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Diversity and evolution of pollinator rewards and protection by *Macaranga* (Euphorbiaceae)

bracteoles

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- 38 Supplemental Figure 1. A female inflorescence of *Macaranga vedeliana*.

39 **Abstract**

40 Flowering plants have modified their floral organs in remarkably diverse ways to optimize
41 their interaction with pollinators. Although floral organs represent a major source of floral
42 diversity, many plants also use extrafloral organs, such as bracts and bracteoles, in interacting
43 with pollinators; however, the evolutionary dynamics of non-floral organs involved in
44 pollination are poorly studied. The genus *Macaranga* is characterized by protective
45 mutualisms with ants that potentially interfere with pollinators on flowers. *Macaranga*
46 flowers lack perianths and, notably, bracteoles serve the dual function of rewarding
47 pollinators and protecting them from guarding ants; in one group of species, bracteoles
48 provide a nectar reward to generalist pollinators, while in another group, bracteole
49 “chambers” protect thrips or hemipteran pollinators that use these structures as feeding and
50 breeding sites. We examined the diversity and evolutionary dynamics of inflorescence
51 morphology in *Macaranga*, focusing on bracteoles. We recognized three inflorescence types
52 based on examination of herbarium materials: Discoid-gland, which possess disc-shaped
53 glands on the bracteole surfaces (including all the generalist-pollinated species); Enclosing, in
54 which bracteoles cover flowers (including all the thrips- and hemipteran-pollinated species);
55 and Inconspicuous, in which bracteoles are small, narrow or absent. Ancestral state
56 reconstruction indicated that inflorescence morphologies have changed multiple times in the
57 genus. These findings suggest that morphological changes in non-floral characters
58 (bracteoles) of *Macaranga* species have occurred as frequently as in the floral structures of
59 many flowering plants. The multiple evolutions of the Enclosing bracteoles, which protect
60 pollinators, might have been facilitated by pollination interference from mutualistic ants.

61

62 **Introduction**

63 Flowering plants exhibit enormously diverse floral traits, many of which are useful for animal
64 pollination (Ollerton et al. 2011). For example, showy perianths and odors can efficiently
65 attract pollinators, and nectars, oils, resins, and other floral secretions can reward pollinators
66 (Fægri and van der Pijl 1979). Therefore, biologists have studied how flower visitors
67 influence the evolution of floral traits in various plant groups by combining phylogenetic
68 relationships among focal plants, and their floral traits and pollination systems (Johnson et al.
69 1998; Beardsley et al. 2003; Whittall and Hodges 2007; Wilson et al. 2007; Okuyama et al.
70 2008; Kawakita and Kato 2009; Sakai et al. 2013). Overall, evidence suggests that the same
71 pollination systems have evolved repeatedly among plant groups, altering floral traits and
72 confirming their flexibility.

73 Although both floral and extrafloral organs are used for attracting and/or rewarding
74 pollinators, floral organs such as petals, sepals, and nectaries within flowers play more
75 important roles in pollination than extrafloral organs in most flowering plants. However, in
76 some plant species, especially in those which have lost conspicuous petals or sepals,
77 extrafloral organs are more important for interactions with pollinators; in most of these cases,
78 bracts and bracteoles play major roles (Ehrenfeld 1979; Armbruster 1997; Wragg and
79 Johnson 2011; Bröderbauer et al. 2012). The roles of bracts or bracteoles have been well
80 described in the pollination systems of the Araceae, and in the *Euphorbia*, *Dalechampia*
81 (*Euphorbiaceae*), and some *Cyperus* (*Cyperaceae*) species (Ehrenfeld 1979; Armbruster
82 1997; Wragg and Johnson 2011; Bröderbauer et al. 2012), but it is unclear whether bracts or
83 bracteoles in these plants change as flexibly as floral organs, or are under similar
84 evolutionary constraints. Some studies have suggested frequent changes in pollination
85 systems without large morphological changes; Armbruster (1993) and Armbruster (1997)
86 reported frequent pollinator reward shifts between resin and pheromone precursors or pollen

87 in *Dalechampia*, while the structure of bracts, the primary organ used to attract pollinators,
88 remained consistent. In Araceae, evolution of pollinator-trapping chambers that enclose the
89 spadices has occurred at least 10 times from inflorescences in which bracts do not cover the
90 spadices completely. However, bract shape does not change drastically in many species in
91 which bracts surround the spadices and form a chamber around the flowers (Bröderbauer et al.
92 2012).

93 It has been said that pollinators play important roles in driving plant traits involved
94 in pollination (Whittal and Hodges 2007; van der Niet and Johnson 2012). However, flowers
95 are visited not only by pollinators but also by unfavorable animals, such as florivores,
96 worthless pollinators, or pollinator-excluding animals (primarily ants) (Willmer and Stone
97 1997; Irwin et al. 2004). These unfavorable visitors can also have a selective effect on
98 floral/inflorescence traits; for example, long corollas exclude insects with short mouthparts,
99 and pendulous flowers exclude all but hovering insects and those with strong legs (Proctor et
100 al. 2006).

101 In this study, we investigated the evolution of inflorescence morphologies in the
102 dioecious tree genus *Macaranga* (Euphorbiaceae), distributed in tropical to subtropical
103 regions (Whitmore 2008). Recent studies have reported two distinct bracteole morphologies
104 in the genus associated with different pollination systems. The first consists of paddle-shaped
105 bracteoles with one to several disc-shaped nectaries on the adaxial (upper) surfaces (Fig. 1a;
106 Yamasaki et al. 2013a). Pollinator insects visit the inflorescences in search of nectar secreted
107 from the nectaries. The number and size of the bracteoles do not differ between male and
108 female inflorescences. The second consists of bracteoles enclosing flowers associated with
109 pollination by thrips, *Dolichothrips* spp. (Phlaeothripidae) or by hemipterans of the
110 Anthocoridae and Miridae (Fig. 1b; Moog et al. 2002; Ishida et al. 2009; Fiala et al. 2011).
111 The pollinators breed in bracteole chambers and feed on trichome- and/or ball-shaped

112 nectaries on the adaxial surfaces of the bracteoles. Although the shape is similar between
113 male and female inflorescences, the bracteoles are stouter and fewer in number in female than
114 in male inflorescences (Davies and Ashton 1999).

115 *Macaranga* is well known for its protective mutualisms with ants. Most of the ca.
116 260 species in the genus possess extrafloral nectaries and/or food bodies (nourishing small
117 particles) on leaves and potentially attract ants that can exclude herbivores from flowers
118 (Whalen and Mackay 1988; Fiala and Maschwitz 1991; Mackay and Whalen 1991; Whitmore
119 2008). On the other hand, ca. 30 species offer not only foods for ants but also nesting sites for
120 them (Davies et al. 2001). In this paper, we call these species “ant-plants.” The ant-plant
121 *Macaranga* species are intensely protected by the almost species-specific resident ants,
122 compared to species that are facultatively protected by ants attracted to extrafloral nectaries
123 and/or food bodies (Quek et al. 2004; Itioka 2005). While ant-plant *Macaranga* species are
124 pollinated by *Dolichothrips* spp. (Fiala et al. 2011), our previous study indicated that
125 pollinator thrips are not excluded by the guard ants (Yamasaki et al. 2013b). This may be
126 because pollinators are protected from the guard ants by bracteoles (Fiala et al. 2011;
127 Yamasaki et al. 2013b); although ant guards are often seen on the surface of the
128 inflorescences, we have never observed the guard ants crawling into the chambers, where
129 pollinator thrips spend most of their lifetime, maybe because the gaps of the bracteoles are
130 too narrow for the ants.

131 While some previous studies have examined the evolution of inflorescence
132 morphology in *Macaranga* (Davies 2001; Fiala et al. 2011), they mainly focused on the sister
133 sections *Pachystemon* and *Pruinosae*, which have flower-enclosing bracteoles, no clear
134 overview of the evolutionary pattern in the genus has been elucidated. The aim of this study
135 was to examine whether inflorescence morphologies have changed repeatedly and, if so,
136 whether the repeated evolution is related to interacting animals such as pollinators and

137 bodyguard ants in *Macaranga*. For this purpose, we first examined interspecific variation in
138 inflorescence morphologies and estimated pollination systems of each morphology type by
139 examining the inflorescence characteristics and previous pollination studies in *Macaranga*.
140 Next, we mapped the inflorescence types on a molecular phylogenetic tree and estimated
141 ancestral inflorescence morphologies to examine whether inflorescence morphologies have
142 repeatedly changed in *Macaranga*.

143

144 **Materials and Methods**

145 *Observation of inflorescence/floral morphologies*

146 We observed the inflorescences of dry specimens of 53 taxa in the genus *Macaranga* (52
147 species and one variety) in herbaria (Royal Botanic Gardens, Kew (K), Leiden Naturalis
148 Biodiversity Center (L), Kyoto University (KYO) and Forest Department, Sarawak (SAR)).
149 We also obtained morphological data for *Macaranga lamellata* and *Macaranga umbrosa*
150 from Fiala et al. (2011). We recorded (1) the presence/absence of disc-shaped glands on
151 bracteole surfaces, (2) internode distances between adjacent bracteoles, (3) length, and (4)
152 width of bracteoles in male specimens, and (5) style length in female specimens (Fig. 2).
153 Traits (1)–(4) were not measured in female specimens because those with bracteoles were
154 absent in many of the species, while most of the bracteoles in male inflorescences remained.
155 For each trait, we looked at two to five samples from each of one to five specimens. For trait
156 (1), we judged disc-shaped glands to be present when at least one bracteole possessed them,
157 and we determined that specific taxa possessed the glands if they occurred in at least one
158 specimen. The presence/absence of the glands was consistent among specimens in most
159 species, with the exception of *Macaranga denticulata*. Some specimens of this species lack
160 disc-shaped glands on their bracteoles, but in all other respects, their bracteoles were similar
161 to those of other specimens. Thus, we regarded it as a species with disc-shaped glands. For

162 quantitative traits (2)–(5), average values were calculated for each specimen and averaged
163 across specimens to obtain species values.

164 To determine which traits to use in categorizing inflorescence morphologies, we
165 examined the differences in inflorescence morphologies among the species by principal
166 component analysis (PCA) using *Z*-score standardized values of the four quantitative traits
167 (traits (2)–(5)). Thirty-two taxa in which all four variables were available were included in
168 the analysis. We used the *prcomp* function in R 3.0.2 (R Development Core Team 2013). The
169 first principal component (PC 1) clearly separated species that did not contain disc-shaped
170 glands into two groups, and bracteole length and width were major components (see Results).
171 Therefore, we classified all 55 taxa into three inflorescence types based on the
172 presence/absence of disc-shaped glands, and bracteole shape and size (see Results). Style
173 length was not used because we were unable to measure it in many species, mostly due to a
174 lack of specimens containing flowering female inflorescences.

175

176 *Molecular phylogenetic analysis*

177 We constructed a molecular phylogeny based on the DNA sequence and indel data on one
178 plastid (*trnL-F*) and three nuclear markers (ITS, *ncpGS*, and *phyC*) of 59 taxa in the genus
179 *Macaranga* using species of the related genus *Mallotus* (Euphorbiaceae) as outgroups
180 (*Mallotus griffithianus*, *Mallotus claoxyloides*, and *Mallotus paniculatus*). Alignment and
181 indel data for all taxa other than *Macaranga sinensis* were acquired from Kulju et al. (2007);
182 those of *M. sinensis* were obtained via the following procedures. First, DNA was extracted
183 from silica gel-dried leaves following a modified CTAB procedure (Doyle and Doyle 1987;
184 Okuyama and Kawakita 2012). Regions were amplified by different primer pairs (Kulju et al.
185 2007). Polymerase chain reaction (PCR) amplifications were carried as follows: initial
186 denaturation step at 94°C for 5 min; 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1

187 min; and a final extension at 72°C for 7 min. Products were sequenced on an ABI 3100
188 automated sequencer using BigDye chain termination chemistry (Applied Biosystems, Foster
189 City, CA, USA), and obvious sequence errors were manually corrected using MEGA 5.05.
190 Indel information was incorporated based on Kulju et al. (2007). A phylogenetic tree was
191 constructed by Bayesian inference methods using MrBayes version 3.2 (Ronquist and
192 Huelsenbeck 2003). The substitution models were chosen separately for each marker based
193 on Akaike Information Criterion using Kakusan 4 (Tanabe 2011) as follows: GTR+G for *trnL*
194 and ITS and HKY85+G for *ncpGS* and *phyC*. We employed the binary model for gap
195 characters (Ronquist and Huelsenbeck 2003). Two independent runs for four Markov chains
196 were conducted for four million generations, and the tree was sampled every 100 generations.
197 The first 4001 trees were discarded as burn-in. A majority-rule consensus tree was
198 constructed from the remaining 36000 trees.

199

200 *Reconstruction of ancestral inflorescence morphologies*

201 To investigate how inflorescence type has shifted in the genus, we estimated ancestral
202 inflorescence morphologies (the inflorescence morphology of each node) using 1000 trees
203 randomly chosen from the Bayesian phylogenetic analysis. Inflorescence morphologies of the
204 outgroups are not included in the analysis. The Markov chain Monte Carlo method (Ronquist
205 2004) was applied for this analysis using the BayesMultiState function in BayesTraits (Pagel
206 and Meade 2006). The reverse jump hyper prior function with a gamma prior of 0, 10, 0, 10
207 (minimum and maximum of priors for both mean and variance parameters) was used. Rate
208 deviation was set to 50 to achieve the recommended 20–40% acceptance rates. Inflorescence
209 morphology types were treated as categorical variables with three states (Fig. 1; see Results).
210 Alternative ancestral states were compared on 49 nodes with >0.5 posterior probabilities
211 using the ‘fossil’ (fixing) command. Because harmonic means in the analyses can be unstable

212 (Pagel and Meade 2006), we ran the analyses five times to check the consistency of the
213 results. Bayes Factors ($2 [\log(\text{harmonic mean of model 1}) - \log(\text{harmonic mean of model 2})]$)
214 >5 indicated strong evidence of support for the best model (Pagel et al. 2004).

215

216 **Results**

217 *Observation of inflorescence/floral morphologies*

218 Based on the PCA results, inflorescence types were classified into three categories using
219 bracteole shape and size and the presence/absence of disc-shaped glands: Discoid-gland,
220 Inconspicuous, and Enclosing (Fig. 1; Supplemental Table 1). In the PCA results, the first
221 and second principal components (PC 1 and PC 2) contributed 54.1% and 24.6% of the total
222 variance of the measured data, respectively (Table 1). Bracteole size (length and width) and
223 style length had substantial loading in PC 1 (Table 1, Fig. 3). PC 1 distinctly separated
224 species not possessing disc-shaped glands into two groups: one with relatively large
225 bracteoles and short styles, and one with small bracteoles and long styles. PC 2 mainly
226 represented internode distances between bracteoles and bracteole length, and some species
227 with disc-shaped glands had extremely low values. The two groups distinguished by PC 1
228 were not represented by PC 2.

229 The three categories are defined as follows based on the PCA and presence/absence of
230 disc-shaped glands:

231 (1a) Disc-shaped glands on the bracteole surfaces presentDiscoid-gland

232 (1b) Disc-shaped glands on the bracteole surfaces absent (2)

233 (2a) Bracteoles very small (length and width < 1.6 mm) or narrow (length/width > 1.8),
234 or absent.....Inconspicuous

235 (2b) Bracteoles relatively large, enclosing flower clusters.....Enclosing

236 We also observed fresh inflorescences of 11 species in which all three inflorescence types

237 were represented. The shape did not differ between dry specimens and fresh inflorescences,
238 although the size was smaller in the dry specimens. All species mainly visited by thrips or
239 hemipterans were of the Enclosing type (Moog et al. 2002; Ishida et al. 2009; Fiala et al.
240 2011; Figs. 3 and 4), as were the ant-plant species (Davies 2001; Davies et al. 2001; Fig. 4).
241 *M. sinensis*, pollinated by generalist insects attracted to the disc-shaped nectaries on
242 bracteoles (Yamasaki et al. 2013a), and *M. denticulata* and *Macaranga indica*, whose male
243 inflorescences are mainly visited by generalist insects (bees, flies, wasps, and beetles) (Fiala
244 et al. 2011), were of the Discoid-gland type (Fig. 4).

245

246 *Molecular phylogenetic analysis*

247 Molecular analysis revealed two well-supported basal clades (B1 and B2) and three crown
248 clades (C1, C2, and C3), as in Kulju et al. (2007), who analyzed the phylogeny by Bayesian
249 and maximum parsimony methods (Fig. 4). While the Bayesian tree in Kulju et al. (2007)
250 united the C1 and C2 clades, our tree united the C2 and C3 clades, as in the maximum
251 parsimony tree in Kulju et al. (2007).

252

253 *Reconstruction of ancestral bracteole morphologies*

254 All observed species in basal clades B1 and B2 were classified into the Inconspicuous
255 category (Fig. 4). Conversely, we detected all three inflorescence types in the crown clades.
256 No inflorescence type was determined to be monophyletic. Among the 49 focal nodes, six,
257 five, and 14 nodes were strongly supported as Inconspicuous, Discoid-gland, and Enclosing
258 types, respectively (Bayes Factors > 5; Fig. 4). When Bayes factor >5 is considered to
259 indicate a significant occurrence of the focal inflorescence type, ancestral state reconstruction
260 indicated at least two shifts from the Discoid-gland to the Enclosing type and at least one
261 shift from the Enclosing to the Inconspicuous type (Fig. 4, Table 2).

262

263 **Discussion**

264 All *Macaranga* species in this study were classified into three inflorescence types based on
265 bracteole morphological characteristics: Discoid-gland, Inconspicuous, and Enclosing (Fig. 1,
266 see Results for detailed characteristics). Considering the morphology and existing pollination
267 studies in *Macaranga*, we propose that the three types of inflorescence morphology are
268 related to different pollination systems. Because this study lacks empirical pollination surveys
269 for many of the species, we cannot yet test this hypothesis. However, it is often said that
270 floral morphologies are generated by functional groups of similar pollinators (Fægri and van
271 der Pijl 1979; Fenster et al. 2004). Wind may contribute, at least in part, to pollination in the
272 Inconspicuous species, which have exposed flowers, inflorescences that penetrate through the
273 leaf mass, and sometimes extremely long (up to 5 cm) styles (Bullock 1994; Culley et al.
274 2002). We observed that *Macaranga vedeliana*, with Inconspicuous inflorescences (not
275 included in this study), did not secrete nectar and were wind-pollinated (Yamasaki et al.
276 unpublished data; Supplemental Figure 2).

277 In Discoid-gland species, the glands on bracteoles, which are located adjacent to flowers,
278 may attract insects for pollination in the manner of *M. sinensis*, which is pollinated by
279 generalist insects that forage on the disc-shaped nectaries (Yamasaki et al. 2013a). Similarly,
280 male inflorescences of two other Discoid-gland species, *M. denticulata* and *M. indica*, are
281 visited by generalist insects such as bees, wasps, flies, and beetles (Fiala et al. 2011). On the
282 other hand, the nectaries on bracteoles may also attract ants, which may exclude pollinators
283 or contribute pollination. Further experimental studies are needed to confirm whether the
284 nectaries on the bracteoles attract pollinators and how the plants avoid disadvantage from
285 ants if the glands also attract ants.

286 All thrips- or hemipteran-pollinated species (Moog et al. 2002; Ishida et al. 2009; Fiala et

287 al. 2011) were classified as Enclosing species (Fig. 4). Pollination by wind or generalist
288 insects such as bees and flies is unlikely for this inflorescence type because such pollen
289 vectors are impeded by the bracteoles. In addition to providing chambers for pollinators, the
290 bracteoles of the Enclosing type may also physically protect pollinators against their natural
291 enemies, such as ants (Fiala et al. 2011; Yamasaki et al. 2013b).

292 Ancestral state reconstruction indicated at least two shifts from the Discoid-gland to the
293 Enclosing type and at least one shift from the Enclosing to the Inconspicuous type (Fig. 4,
294 Table 2). Additionally, while direction is ambiguous, among the three inflorescence types,
295 other shifts seem to have occurred several times (Fig. 4, Table 2). The present study is the
296 first to describe multiple drastic evolutionary changes in extrafloral plant organs.

297 Repeated evolution of the Enclosing inflorescence type might be due to pollination
298 interference by ants. Because bracteoles can physically separate ants and pollinators on the
299 inflorescences, pollination interference by ants may have selected for the evolution of
300 flower-covering bracteoles that eliminate ants from flowers. Therefore, the flower-enclosing
301 bracteoles in the Enclosing inflorescences may act as “barriers” against
302 pollination-interfering animals (Santamaría and Rodríguez-Gironés 2007). To explore these
303 possibilities, the functions of bracteoles should be examined in Enclosing species from
304 various clades. It would also be instructive to investigate why species with other
305 inflorescence types do not need to exclude guard ants from their flowers. Because ant-plants
306 are likely to have occurred more than once in an Enclosing-type clade (Blattner et al. 2001;
307 Davies et al. 2001), the evolution of ant-plants and the development of the Enclosing
308 inflorescences may be related. Not only mutualisms with ants but also the lack of perianths
309 may be related to the multiple evolutions of the Enclosing type; because *Macaranga* lacks
310 perianths that visually attract generalist pollinators such as bees, specialization for pollination
311 by thrips or hemipterans, which infest the flowers of various plants (Lewis 1973; Wheeler

312 2001), may have occurred relatively easily.

313 In conclusion, our study found high variability in bracteole morphology compared with
314 that of flowers. *Macaranga* species have repeatedly evolved unusual traits involved in
315 pollination, including flower-enclosing bracteoles. The repeated evolution of
316 flower-enclosing bracteoles may indicate a recurring need for floral barriers against
317 bodyguard ants. The genus *Macaranga* may be a good model for studying the factors
318 underlying the acquisition of floral barriers.

319

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483 Figure Legends

484

485 **Fig. 1.** Examples of species exhibiting the three inflorescence types, categorized based on
486 bracteole morphology: (a) *Macaranga sinensis* (Discoid-gland type), (b) *Macaranga*
487 *gigantea* (Enclosing type), and (c) *Macaranga coriacea* (Inconspicuous type) (not included in
488 the phylogeny). Scale bar = 1 cm.

489

490 **Fig. 2.** The inflorescence/floral traits investigated. See (1)–(5) in the *Observation of*
491 *inflorescence/floral morphologies* section of Materials and Methods.

492

493 **Fig. 3.** Scatterplot of the first and second principal components (PC 1 and PC 2) of a
494 principal components analysis (PCA) using four inflorescence and floral traits (internode
495 distances between adjacent bracteoles, lengths and widths of bracteoles, internodes between
496 two bracteoles, and style lengths). Different colors indicate inflorescence types (see text for
497 classifications). Species visited mainly by thrips or hemipterans and those visited by other
498 insects are indicated by diamond and triangle symbols, respectively. The information on
499 flower visitors was obtained from Moog et al. (2002), Ishida et al. (2009), Fiala et al. (2011),
500 and Yamasaki et al. (2013).

501

502 **Fig. 4.** Phylogenetic tree of *Macaranga* species constructed with the Bayesian inference
503 method. The pies indicate estimated ancestral morphologies by Bayesian inference methods.
504 Bayes factors >5 are indicated with an asterisk. Numbers above branches indicate posterior
505 probabilities of the branches obtained from reconstruction of the tree (>0.5). The mapped
506 information, with the exception of inflorescence morphologies, was obtained from the
507 following literature: main flower visitors, Moog et al. (2002), Ishida et al. (2009), Fiala et al.

508 (2011), and Yamasaki et al. (2013a); whether the species are ant-plants, Davies (2001) and
509 Davies et al. (2001). Clade grouping (B1, B2, C1, C2, and C3) was done according to Kulju
510 *et al.* (2007).

511

512 **Table 1.** The proportion of variance and factor loadings of principal components analysis
513 axes using four inflorescence traits.

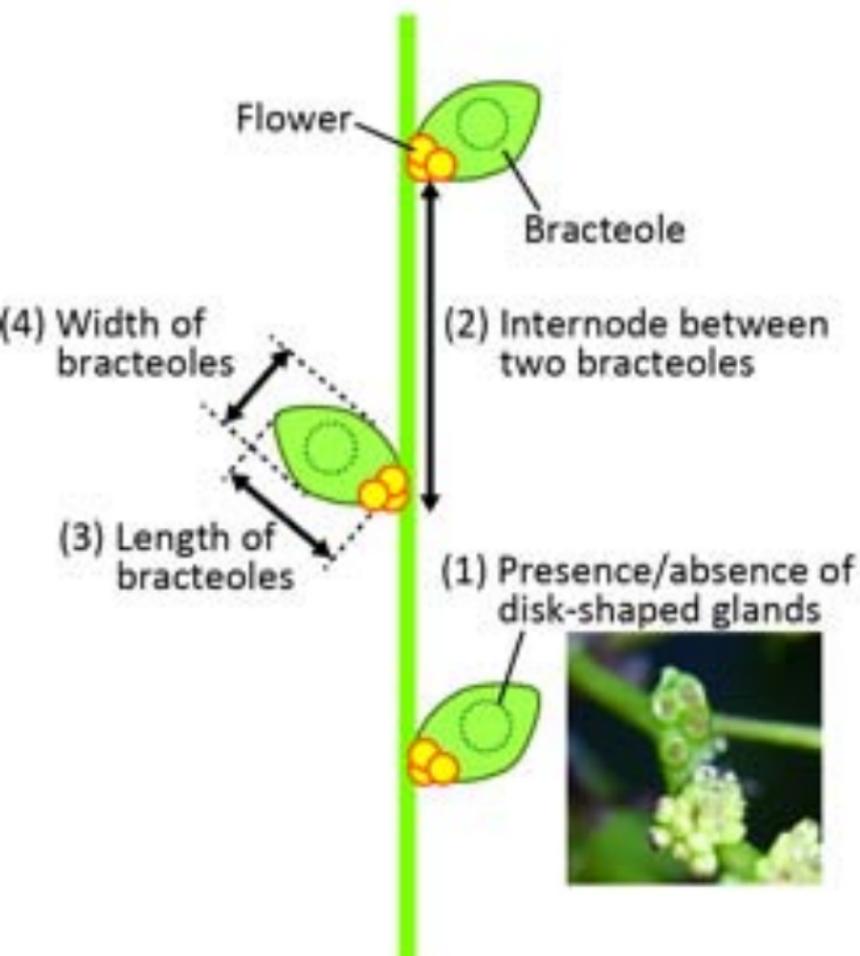
| | PC 1 | PC 2 |
|------------------------------|--------|--------|
| Proportion of variance | 54.11% | 24.60% |
| Internode between bracteoles | -0.20 | -0.96 |
| Length of bracteole | 0.61 | -0.26 |
| Width of bracteole | 0.65 | 0.00 |
| Style length | -0.41 | 0.08 |

514

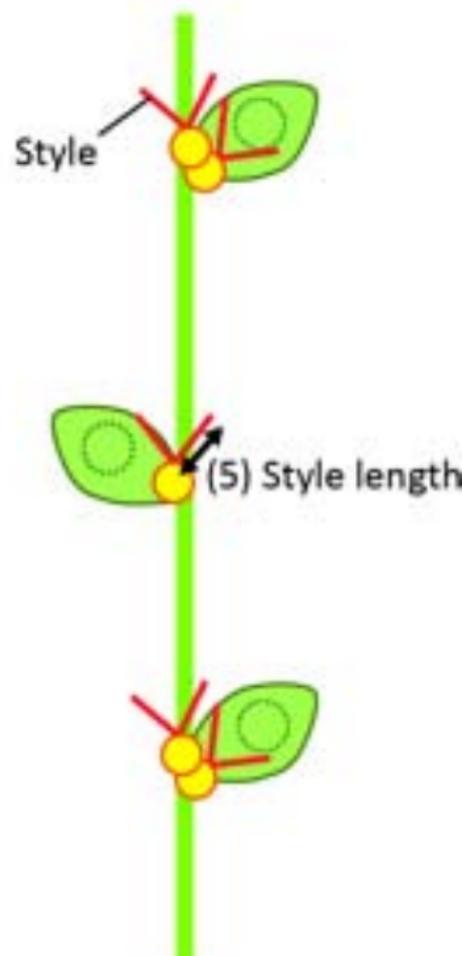
515

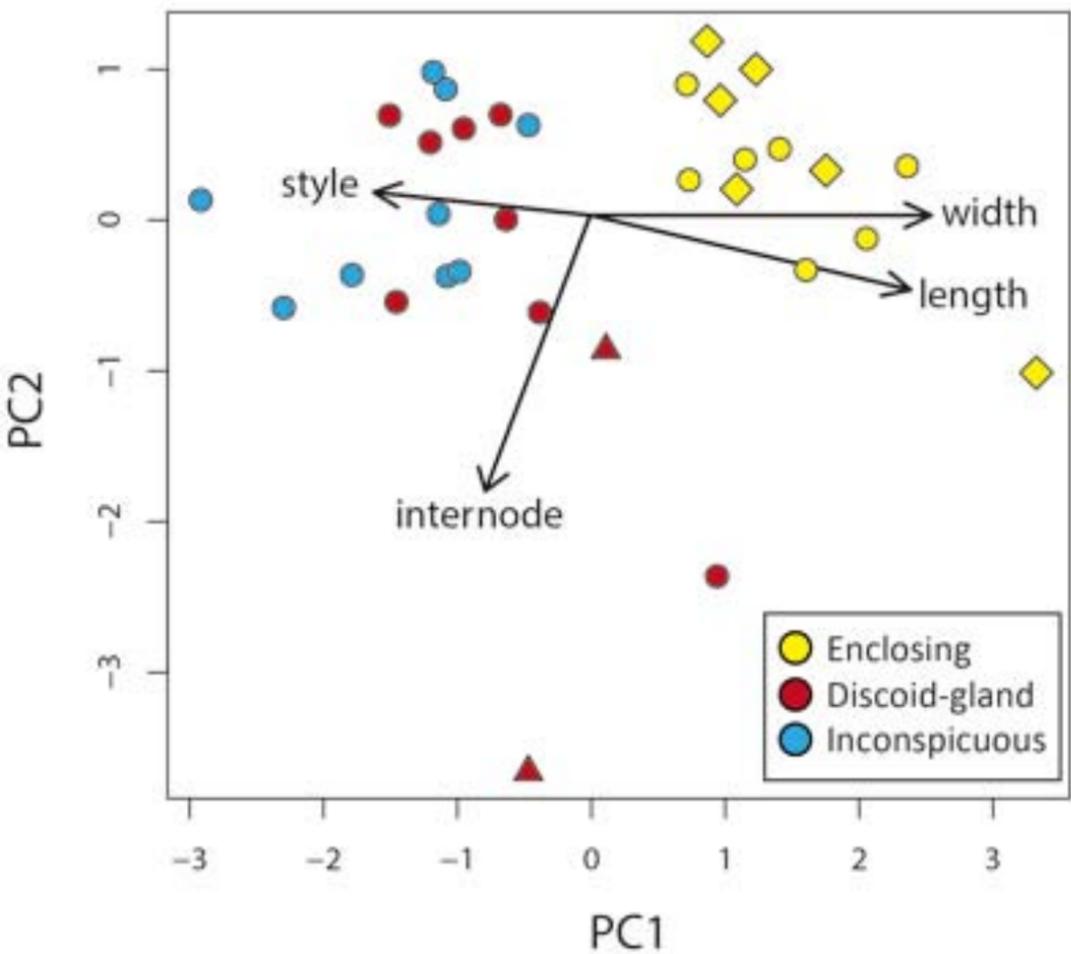


Male inflorescence



Female inflorescence





Supplemental Table 1 Inflorescence/floral traits assessed on herbarium specimens. Inflorescence morphology types of Enclosing, Discoid-gland and Inconspicuous are shown as E, D and I, respectively. *N* indicates numbers of specimens observed (numbers of male and female specimens are shown separately). Presence/absence of disk-shaped glands on the middle part of the bracteole surfaces are indicated by “+” and “-”. Data not available are indicated by NA.

| Species | Bracteole type | <i>N</i> | | Disk-shaped glands | Length of bracteoles (mm) | Width of bracteoles (mm) | Internode between two bracteoles (mm) | Length of stigma (mm) |
|-------------------------|----------------|----------|---|--------------------|---------------------------|--------------------------|---------------------------------------|-----------------------|
| | | ♂ | ♀ | | | | | |
| <i>M. albescens</i> | I | 3 | 3 | - | 1.28 | 0.55 | 2.78 | 2.63 |
| <i>M. alchorneoides</i> | I | 3 | 0 | - | 1.14 | 1.12 | 2.76 | NA |
| <i>M. aleuritoides</i> | D | 3 | 3 | + | 1.19 | 0.39 | 2.00 | 6.43 |
| <i>M. alnifolia</i> | D | 2 | 2 | + | 1.43 | 0.37 | 3.58 | 4.37 |
| <i>M. angustifolia</i> | D | 3 | 2 | + | 4.19 | 2.18 | 5.23 | 0.34 |
| <i>M. auriculata</i> | I | 2 | 1 | - | 1.27 | 1.07 | 1.75 | 6.73 |
| <i>M. barteri</i> | E | 2 | 2 | - | 4.65 | 3.35 | 1.38 | 0.53 |
| <i>M. bicolor</i> | E | 4 | 0 | - | 2.33 | 2.17 | 0.99 | NA |
| <i>M. bifoveata</i> | D | 3 | 3 | + | 1.90 | 1.66 | 3.51 | 2.92 |
| <i>M. brachytricha</i> | NA | 0 | 0 | NA | NA | NA | NA | NA |
| <i>M. clavata</i> | E | 3 | 3 | - | 2.94 | 2.88 | 1.81 | 1.60 |
| <i>M. conifera</i> | E | 3 | 0 | - | 1.41 | 1.77 | 0.80 | NA |
| <i>M. densiflora</i> | I | 3 | 0 | - | 1.67 | 0.96 | 1.83 | 1.30 |
| <i>M. denticulata</i> | D | 5 | 4 | + | 1.01 | 1.03 | NA | 0.69 |
| <i>M. diepenhorstii</i> | E | 3 | 1 | - | 2.84 | 2.70 | 1.01 | 0.43 |
| <i>M. domatiosa</i> | I | 3 | 3 | - | 1.43 | 0.22 | 3.41 | 7.33 |

Supplemental Table 1 Continued.

| Species | Bracteole type | N | | Disk-shaped glands | Length of bracteoles (mm) | Width of bracteoles (mm) | Internode between two bracteoles (mm) | Length of stigma (mm) |
|--|----------------|---|---|--------------------|---------------------------|--------------------------|---------------------------------------|-----------------------|
| | | ♂ | ♀ | | | | | |
| <i>M. ducis</i> | I | 2 | 0 | – | 2.43 | 1.27 | 1.88 | NA |
| <i>M. echinocarpa</i> | NA | 0 | 0 | NA | NA | NA | NA | NA |
| <i>M. gabunica</i> | E | 1 | 1 | – | 2.33 | 2.53 | 2.13 | 0.47 |
| <i>M. gigantea</i> | E | 3 | 0 | – | 3.40 | 3.43 | 1.91 | NA |
| <i>M. grallata</i> | I | 1 | 1 | – | 0.00 | 0.00 | 4.00 | 3.47 |
| <i>M. grandifolia</i> | E | 3 | 1 | – | 4.21 | 3.34 | 2.15 | 0.30 |
| <i>M. heterophylla</i> | E | 2 | 0 | – | 7.86 | 4.73 | 2.20 | NA |
| <i>M. heynei</i> | E | 4 | 0 | – | 5.69 | 4.39 | 1.97 | NA |
| <i>M. hispida</i> | D | 5 | 3 | + | 2.61 | 1.10 | 2.57 | 7.60 |
| <i>M. hullettii</i> | E | 3 | 0 | – | 3.61 | 2.69 | 2.07 | NA |
| <i>M. hurifolia</i> | E | 2 | 0 | – | 3.05 | 2.72 | 1.13 | NA |
| <i>M. inamoena</i> | I | 2 | 1 | – | 0.00 | 0.00 | 4.73 | 8.00 |
| <i>M. indica</i> | D | 5 | 2 | + | 3.19 | 1.33 | 7.30 | 2.03 |
| <i>M. induta</i> | NA | 0 | 2 | – | NA | NA | NA | 3.33 |
| <i>M. involucrata</i> | D | 2 | 2 | + | 0.97 | 0.60 | 2.20 | 2.77 |
| <i>M. klaineana</i> | I | 2 | 0 | – | 1.53 | 1.53 | 1.73 | NA |
| <i>M. lamellata</i> | E | 0 | 0 | – | NA | NA | NA | NA |
| <i>M. lowii</i> var. <i>kostermansii</i> | I | 3 | 2 | – | 1.39 | 0.72 | 1.56 | 6.57 |
| <i>M. lowii</i> var. <i>lowii</i> | I | 2 | 0 | – | 1.17 | 0.93 | 1.93 | NA |
| <i>M. mauritiana</i> | I | 2 | 0 | – | 2.15 | 2.20 | 3.55 | NA |

Supplemental Table 1 Continued.

| Species | Bracteole type | N | | Disk-shaped glands | Length of bracteoles (mm) | Width of bracteoles (mm) | Internode between two bracteoles (mm) | Length of stigma (mm) |
|----------------------------|----------------|---|---|--------------------|---------------------------|--------------------------|---------------------------------------|-----------------------|
| | | ♂ | ♀ | | | | | |
| <i>M. monandra</i> | E | 3 | 0 | – | 4.71 | 3.42 | 1.22 | NA |
| <i>M. novo-guineensis</i> | E | 3 | 0 | – | 1.99 | 1.54 | 1.03 | NA |
| <i>M. oblongifolia</i> | I | 1 | 0 | – | 2.30 | 2.37 | 1.25 | NA |
| <i>M. obovata</i> | D | 2 | 1 | + | 1.85 | 0.80 | 1.75 | 3.60 |
| <i>M. pachyphylla</i> | E | 5 | 1 | – | 2.46 | 2.36 | 0.85 | 1.03 |
| <i>M. pearsonii</i> | E | 3 | 0 | – | 2.54 | 2.16 | 1.72 | NA |
| <i>M. poggei</i> | E | 2 | 0 | – | 2.30 | 2.37 | 1.25 | NA |
| <i>M. puncticulata</i> | E | 4 | 1 | – | 3.58 | 2.70 | 1.53 | 1.37 |
| <i>M. praestans</i> | I | 3 | 4 | – | 1.68 | 0.73 | 3.08 | 24.24 |
| <i>M. quadriglandulosa</i> | E | 3 | 3 | – | 2.72 | 2.42 | 1.23 | 3.40 |
| <i>M. repando-dentata</i> | NA | 0 | 3 | NA | NA | NA | NA | 38.11 |
| <i>M. rhizinoides</i> | I | 3 | 3 | – | 1.12 | 0.82 | 3.37 | 1.78 |
| <i>M. saccifera</i> | E | 1 | 0 | – | 2.97 | 3.17 | 1.13 | NA |
| <i>M. schweinfurthii</i> | E | 2 | 0 | – | 2.53 | 3.17 | 1.58 | NA |
| <i>M. siamensis</i> | E | 4 | 2 | – | 3.79 | 3.14 | 2.58 | 1.20 |
| <i>M. sinensis</i> | D | 3 | 3 | + | 3.17 | 1.09 | 3.46 | 0.68 |
| <i>M. subdentata</i> | I | 2 | 1 | – | 1.07 | 1.35 | 3.38 | 3.67 |
| <i>M. tanarius</i> | E | 3 | 3 | – | 6.00 | 4.97 | 2.98 | 3.83 |
| <i>M. tessellata</i> | D | 2 | 1 | + | 1.38 | 0.90 | 2.02 | 4.40 |
| <i>M. trichocarpa</i> | E | 4 | 3 | – | 4.28 | 2.63 | 1.51 | 0.88 |

Supplemental Table 1 Continued.

| Species | Bracteole type | N | | Disk-shaped glands | Length of bracteoles (mm) | Width of bracteoles (mm) | Internode between two bracteoles (mm) | Length of stigma (mm) |
|--------------------|----------------|---|---|--------------------|---------------------------|--------------------------|---------------------------------------|-----------------------|
| | | ♂ | ♀ | | | | | |
| <i>M. triloba</i> | E | 4 | 1 | – | 2.62 | 2.42 | 1.29 | 0.30 |
| <i>M. umbrosa</i> | E | 0 | 0 | – | NA | NA | NA | NA |
| <i>M. winkleri</i> | E | 4 | 3 | – | 2.83 | 2.70 | 2.08 | 0.26 |

Supplemental Figure 1 A female inflorescence of *Macaranga vedeliana*, not included in the analysis, classified into the Inconspicuous type.

