocnide_meyniana_D2	A→D	0.2 (0-0.6)-present	diecious	non-epiphytic	wet	semi-disturbed
Clade III	*Dendrocnide_sp_W1*	A→D	8.6 (3.3-14.5)-present	diecious	non-epiphytic	dry	semi-disturbed
	Urtica ferox_Q10	A→E	15.8 (10.9-21.5)-present	diecious	non-epiphytic	dry	semi-disturbed
	Urtica_pilulifera_Q20	A→B	26.2 (18.7-33.8)-present	monecious	non-epiphytic	dry	completely disturbed
	U. sp_U18	A→B	5.7 (2.3-9.5)-present	diecious	non-epiphytic	dry	semi-disturbed
	Urtica urens_U25+U. urens_U27+U. sp_U19	A→E	5.5 (2.5-9.1)-2 (0.5-3.9)	diecious	non-epiphytic	dry	completely disturbed
	Urtica mollis_Q16+U. aquatica_Q6	A→G	4.1 (1.8-6.8)-1.5 (0.3-3.1)	diecious	non-epiphytic	wet	semi-disturbed

Urtica andicola_Q5+U. leptophylla_Q13+U. macbridei_Q14+U. magellanica_Q15+U. peruviana_Q19_U. magellanica_U33	A→G	7.0 (6.5-13.6)-6.4 (4.3-9.1)	diecious	non-epiphytic	wet	semi-disturbed
Urtica echinata_U34	A→G	2.4 (0.9-3.8)--present	diecious	non-epiphytic	wet	semi-disturbed
Urtica dioica subsp. dioica_J10+U21	A→B&F	2.4 (0.9-3.8)-2.0 (0.7-3.3)	diecious	non-epiphytic	wet	completely disturbed
Urtica sp_U21	A→G	1.0 (0.2-2.0)-present	monecious	non-epiphytic	dry	semi-disturbed
Urtica trichantha_Q24+Urtica echinata_Q9	A→G	8.6 (5.7-12.0) -6.7 (4.3-9.1)	diecious	non-epiphytic	wet	semi-disturbed
Hesperocnide tenella_331A	A→F	7.0 (4.3-9.1)-present	monecious	non-epiphytic	wet	semi-disturbed
Utica_angustifolia_U1	A→D	3.2 (2.8-7.1)-present	diecious	non-epiphytic	wet	semi-disturbed
Touchardia latifolia_T1+Urera (Ur1+Ur2+Ur18+B30)	B→H	12.8 (9.8-24.0)-10. (5.1-15.6)	diecious	non-epiphytic	dry	semi-disturbed
Laportea_aestuans_L31+L30	B→A& C& D& G	12.8-0.6 (0.03-1.46)	monecious	non-epiphytic	wet	semi-disturbed
Laportea_bulbifera_L5	A→D	3.4 (0.7-7.0)-present	monecious	non-epiphytic	dry	completely disturbed
Laportea_mooreana_L12	A→B	3.4 (0.7-7.0)-present	diecious	non-epiphytic	wet	semi-disturbed
Laportea_alatipes_L10	A→B	10.0 (3.2-18.2)-present	diecious	non-epiphytic	dry	semi-disturbed
Urera nitida_Ur26+Urera sp._Ur25+Urera baccifera_Ur21+Urera baccifera_C4A	B→G	17.3 (11.1-25.6)-6.7 (3.0-10.9)	diecious	non-epiphytic	dry	semi-disturbed
Poikilospermum suaveolens_Pi3+Poikilospermum lanceolatum_Pi1+Poikilospermum_lanceolatum_Poi8	B→A	17.3-4.1 (1.0-8.6)	diecious	non-epiphytic	dry	semi-disturbed
Poikilospermum suaveolens_Pi3	A→D	0.5 (0.04-1.2)-present	diecious	non-epiphytic	dry	semi-disturbed

	Taxon	Transition	Value	Breeding	Epiphytic	Moisture	Disturbance
	Urera alceifolia_C11A+Urera lianoides_313A+Poikilospermum tangaum_Poi5+Urera caracasana_21+Urera caracasana_23561	B→G	18.7 (11.1-27.6)-8.2 (3.5-13.4)	diecious	non-epiphytic	dry	semi-disturbed
	Poikilospermum tangaum_Poi5	G→D	4.7 (1.9-8.0)-present	diecious	non-epiphytic	dry	semi-disturbed
	Obetia_pinnatifida_Ob1	B→C	2.7 (0.5-5.6)-present	diecious	non-epiphytic	dry	semi-disturbed
	Obetia_radula_Ob2	B→C	4.6 (1.4-8.3)-present	diecious	non-epiphytic	dry	semi-disturbed
	Girardinia_diversifolia_subsp_diversifolia_G9	A→B& C& D	14.0 (4.8-25.0)-present	monecious	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)	monecious	non-epiphytic	wet	completely disturbed
	2A	A→G	51.6-28.8 (16.7-42.8)	diecious	non-epiphytic	dry	semi-disturbed
	Pilea tetraphylla_P90	A→B&C	16.3 (6.7-27.0)-present	diecious	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)	diecious	non-epiphytic	wet	semi-disturbed
	Pilea bemarivensis_P91+P92	A→C	18.2 (11.1-25.8)-1.6 (0.3-3.5)	diecious	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)	monecious	non-epiphytic	wet	completely disturbed
	Pilea_peploides_var_major_P62	A→D	4.1 (1.0-8.4)-present	monecious	non-epiphytic	wet	semi-disturbed
	Pilea_melastomoides_P20	A→D	4.9 (2.3-7.8)-present	monecious	non-epiphytic	wet	semi-disturbed
	Pilea_pumila_P64	A→F	24.5 (17.3-32.5)-present	monecious	non-epiphytic	dry	semi-disturbed

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

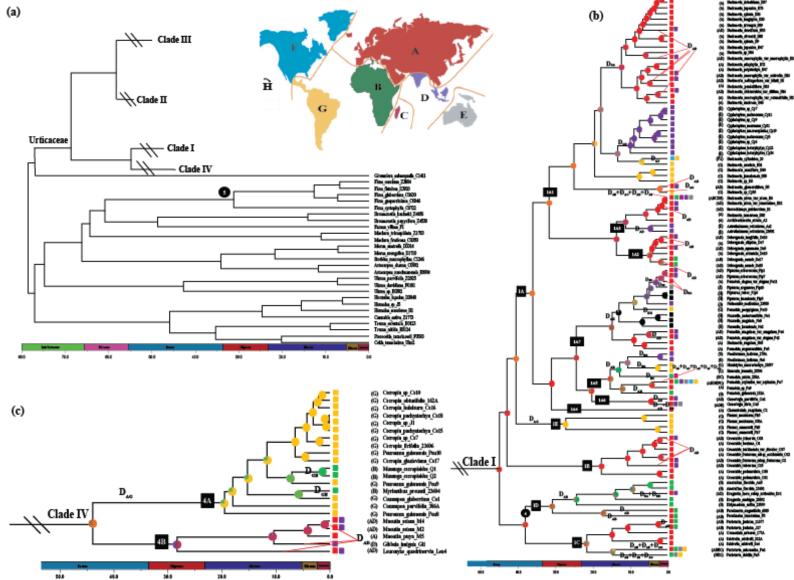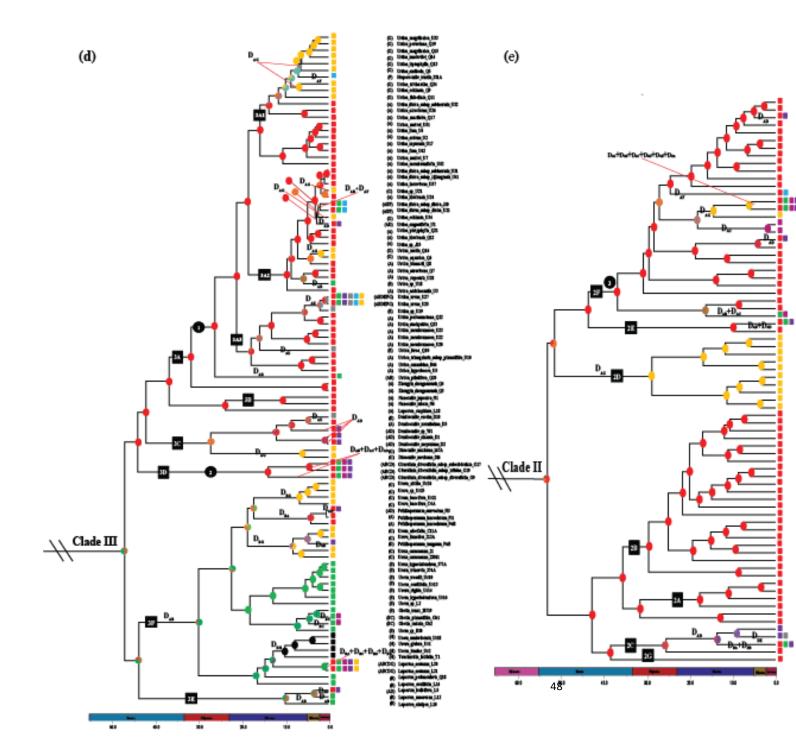| Species	Transition	Value	Sexual system	Epiphytism	Habitat	Disturbance
Boehmeria_nivea_var_nivea_B6+Boehmeria_nivea_var_tenacissima_B32	A→B& C&D& E	1.26 (0.36-2.35)-0.5 (0.04-1.1)	monecious	non-epiphytic	wet	completely disturbed
Sarcochlamys_pulcherrima_S1	A→D	1.3 (0.4-2.4)-present	diecious	non-epiphytic	dry	semi-disturbed
Cypholophus	G→D	13.2 (8.6-18.5)-9.9 (5.6-14.5)	monecious	non-epiphytic	dry	semi-disturbed
Astrothalamus reticulatus (As2+23592)	A→D	11.1 (5.5-17.5)-4.5 (1.4-8.0)	monecious	non-epiphytic	wet	semi-disturbed
Debregeasia saeneb_De17+De25	A→B	4.5 (2.1-7.2)-1.7 (0.4-3.5)	diecious	non-epiphytic	wet	completely disturbed
Debregeasia_longifolia_De10	A→D	1.7 (0.3-3.5)-present	diecious	non-epiphytic	wet	completely disturbed
Debregeasia_squamata_De5	A→D	3.1 (1.2-5.5)-present	monecious	non-epiphytic	wet	completely disturbed
Neraudia+its sistergroups	A→H	14.8 (9.0-21.2)-12.9 (7.9-18.2)	diecious	non-epiphytic	wet	semi-disturbed
Pouzolzia_poeppigiana_Po10	H→G	9.0 (4.3-14.1)-present	monecious	non-epiphytic	dry	semi-disturbed
Pouzolzia_sanguinea_var_sanguinea_Po6+Po2	A→D	8.9 (4.0-14.1)-5.1 (1.4-9.4)	diecious	non-epiphytic	dry	semi-disturbed
Pouzolzia_zeylanica_var_zeylanica_Po7	A→B& D& E& F& G	4.1 (1.3-7.6)-present	diecious	non-epiphytic	wet	completely disturbed
Gonostegia_parvifolia_Go1	A→D	4.2 (1.1-8.2)-present	monecious	non-epiphytic	dry	semi-disturbed
Gonostegia_hirta_Go3	A→D& E	4.2 (1.1-8.2)-present	monecious	non-epiphytic	dry	completely disturbed
Pouzolzia_elegans_var_elegans_Po11	D→A	1.0 (0.1-2.7)-present	monecious	non-epiphytic	dry	semi-disturbed
Pipturus arborescens_Pip1+Pip7	D→A	1.0 (0.05-2.66)-0.4 (0.02-1.12)	diecious	non-epiphytic	dry	semi-disturbed

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Pouzolzia_elegans_var_elegans_Po11+Pipturus (Pip1+Pip7+Pip10)	H→D	4.8-2.9 (1.0-5.2)	monecious	non-epiphytic	dry	semi-disturbed
Hemistylus macrostachya_23597+Rousselia humulis_23596	B→G	10.6 (5.2-16.0)-2.5 (0.2-5.7)	monecious	non-epiphytic	dry	semi-disturbed
Hemistylus macrostachya_23597+Rousselia humulis_23596+Neodistemon indicum (279A+Ne6)+Pouzolzia maxta_288A	A→B	15.3 (9.7-21.2)-12.7 (7.3-18.2)	monecious	non-epiphytic	dry	semi-disturbed
Neodistemon indicum (279A+Ne6)	B→D	10.6 (5.2-16.0)-1.7 (0-4.9)	monecious	non-epiphytic	wet	semi-disturbed
Pouzolzia maxta_288A	B→C	12.7 (7.3-18.2)-present	monecious	non-epiphytic	dry	semi-disturbed
Pouzolzia guineensis_282A	A→B	12.3 (6.7-18.2)-present	monecious	non-epiphytic	dry	semi-disturbed
Oreocnide_trinervis_O33	A→D	2.3-present	diecious	non-epiphytic	dry	undisturbed
Oreocnide_frutescens_subsp_frutescens_O2	A→D	1.8 (0.2-3.9)-present	diecious	non-epiphytic	dry	undisturbed
Oreocnide_rubescens_O15	A→D	10.6 (4.9-17.4)-present	diecious	non-epiphytic	dry	undisturbed
Didymodoxa caffra_23599+its sister groups	A→B	30.7 (20.6-41.1) -24.3 (14.5-33.9)	monecious	non-epiphytic	dry	semi-disturbed
Parietaria_judaica_11077+J7	A→B	10.4 (4.5-17.1)-2.4 (0.4-5.1)	monecious	non-epiphytic	dry	completely disturbed
Forsskaolea angustifolia_6515	A→B	17.1 (6.6-27.3)-present	monecious	non-epiphytic	dry	semi-disturbed
Forsskaolea tenacissima_F5	A→B	17.1 (6.6-27.3)-present	monecious	non-epiphytic	dry	semi-disturbed

	Parietaria micrantha_Pa1	A→B& E& G	(5.4-24.6) 14.7-present	monecious	non-epiphytic	dry	completely disturbed
	Parietaria debilis_Pa5	A→E& G	(5.4-24.6) 14.7-present	monecious	non-epiphytic	dry	completely disturbed
	Drouguetia_iners_subsp_urticoides_Dr1	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
	Musanga	G→B	7.1-1.4 (0-4.3)	diecious	non-epiphytic	dry	semi-disturbed
	Myrianthus	G→B	5.7-present	diecious	non-epiphytic	dry	semi-disturbed
Clade IV	*Gibbsia insignis_Gi1*	A→D	10.4 (3.4-19.2)-present	monecious	non-epiphytic	dry	semi-disturbed
	Maoutia_setosa_M4+M2	A→D	4.0 (0.7-8.8)-0.7 (0-2.2)	monecious	non-epiphytic	dry	semi-disturbed
	Leucosyke_quadrinervia_Leu4	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.

Seed viability percentage (VP) (%)

Seed soaking time (days)

(a) *Debregeasia longifolia*
(b) *Boehmeria penduliflora*
(c) *Droguetia iners* subsp. *urticoides*
(d) *Parietaria micrantha*
(e) *Pilea pumila*
(f) *Elatostema stewardii*
(g) *Gonostegia hirta*
(h) *Poikilospermum suaveolens*
(i) *Laportea bulbifera*
(j) *Dendrocnide basirotunda*
(k) *Maoutia puya*
(l) *Urtica dioica* subsp. *dioica*

Saltwater
concentrations

— 1.0 %
— 3.5 %
— 5.0 %
— 8.0 %

Taxon
- *Dendrocnide basirotunda*
- *Elatostema stewardii*
- *Girardinia diversifolia* subsp. *diversifolia*
- *Gonostegia hirta*
- *Laportea bulbifera*
- *Lecanthus peduncularis*
- *Nanocnide lobata*

Taxon
- *Boehmeria nivea*
- *Boehmeria penduliflora*
- *Chamabainia cuspidata*
- *Debregeasia longifolia*
- *Droguetia iners* subsp. *urticoides*
- *Maoutia puya*
- *Pilea pumila*
- *Pouzolzia sanguinea*
- *Oreocnide integrifolia*
- *Parietaria micrantha*
- *Pellionia yunnanensis*
- *Poikilospermum suaveolens*
- *Procris crenata*
- *Urtica dioica* subsp. *dioica*