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Multiple, independent colonizations of the Hawaiian Archipelago by the family Dolichopodidae (Diptera)

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The family Dolichopodidae forms two of the four largest evolutionary radiations in the Hawaiian Islands across all flies: *Campsicnemus* (183 spp) and the *Eurynogaster* complex (66 spp). They also include a small radiation of *Conchopus* (6 spp). A handful of other dolichopodid species are native to the islands in singleton lineages or small radiations. This study provides a phylogenetic perspective on the colonization history of the dolichopodid fauna in the islands. We generated a multi gene data set including representatives from 11 of the 14 endemic Hawaiian dolichopodid genera to examine the history of colonization to the islands, and analyzed it using Bayesian and maximum likelihood phylogenetic methods. We used a subset of the data that included *Conchopus* and the eight genera comprising the *Eurynogaster* complex to estimate the first phylogenetic hypothesis for these endemic groups, then used Beast to estimate their age of arrival to the archipelago. The *Eurynogaster* complex, *Campsicnemus* and *Conchopus* are clearly the result of independent colonizations. The results strongly support the *Eurynogaster* complex as a monophyletic group, and also supports the monophyly of 4 of the 8 described genera within the complex (*Adachia*, *Arciellia*, *Uropachys* and *Eurynogaster*). Members of the family Dolichopodidae have been dispersing over vast distances to colonize the Hawaiian Archipelago for millions of years, leading to multiple independent evolutionary diversification events. The *Eurynogaster* complex arrived in the Hawaiian Archipelago 11.8 Ma, well before the arrival of *Campsicnemus* (4.5 Ma), and the even more recent *Conchopus* (1.8 Ma). Data presented here demonstrate that the Hawaiian Dolichopodidae both disperse and diversify easily, a rare combination that lays the groundwork for field studies on the reproductive isolating mechanisms and ecological partitioning of this group.

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2

3 **Title:**

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5 Multiple, Independent Colonizations of the Hawaiian Archipelago by the Family Dolichopodidae
6 (Diptera)

7

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43 **ABSTRACT**

44

45 The family Dolichopodidae forms two of the four largest evolutionary radiations in the Hawaiian
46 Islands across all flies: *Campsicnemus* (183 spp) and the *Eurynogaster* complex (66 spp). They
47 also include a small radiation of *Conchopus* (6 spp). A handful of other dolichopodid species are
48 native to the islands in singleton lineages or small radiations. This study provides a phylogenetic
49 perspective on the colonization history of the dolichopodid fauna in the islands. We generated a
50 multi gene data set including representatives from 11 of the 14 endemic Hawaiian dolichopodid
51 genera to examine the history of colonization to the islands, and analyzed it using Bayesian and
52 maximum likelihood phylogenetic methods. We used a subset of the data that included
53 *Conchopus* and the eight genera comprising the *Eurynogaster* complex to estimate the first
54 phylogenetic hypothesis for these endemic groups, then used BEAST to estimate their age of
55 arrival to the archipelago. The *Eurynogaster* complex, *Campsicnemus* and *Conchopus* are clearly
56 the result of independent colonizations. The results strongly support the *Eurynogaster* complex
57 as a monophyletic group, and also supports the monophyly of 4 of the 8 described genera within
58 the complex (*Adachia*, *Arciellia*, *Uropachys* and *Eurynogaster*). Members of the family
59 Dolichopodidae have been dispersing over vast distances to colonize the Hawaiian Archipelago
60 for millions of years, leading to multiple independent evolutionary diversification events. The
61 *Eurynogaster* complex arrived in the Hawaiian Archipelago 11.8 Ma, well before the arrival of
62 *Campsicnemus* (4.5 Ma), and the even more recent *Conchopus* (1.8 Ma). Data presented here
63 demonstrate that the Hawaiian Dolichopodidae both disperse and diversify easily, a rare
64 combination that lays the groundwork for field studies on the reproductive isolating mechanisms
65 and ecological partitioning of this group.

66

67 **Keywords**

68 Colonization history, Diptera, Divergence dating, Dolichopodidae, Evolutionary radiation,

69 Hawaiian Islands, Long distance dispersal

71 **INTRODUCTION**

72 Long distance dispersal from continental populations is critical to the formation of the
73 Hawaiian flora and fauna (Carson & Kaneshiro, 1976; O'Grady *et al.*, 2009), but is considered
74 rare. This infrequent arrival and establishment has led to a flora and fauna that is disharmonic
75 relative to those on the continents that served as sources (Gillespie & Roderick, 2002). Recently,
76 several studies (reviewed in Heaney, 2007; Bellemain & Ricklefs, 2008) have shown that reverse
77 colonization from Hawaii to continental landmasses is observed in birds (Filardi & Moyle,
78 2005), plants (Harbaugh & Baldwin, 2007) and insects (O'Grady & DeSalle, 2008; Lapoint *et al.*,
79 2014), suggesting that dispersal plays a larger role than previously thought and evidence is
80 accumulating to indicate that movement to and from island systems is more common, especially
81 at geological time scales (Heaney, 2007; Cibois *et al.*, 2011; Hembry *et al.*, 2013; Casquet *et al.*,
82 2014). If a lineage is vagile enough to repeatedly colonize an area, there is a reduced chance that
83 it will generate the reproductive isolation necessary to speciate and then radiate. Furthermore, if
84 radiation does occur in a lineage and there is subsequent colonization of the area by close
85 relatives, ecological theory would predict that the existing niches would be pre-empted (Hardin,
86 1960), rendering a second radiation unsuccessful. Thus, clear examples where a lineage
87 colonizes and radiates repeatedly and substantially are rare.

88 The Hawaiian-Emperor Archipelago has a long and dynamic geological history, well
89 isolated in the central Pacific Ocean far from any continental mass. It has been forming by the
90 motion of the Pacific plate over a stationary hotspot (Wilson, 1963), generating an island chain
91 that is at least 80 million years old (Clague & Dalrymple, 1987; Duncan & Keller, 2004; Sharp
92 & Clague, 2006). Island formation during this long history has been episodic, with some periods
93 characterized by only few, low elevation atolls and reduced species diversity and other times
94 with multiple high islands capable of supporting a diverse flora and fauna (Price & Clague,

95 1992). Many of the older islands that are now submerged or heavily eroded to small land masses
96 once provided the kind of high island habitat we are familiar with in the contemporary high
97 islands (Niihau, Kauai, Oahu, Molokai, Lanai, Maui, Kahoolawe and Hawaii), which have been
98 forming very recently – only over the past five million years (Clague & Dalrymple, 1987;
99 Clague, 1996: Figure 1). The current high islands provide a rich array of habitats, ranging from
100 low to high elevation and very dry to very wet vegetation types.

101 All of the flora and fauna arrived to this dynamic archipelago via long distance dispersal
102 in an unlikely sequence of events in which taxa both managed to land on the islands and persist
103 once there (Zimmerman, 2001; Gillespie *et al.*, 2012). Recent phylogenetic studies of Hawaiian
104 insects (Jordan *et al.* 2003; Mendelson & Shaw, 2005; Shapiro *et al.* 2006; Medeiros *et al.* 2009;
105 Lapoint *et al.*, 2011; Medeiros & Gillespie, 2011; O’Grady *et al.*, 2011; Haines & Rubinoff,
106 2012; Bennett & O’Grady, 2013; Bess *et al.*, 2013; Goodman & O’Grady, 2013; Lapoint *et al.*,
107 2013; Goodman *et al.*, 2014; Haines *et al.*, 2014; Lapoint *et al.*, 2014), have begun to reveal the
108 history of colonization to and diversification within the Hawaiian Archipelago, and it appears
109 that history is somewhat idiosyncratic. Some large groups, such as Hawaiian Drosophilidae with
110 an estimated 1,000 species, colonized the Hawaiian Islands tens of millions of years ago. Other
111 diverse groups, such as *Nesophrosyne* leafhoppers, with 72 described and over 100 undescribed
112 species (Bennett & O’Grady, 2011), and *Campsicnemus* flies with about 200 species (Goodman
113 *et al.*, 2014) are young, dating to only a few million years. One thing is clear, however – very
114 few endemic Hawaiian plant or animal families have successfully colonized the islands multiple
115 times (e.g., Araliaceae; Plunkett *et al.* 1997; Costello & Motley 2001) and in no case have any of
116 these generated two radiations of with more than 50 species each.

117 Flies in the family Dolichopodidae are remarkable in that they have colonized the
118 Hawaiian Islands multiple times and still have managed to generate two of the largest
119 evolutionary radiations within the Hawaiian Diptera: *Campsicnemus* Haliday, 183 spp.
120 (Goodman *et al.*, 2014), and the *Eurynogaster* complex, 66 spp. in eight genera (Evenhuis,
121 2005). In addition, they also generated a small radiation of 6 spp., *Conchopus* Takagi. In addition
122 to these three radiations, four other dolichopodid genera contain endemic species: *Asyndetus* (1),
123 *Hydrophorus* (2), *Paraliancalus* (2), and *Thinophilus* (1) (Table 1). Thus, the family
124 Dolichopodidae offers a unique opportunity to examine the timing and frequency of long
125 distance colonization events in the founding of the endemic Hawaiian fauna. While recent
126 molecular phylogenies of Dolichopodidae (e.g., Lim *et al.*, 2010; Bernasconi *et al.*, 2007) have
127 sampled some of these genera (e.g., *Campsicnemus*, *Hydrophorus*, *Thinophilus*), uneven
128 sampling between studies and the lack of Hawaiian exemplars makes it difficult to infer the
129 colonization history in detail. Furthermore, while the biogeography of *Campsicnemus* has been
130 studied (Goodman *et al.* 2014), the evolutionary relationships among the three radiations and the
131 monophyly and biogeography of the large *Eurynogaster* complex have never been examined.

132 The primary goal of this paper is to address the colonization history of the endemic
133 Hawaiian Dolichopodidae and assess how many colonization events have generated the present-
134 day diversity within this lineage. We sampled 11 of the 14 genera with endemic Hawaiian
135 species and included samples from across the family Dolichopodidae. We sequenced a
136 combination of five mitochondrial and two nuclear genes and used these data to estimate
137 colonization times using the Bayesian algorithm implemented in BEAST to infer the colonization
138 history of this family in Hawaii. With our sampling we also provide the first molecular

139 phylogenetic analysis of the *Eurynogaster* complex, with which we assess the monophyly of this
140 lineages and its constituent genera.

141

142 **MATERIALS & METHODS**

143 **Taxonomic sampling**

144 Specimens were collected from 2004 to 2012 from sites across the Hawaiian Islands. The
145 bulk of Hawaiian Dolichopodidae species are endemic to high elevation (900 – 1700 m.) rain
146 forest habitats, and thus collecting efforts were concentrated in these areas. Other habitats (*e.g.*,
147 coastal strand, dry and mesic forests, alpine zone) were also sampled, including rocky beaches,
148 the only known habitat of *Conchopus*, *Thinophilus*, *Asyndetus* and *Hydrophorus*. We succeeded
149 in collecting specimens from 11 of the 14 Hawaiian dolichopodid genera with endemic species
150 known from the islands (*Campsicnemus*, *Conchopus*, *Thinophilus* and eight genera from the
151 *Eurynogaster* complex, Table S1a in Appendix S1 in Supporting Information). Data from the
152 Hawaiian *Campsicnemus* are included here from a previous study from our group, and are
153 described in Appendix A from Goodman *et al.* (2014). Material was collected by general
154 sweeping of vegetation and leaf litter, pan and Malaise trapping, and hand collecting. To
155 evaluate monophyly of and diversity within the *Eurynogaster* complex, we included
156 representatives from each of its eight constituent genera (Table 1; Evenhuis, 2005). No
157 *Eurynogaster* complex lineages were omitted from our sampling. All material was preserved in
158 95% ethanol.

159 All material was identified using the most recent key to species in Tenorio (1969) and
160 Evenhuis (2005). Descriptions of new species from within the *Eurynogaster* complex discovered
161 as a result of this project are in preparation. Unpublished new species included in the study were

162 given letters (e.g., *Eurynogaster* n. sp. A, B, C, etc.). In addition to the extracted specimens,
163 whenever possible, a series of conspecifics from the same site were also preserved in 95%
164 ethanol. Voucher material has been deposited in the Bernice Pauahi Bishop Museum (Honolulu).
165 In addition, new sequences were generated for outgroup specimens from the non-endemic
166 Dolichopodidae: five specimens of *Dolichopus exsul*, two specimens of *Chrysotus longipalpis*,
167 and one specimen each of *Condylostylus* sp. and *Tachytrechus angustipennis*. Finally, sequences
168 from *Hercostomus indonesianus* were also downloaded from GenBank to include in the outgroup
169 (see Table S1a in Appendix S1). Access and collection permits were granted by the State of
170 Hawaii Department of Land and Natural Resources, the National Park Service (Hawaii
171 Volcanoes and Haleakala National Parks), Maui Land and Pineapple, East Maui Irrigation,
172 Parker Ranch, and The Nature Conservancy of Hawaii (Appendix S4).

173

174 **Phylogenetic Analysis**

175 *Relationships within Dolichopodidae and colonization of the Hawaiian Islands*

176 To address the question of whether the endemic dolichopodid fauna, including the three
177 major radiations (*Campsicnemus*, the *Eurynogaster* complex and *Conchopus*) is the result of a
178 single or multiple colonizations, new sequences were generated for the samples described above
179 (and in Table S1a in Appendix S1) and were combined with the entire data matrix generated
180 from the Goodman *et al.* (2014) *Campsicnemus* study. Extraction, amplification, sequencing,
181 editing and alignment followed the same protocols described in Goodman *et al.* (2014). Loci
182 used are described in Table 2. Eleven of the 14 dolichopodid genera with endemic species are
183 represented. This yielded an alignment, referred to as dataset A, containing 183 individuals and

184 seven loci containing 4763 base pairs that was used to assess deep temporal and biogeographic
185 patterns within Hawaiian Dolichopodidae.

186 *Phylogenetic Relationships within the Eurynogaster Complex*

187 To assess the monophyly of the *Eurynogaster* complex and its component genera,
188 seventeen described, four new, and five possible new species (labeled as “sp. nr.”) were included
189 in the phylogenetic analysis (Table 1). This matrix was designated as dataset B. Phylogenetic
190 analyses were performed on a data set consisting of 57 individuals (see Table S1 in Appendix
191 S1) and seven loci containing 5,908 base pairs. Analyses were conducted on each gene
192 individually using maximum likelihood (ML, see below). Dataset B was used to assess
193 biogeographic patterns within the *Eurynogaster* complex of genera.

194 Datasets A and B were both analysed using ML and Bayesian inference (BI) optimality
195 criteria. For each of the ML and the BI analyses, the optimum partitioning schemes were
196 calculated in PartitionFinder (Lanfear *et al.*, 2012). The optimal partitioning scheme for the
197 combined analysis of Hawaiian Dolichopodidae (dataset A), was calculated from 18 original data
198 partitions (16S, 12S and 1st, 2nd, and 3rd codon positions for COI, COII, ND2, CAD, EF1 α and
199 one CAD intron region). Partitioning was calculated for the *Eurynogaster* complex dataset
200 (dataset B) from 20 original data partitions (16S, 12S and 1st, 2nd, and 3rd codon positions for
201 COI, COII, ND2, CAD, EF1 α A and EF1 α B, intron regions for CAD, EF1 α A, EF1 α B and ND2)
202 and selected using Bayesian Information Criterion (Table S2b in Appendix 2). For both datasets,
203 in the BI analyses, the best-fit model of sequence evolution for each data partition was also
204 selected using PartitionFinder (Table S2b in Appendix S2: Lanfear *et al.*, 2012). Selection of
205 models and partitions proceeded as described above and these are reported in Table S1b in
206 Appendix S1. The ML analyses were performed on individual genes and on the concatenated

207 data sets in RAxML 3.7.2 (Stamatakis, 2006) on CIPRES (Miller *et al.*, 2010) under the GTR
208 GAMMA model with 1,000 bootstrap replicates and a final search for the best tree. The BI
209 analyses were performed on the concatenated data sets using MrBayes 3.1.2 (Huelsenbeck &
210 Ronquist, 2001) on CIPRES (Miller *et al.*, 2010), with each analyses run for 30,000,000
211 generations with 2 independent runs each.

212 *MCMC convergence diagnostics:* For the BI analyses, stationarity was assessed within
213 and convergence among each of the runs using several complimentary approaches: (1)
214 convergence metrics provided by MrBayes 3.1.2 were checked (Huelsenbeck & Ronquist, 2001)
215 to ensure that the maximum standard deviation of split frequencies of any of the runs was under
216 0.05 and that the potential scale reduction factor for all parameters approached 1.0, and (2) the
217 log-likelihood values for each run were plotted, the Effective Sample Sizes (ESS) were checked
218 to ensure there were an adequate number of independent samples, and the posterior distributions
219 of all parameters were examined using Tracer v.1.72 (Rambaut & Drummond, 2012). Tracer
220 v.1.72 was also used to determine the burn-in phase by assessing each run's plot of log-
221 likelihood values over generations –stationarity was assumed to have been reached when the log
222 likelihood values reached a stable plateau. Finally, a 50% majority rule consensus trees was
223 created from the resulting post burn-in trees.

224

225 **Divergence Time Estimation in the *Eurynogaster* complex:**

226 To estimate the age of the *Eurynogaster* complex lineage, divergence time estimation
227 was performed on dataset B using a Bayesian relaxed-clock method implemented in BEAST
228 1.7.5 (Drummond *et al.*, 2012) on CIPRES (www.phylo.org: Miller *et al.*, 2010). The age of the
229 *Eurynogaster* complex is unknown as representatives of the genus are not known outside of

230 Hawaii and biota in the Hawaiian Islands does not fossilize well. There is a fossil available for
231 one genus that has an endemic species in the Hawaiian Islands (*Thinophilus* Wahlberg:
232 subfamily Hydrophorinae), but the wide range in ages of the fossils (Baltic amber -
233 Eocene/Oligocene; ca. 35–60 mya) compared with the very young ages of the islands make them
234 unsuitable for use in this analysis. Instead, we used three biogeographic calibrations based on the
235 island ages of Kauai, Maui and Hawaii (see Table S2a, Figure S2a in Supporting Information).
236 We also ran two alternate analyses for comparison based on evolutionary rates, described in
237 Appendix S2.

238 We selected two well-supported nodes for calibration from within a lineage of the genus
239 *Eurynogaster* that exhibit a clear progression from older to younger islands (Oahu to Maui to
240 Hawaii). We also performed a maximum likelihood ancestral state reconstruction in MESQUITE
241 v.2.7.2 (Maddison & Maddison, 2009) to assign ancestral areas to all nodes in the phylogeny.
242 We then selected a third well-supported node for calibration with a clear ancestral range
243 reconstruction to the oldest island of Kauai. All three nodes were calibrated with island dates
244 from Carson & Clague (1995) (Table S2a, Figure S2a). While island calibrations have been
245 widely used for the estimation of divergence times in Hawaiian lineages (*e.g.*, (Rubinoff &
246 Schmitz, 2010; Lerner *et al.*, 2011), it is plausible that divergence among populations occurred
247 prior to island emergence and was thus unrelated, or that it occurred well after the emergence of
248 the younger island (Heads, 2005). Standard deviations were chosen to accommodate some of this
249 uncertainty, including a biologically relevant timeframe during which habitat was likely
250 available on the islands, and the fact that the insects may have colonized the islands well before
251 or after they reached their peak heights (Table S2a).

252 Divergence time estimation was performed on dataset B described above. The same
253 seven gene concatenated data set (COI, COII, ND2, 12S, 16S, EF1 α and CAD) was analysed in
254 each of the analyses described here and in Appendix D. Partitions and the best fit models of
255 evolution for each partition were selected using BIC in PARTITIONFINDER (Lanfear *et al.*, 2012).
256 Initial analyses indicated that these models overparameterized the data in that the ESS values
257 were extremely low for some parameters, despite being run with very long chains (beast-users
258 Google group discussions). For the final runs, all GTR models were changed to HKY (Table
259 C.2) and ESS increased significantly while divergence times and tree topology did not change.
260 Base frequencies were estimated from the data. The partitioning scheme in the divergence rate
261 analyses differed only slightly from the island calibration analyses in that COI was assigned its
262 own partition (Table S1b). Site and clock models were unlinked and all partitions were analysed
263 using an uncorrelated lognormal relaxed clock except for the partition comprised of CAD
264 (positions 1 & 2) and the EF1 α intron, for which a strict clock could not be rejected and was thus
265 applied. The tree-shape prior was linked across partitions and the tree-shape prior was specified
266 as a Yule Process. The xml file was hand edited to include a starting tree, generated using
267 maximum likelihood in RAxML 3.7.2 (Stamatakis, 2006). Two independent MCMC searches
268 were conducted, each running for 50 million generations and sampled every 1000 generations.
269 The number of generations was selected to generate ESS values greater than 200 for each of the
270 parameters (Drummond *et al.*, 2007). Convergence was assessed using TRACER v. 1.7.5 and trees
271 were summarized to one Maximum Clade Credibility (MCC) tree using TREE ANNOTATOR v.
272 1.7.5 after removing a burn-in phase.

273

274 **RESULTS & DISCUSSION**

275 **Phylogenetic relationships within the endemic Hawaiian Dolichopodidae**

276 The family Dolichopodidae includes more than 6,800 described species (Yang *et al.*,
277 2006) in 232 genera worldwide (Pape & Thompson, 2013). A total of 29 genera are found in the
278 Hawaiian Islands. Of these, fifteen have been introduced in the past 150 years, most likely
279 through human activity, while the remaining fourteen genera present in the archipelago are
280 known to contain endemic Hawaiian taxa (Table 1). The relationships between *Campsicnemus*
281 and the *Eurynogaster* complex and the colonization history of these genera have remained an
282 open question, largely due to the difficulty of placing both in a subfamilial context. While
283 *Campsicnemus* is clearly placed in the subfamily Sympycninae, the placement of the
284 *Eurynogaster* complex has been more difficult to ascertain (see Appendix S3 in Supporting
285 Information). Individual taxa have previously been described as members of the subfamilies
286 Sympycninae, Hydrophorinae, and Thinophilinae. Hardy & Kohn (1964) considered
287 *Eurynogaster* and associated genera as part of the Sympycninae (see Figure S3a in Appendix 3).
288 Later, Evenhuis (2005) transferred the entire *Eurynogaster* complex to the Hydrophorinae. If the
289 current taxonomy placing these lineages in two separate subfamilies is correct, *Campsicnemus*
290 and the *Eurynogaster* complex represent independent colonizations to the Hawaiian Islands.

291 Molecular evidence demonstrates that the endemic Hawaiian dolichopodid fauna is
292 clearly the result of multiple colonizations to the archipelago (Figure 2, Figures S1a & S1b in
293 Appendix S1). Several key nodes are well supported and allow us to infer the history of the
294 Hawaiian Dolichopodidae. *Conchopus* (posterior probability (PP)=1, bootstrap (BS)=100: node
295 A, Figure 2), the *Eurynogaster* complex (PP=1, BS=100: node B, Figure 2), and *Campsicnemus*
296 (PP=1, BS=98: node C, Figure 2) are each strongly supported as monophyletic with respect to
297 other dolichopodid genera. Another key node that is strongly supported in both analyses (PP=1,

298 BS=99: node D, Figure 2) is the large clade that includes *Campsicnemus* and a number of non-
299 Hawaiian genera in the subfamily Sympycninae (e.g., *Sympycnus*, *Teuchophorus*) and does not
300 include the *Eurynogaster* complex. This demonstrates that there were at least three colonizations
301 to Hawaii by the family Dolichopodidae, one each by the three radiations: *Campsicnemus*, the
302 *Eurynogaster* complex, and *Conchopus*.

303 There is little support for the placement of *Thinophilus*, so its history of arrival to Hawaii
304 remains enigmatic (Figure 2, Figures S1a & S1b in Appendix S1). This genus is known primarily
305 from the Indo-Pacific, with one species each known from the Galapagos Islands and the
306 Hawaiian Islands. Previously it has only been collected from rocky, wet sand on the south shores
307 of Oahu (Carlton & Eldredge, 2009). The specimen included in this study represents the first
308 record from Hawaii Island and suggests that directed collecting on the south shores of Maui and
309 Kauai may turn up additional populations.

310 Three genera that contain endemic taxa were not included in this study because they are
311 difficult to collect and we did not recover them in our sampling. While their placement must
312 await future work, their omission here does not change the result that the Hawaiian Islands have
313 been colonized multiple times. An additional issue is that support at many nodes in this
314 phylogeny is poor, owing partially to the large divergences between the subfamilies and the
315 incomplete taxon sampling with this enormous family. These issues are also seen in previously
316 published phylogenetic studies of dolichopodid relationships (Lim *et al.*, 2010; Bernasconi *et al.*,
317 2007). The lack of support and long branches across most of the rest of this phylogeny preclude
318 identifying the specific sister lineages to the Hawaiian taxa (Figure 2).

319

320 **Phylogenetic Relationships in the *Eurynogaster* Complex**

321 The *Eurynogaster* complex, with 66 described species (Yang *et al.*, 2006) and about a dozen
322 awaiting description, comprises the fourth most species-rich radiation of Hawaiian flies, after the
323 Drosophilidae, *Campsicnemus*, and *Lispocephala* (Muscidae). Although the *Eurynogaster*
324 complex is one of the largest radiations of Diptera in Hawaii, phylogenetic relationships in this
325 group have never been studied. This collection of genera are hypothesized to have derived from a
326 single colonization to the Hawaiian Archipelago (Evenhuis, 2005). Little is known about the
327 biology of these species, but collecting observations suggest that species found on the forest
328 floor and on vegetation tend to be dull coloured, while species found in wet habitat, along seeps,
329 streams and on wet banks tend to have shiny metallic thoraces and/or abdomens.

330 Molecular phylogenetic results presented here show strong support for Evenhuis's (2005)
331 hypothesis of a monophyletic complex of related genera (PP=1, BS=100: Figure 2), as well as
332 strong support for several of the genera within this radiation. We focused on the smaller dataset
333 (dataset B) to address phylogenetic and biogeographic questions within the *Eurynogaster* genus
334 complex. Analyses of individual genes are presented in Figures S1e-S1l in Appendix S1, and
335 final data partitions and evolutionary models are reported in Table S1b in Appendix S1. Tree
336 topologies generated using ML and BI approaches of the concatenated dataset B were very
337 similar; at well-supported nodes, they are identical (Figures S1c & S1d).

338 In Figure 1, the maximum clade credibility tree from the Bayesian analysis performed in
339 BEAST is used to display the patterns within the *Eurynogaster* complex, and the following PP
340 and BS supports are from the BI performed in MrBayes and ML analysis performed in RAxML
341 (shown in Figures S1c & S1d). The *Eurynogaster* complex is split into two clades: Clade A
342 (*Adachia* + *Elmoia* + *Sigmatineurum* + *Major*, PP=1, BS=100) and Clade B (*Sweziella* +
343 *Arciellia* + *Uropachys* + *Eurynogaster*, PP=1, BS=100). Current sampling indicates that the

344 genus *Adachia* is monophyletic (PP=1, BS=100) and sister to a well-supported clade (PP=1,
345 BS=100) composed of the genera *Elmoia*, *Sigmatineurum* and *Major* (ESM Clade). Sampling
346 within the ESM clade is not extensive, with only a single representative each of *Sigmatineurum*
347 and *Major*. Two representatives of the genus *Elmoia* were sampled and our results indicate that
348 this genus is paraphyletic with respect to *Sigmatineurum* and *Major*. Denser sampling with the
349 ESM clade will be necessary to resolve the placement of the *Elmoia* taxa.

350 Clade B includes the large genus *Eurynogaster*, along with *Arciellia*, *Uropachys* and
351 *Sweziella*. *Sweziella*, represented by *S. tergoprolixa* from Maui, is the basal lineage within clade
352 B and sister to the lineage formed by *Arciellia*, *Uropachys* and *Eurynogaster* (PP=1, BS=91:
353 Figure 2). Current sampling indicates that the genus *Arciellia* and *Uropachys* are each
354 monophyletic (PP = 1, BS = 100 and PP=1, BS=100, respectively) and sister to one another
355 (PP=1, BS=100). *Eurynogaster* is strongly supported as monophyletic (PP=1, BS=100). This
356 genus is confusing taxonomically and is in need of revision. There are three undescribed
357 *Eurynogaster* species that were discovered as part of this work, *E. n. spp.* A–C. There are also a
358 number of taxa that, while morphologically similar to named taxa, show significant sequence
359 divergence from the described species. This sometimes corresponds to samples having been
360 taken from different islands. For example, *E. maculata* from Oahu is quite different from the *E.*
361 sp. nr. *maculata* samples collected from Maui (*E. sp. nr. maculata 141*) and Hawaii Island (*E. sp.*
362 nr. *maculata 115* and *126*) – they are 3.9% and 3.6% divergent at COI, respectively.
363 Furthermore, one exemplar of *E. maculata* from Maui is quite similar to *E. sp. nr. maculata 141*
364 – it is identical at COI – suggesting that cryptic species may exist within the concept of what we
365 currently recognize as *E. maculata*. This phenomenon is common in large evolutionary
366 radiations in Hawaii (e.g., Bennett & O’Grady, 2011). Another species we sampled, *E.*

367 *cilifemorata*, also seems to be a complex of species sampled from Maui and Oahu. Additional
368 sampling within *Eurynogaster*, as well as thorough taxonomic revisions of the genera within this
369 complex, will be necessary to better delineate species within this rapidly evolving clade.

370 Finally, four new species within the *Eurynogaster* complex were discovered as a result of
371 this project, three within *Eurynogaster* and one within *Sigmatineurum*. An additional five
372 possible new species (*Adachia* - 1 species; *Eurynogaster* - 4 species) were identified (labeled as
373 “sp. nr.”) and are in the process of examination to confirm their taxonomic status.

374

375 **Arrival times and biogeography**

376 We estimate that the *Eurynogaster* complex arrived in the Hawaiian Archipelago
377 11.83 (9.08-15.04) Ma, approximately within the timeframe that the Northwest Hawaiian Islands
378 of La Perouse, Necker, and Gardner were providing substantial high island habitat (Price &
379 Clague, 2002). This ancient lineage arrived well before the formation of the current high islands
380 about 5 Ma and the arrival of *Campsicnemus*, which is estimated to have occurred approximately
381 4.6 Ma (Goodman *et al.*, 2014). Early diversification into five of the eight contemporary genera
382 took place in the older, now eroded, northwest Hawaiian Islands, and five colonizations of these
383 ancestral lineages into the current main (high) islands are needed to explain the contemporary
384 patterns of diversity. All of the diversification within the crown groups has occurred within the
385 past 5 million years (Myr), the timeframe of the current high islands. The most speciose lineage
386 within the *Eurynogaster* complex, the genus *Eurynogaster*, began diversifying approximately 2.6
387 (95% HPD: 1.94–3.26) Ma, about the time Oahu and Maui Nui were forming. We estimate that
388 the small endemic dolichopodid genus *Conchopus* arrived quite recently – 1.77 (95% HPD:
389 1.09–2.6) Ma (Figure 1).

391 Within the *Eurynogaster* complex, a number of classic biogeographic patterns are
392 evident, some of which are significantly different from what is observed in other large radiations.
393 First, a progression rule pattern (Hennig, 1966) is common in hotspot archipelagos where islands
394 appear along a chronosequence. The typical progression rule pattern seen in Hawaii occurs when
395 the most basally branching taxon is present on Kauai, the oldest island, with more recently
396 branching taxa present on the progressively younger islands of Oahu, Molokai, Maui and Hawaii
397 (Wagner & Funk, 1995). While the progression rule is commonly observed in both the Hawaiian
398 *Drosophila* (Bonacum *et al.*, 2005) and *Campsicnemus* (Goodman *et al.*, 2014) lineages, it is less
399 prevalent in *Eurynogaster*. Only a single lineage of the genus *Eurynogaster* shows a clear
400 progression from Oahu to Maui to Hawaii (Figure 1).

401 Another phenomenon observed in Hawaiian lineages is within-island diversification,
402 where species break up to diversify into new populations and eventually sibling species on the
403 same island. This has been thought to be an uncommon occurrence, in part because it is fairly
404 uncommon across the historically best-studied group in the islands, the Hawaiian *Drosophila* –
405 for whom diversification primarily occurs following inter-island dispersal. However, even within
406 this iconic group, there are examples and it has been very well studied in the sympatric sibling
407 pair *D. silvestris* and *D. heteroneura* (Carson, 1982; DeSalle *et al.*, 1987; Price & Boake, 1995).
408 Newer examples are now accumulating across taxonomic groups (*e.g.*: Goodman *et al.* 2012;
409 Eldon *et al.* 2013; Bennett & O’Grady 2013; Liebherr, 2015), exposing how variable a process
410 diversification can be, and how dependent it is on the dispersal capabilities of the groups studied
411 (Price & Wagner, 2004). The *Eurynogaster* complex shows at least five clear instances of
412 within-island diversification. *Uropachys* is a genus of 6 species only known from Kauai. Three
413 *Uropachys* species were sampled for this study and are strongly supported as a monophyletic

414 clade, indicating they diversified there. This pattern is also observed in *Adachia*, where *A.*
415 *hispidata* and *A. apicenigra* have both formed on Hawaii, and in several clades of the genus
416 *Eurynogaster* where diversification has occurred on Oahu and Hawaii. Within-island
417 diversification is also observed in some lineages of the other major dolichopodid radiation in
418 Hawaii, the *Campsicnemus* (Goodman *et al.*, 2014).

419

420 **Colonization of and diversification within the Hawaiian Islands**

421 It is clear that the endemic Dolichopodidae of Hawaii arrived to the archipelago in at
422 least three successful colonization to radiation sequences over the last 12 Myr (*Eurynogaster*
423 complex, 11.8 Ma; *Campsicnemus*, 4.6 Ma; *Conchopus*, 1.8 Ma) –demonstrating that dispersal to
424 and establishment within this remote island group is more common than has been documented in
425 other groups. This is fascinating because it means that three separate radiations occurred despite
426 the excellent dispersal capabilities of these animals. In order to multiply into radiations, they
427 must have been able to generate reproductive isolation rapidly enough to overcome gene flow
428 from their highly vagile conspecifics. Members of this family are known to have complicated
429 courtship behavior (Zimmer *et al.* 2003). Though this has never been studied in the Hawaiian
430 fauna, it may be a contributing factor to the development of reproductive isolation as has been
431 shown with the Hawaiian *Drosophilidae* (Kaneshiro, 1976; Price & Boake, 1995), *Laupala*
432 (Grace & Shaw, 2011) and *Nesosydne* (Goodman *et al.* 2015), and suggests fruitful research
433 directions. There seems to be no correlation between the age of colonization and the diversity of
434 each lineage.

435 MacArthur & Wilson (1967) stated that “an island is closed to a particular species when
436 the species is excluded ... by competitors already in residence...”. The Hawaiian Islands were

437 clearly not closed to dolichopodid flies that arrived after the first wave 12 Ma. This suggests that,
438 at the arrival of each new lineage, there was still plenty of ecological opportunity available or
439 these insects are ecologically labile and able to adapt easily when faced with niches already
440 occupied by competitors. Both statements may be true.

441 Very little is known about the ecology of the Dolichopodidae in Hawaii, but they are
442 known to be predatory from observations elsewhere in the world (Ulrich, 2005). For the
443 *Eurynogaster* complex (which only occur in Hawaii), there is only a single published account in
444 the literature that includes ecological observations (Williams, 1938). Despite the dearth of
445 ecological data available, we have some evidence to support the idea that the dolichopodids seem
446 to adapt easily. In our 2014 study, we used morphological colouring together with field
447 observations to infer that the Hawaiian *Campsicnemus* have rapidly diversified into three
448 ecological types: (1) brown, low vegetation and litter dwellers, (2) black water skaters and (3)
449 yellow canopy dwellers. Two of these are not known outside the Pacific, and one is not known
450 outside Hawaii (Goodman *et al.* 2014). The Hawaiian *Conchopus* may have also undergone a
451 shift in ecological type. This lineage can be traced back to East Asia (Takagi, 1965), where they
452 are known primarily from barnacle colonies in the marine tidal zone, living in the interstices or
453 in nearby cracks in the rocks and feeding on tiny invertebrates (Sunose & Sato, 1994). There are
454 no native barnacles in the Hawaiian Islands, and *Conchopus* there are known from *puka* (holes)
455 in beach rocks deriving from volcanic flows. Once established in this habitat, they radiated into
456 six known species.

457 Prevailing dogma among Hawaiian evolutionary biologists in the past 30 years has been
458 that colonization events to the archipelago are rare and colonization within the islands follow a
459 few well-defined patterns, such as the progression rule (Wagner & Funk 1995). Recent

460 molecular phylogenetic studies are beginning to overturn these overly simplified notions
461 (Heaney 2007; Bellemain & Rickliffs 2008), finding that colonization and diversification are
462 based on a combination of factors. These include characters linked to the dispersal and
463 adaptability of the lineage in question and the ecological and environmental context of the
464 islands when that lineage arrives. The current study highlighting the multiple colonizations that
465 Dolichopodidae have undergone in the past and the specific patterns of diversification within the
466 *Eurynogaster* complex further demonstrate that there are no simple “rules” and each colonization
467 event should be considered an independent event. The Hawaiian Dolichopodidae are an
468 intriguing example of repeated, overlapping evolutionary radiations, ripe for field studies that
469 can begin to untangle their propensity to speciate and ecological lability.

470

471

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482

484 **REFERENCES**

- 485 Bellemain, E. & Ricklefs, R.E. (2008) Are islands the end of the colonization road?
 486 *Trends in Ecology and Evolution*, **23**, 461-468.
- 487 Bennett, G.M. & O’Grady, P.M. (2011) Review of the native Hawaiian leafhopper
 488 genus *Nesophrosyne* (Hemiptera: Cicadellidae: Deltocephalinae) with
 489 descriptions of eight new species associated with *Broussaisia arguta*. *Zootaxa* **2805**, 1-
 490 25.
- 491 Bennett, G.M. & O’Grady, P.M. (2013) Historical biogeography and ecological
 492 opportunity in the adaptive radiation of native Hawaiian leafhoppers (Cicadellidae:
 493 *Nesophrosyne*). *Journal of Biogeography*, **40(8)**, 1512-1523.
- 494 Bernasconi, M.V., Pollet, M. & Ward, P.I. (2007) Molecular systematics of
 495 Dolichopodidae (Diptera) inferred from COI and 12S rDNA gene sequences based on
 496 European exemplars. *Invertebrate Systematics*, **21**, 453-470.
- 497 Bess, E.C., Catanach, T.A. & Johnson, K.P. (2013) The importance of molecular dating
 498 analyses for inferring Hawaiian biogeographical history: a case study with bark lice
 499 (Psocidae: *Ptycta*). *Journal of Biogeography*, **41**, 158-167.
- 500 Bonacum, J., DeSalle, R., O’Grady, P.M., Olivera, D.S.C.G., Wintermute, J. & Zilversmit, M.
 501 (2001) New nuclear and mitochondrial primers for systematics and comparative
 502 genomics in Drosophilidae. *Drosophila Information Service*, **84**, 201-204.
- 503 Bonacum, J., O’Grady, P.M., Kambysellis, M. & DeSalle, R. (2005) Phylogeny and age of
 504 diversification of the planitibia species group of the Hawaiian *Drosophila*. *Molecular*
 505 *Phylogenetics and Evolution*. **37**, 73–82.
- 506 Carlton, J.T. & L.G. Eldredge (2009). Marine Bioinvasions of Hawai‘i: The Introduced
 507 and Cryptogenic Marine and Estuarine Animals and Plants of the Hawaiian
 508 Archipelago. *Bishop Museum Bulletin in Cultural and Environmental Studies* 4,
 509 pp. 202. Bernice P. Bishop Museum, Honolulu.
- 510 Carson, H. L. 1982. Evolution of *Drosophila* on the newer Hawaiian volcanoes. *Heredity*, **48**, 3–
 511 25.
- 512 Carson, H.L. & Kaneshiro, K.Y. (1976) *Drosophila* of Hawaii - systematics and ecological
 513 genetics. *Annual Review of Ecology and Systematics*, **7**, 311-345.
- 514 Carson, H.L. & Clague, D.A. (1995) Geology and biogeography of the Hawaiian Islands.
 515 *Hawaiian biogeography: evolution on a hotspot archipelago* (ed. by W.L. Wagner and
 516 V.A. Funk), pp. 14-29. Smithsonian Institution Press, Washington, D.C.
- 517 Casquet, J., Bourgeois, Y.X., Cruaud, C., Gavory, F., Gillespie, R.G. & Thebaud, C. (2015)
 518 Community assembly on remote islands: a comparison of Hawaiian and Mascarene
 519 spiders. *Journal of Biogeography*, **42**, 39-50.
- 520 Cibois, A., Beadell, J.S., Graves, G.R., Pasquet, E., Slikas, B., Sonsthagen, S.A., Thibault, J., &
 521 Fleischer, R.C. (2011) Charting the course of reed-warblers across the Pacific islands.
 522 *Journal of Biogeography*, **38**, 1963-1975.
- 523 Clague, D.A. (1996) The growth and subsidence of the Hawaiian-Emperor volcanic chain. *The*
 524 *origin and evolution of Pacific island biotas, New Guinea to eastern Polynesia: patterns*
 525 *and processes* (ed. by A. Keast and S.E. Miller), pp. 35-50. SPB Academic Publishing
 526 Amsterdam.
- 527 Clague, D.A. & Dalrymple, G.B. (1987) The Hawaiian-Emperor volcanic chain: part 1. Geologic
 528 evolution. *United States Geological Survey Professional Paper*, **1350**, 5-54.

- 529 Clary, D.O. & Wolstenholme, D.R. (1985) The mitochondrial DNA molecule of *Drosophila*
530 *yakuba*: Nucleotide sequence, gene organization and genetic code. *Journal of Molecular*
531 *Evolution*, **22**, 252-271.
- 532 Collins, K.P. & Wiegmann, B.M. (2002) Phylogenetic relationships and placement of the
533 Empidoidea (Diptera: Brachycera) based on 28S rDNA and EF-1alpha sequences. *Insect*
534 *Systematics and Evolution*, **33**, 421-444.
- 535 Costello, A. & Motley, T. J. (2001) Molecular systematics of *Tetraplasandra*, *Munroidendron*
536 and *Reynoldsia sandwicensis* (Araliaceae) and the evolution of superior ovaries in
537 *Tetraplasandra*. *Edinburgh Journal of Botany*, **58**, 229-242.
- 538 DeSalle, R. (1992) The origin and possible time of divergence of the Hawaiian Drosophilidae -
539 evidence from DNA-sequences. *Molecular Biology and Evolution*, **9**, 905.
- 540 DeSalle, R., Freedman, R., Prager, E.M. & Wilson, A.C. (1987) Tempo and mode of sequence
541 evolution in mitochondrial DNA of Hawaiian *Drosophila*. *Journal of Molecular*
542 *Evolution*, **26**, 157-164.
- 543 Drummond, A.J., Ho, S.Y.W., Rawlence, N. & Rambaut, A. (2007) A Rough Guide to BEAST
544 1.4 (Program Manual). In:
- 545 Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with
546 BEAUTi and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969-1973.
- 547 Duncan, R.A. & Keller, R.A. (2004) Radiometric ages for basement rocks from the Emperor
548 Seamounts, ODP Leg 197. *Geochemistry Geophysics Geosystems* **5**, Q08L03,
549 doi:10.1029/2004GC000704.
- 550 Eldon, J., Price, J.P., Magnacca, K. & Price, D.K. (2013). Patterns and processes in complex
551 landscapes: testing alternative biogeographical hypotheses through intergrated analysis of
552 phylogeography and community ecology in Hawaii. *Molecular Ecology*. **22**, 3613-3628.
- 553 Evenhuis, N.L. (2005) A review of the genera comprising species of the genus *Eurynogaster*
554 *sensu* Hardy & Kohn, 1964 in Hawaii (Diptera: Dolichopodidae). *Zootaxa*, **1017**, 39-60.
- 555 Filardi, C.E. & Moyle, R.G. (2005) Single origin of a pan-Pacific bird group and upstream
556 colonization of Australasia. *Nature*, **438**, 216-219.
- 557 Gillespie R.G. & Roderick G.K. (2002) Arthropods on islands: colonization, speciation, and
558 conservation. *Annual Review of Entomology* **47**, 595-632.
- 559 Gillespie, R.G., Baldwin, B.G., Waters, J.M., Fraser, C., Nikula, R., & Roderick, G.K. (2012)
560 Long-distance dispersal – a framework for hypothesis testing. *Trends in Ecology &*
561 *Evolution*, **27(1)**, 47-56.
- 562 Goodman, K.R., Welter, S.C. & Roderick, G.K. (2012) Genetic divergence is decoupled from
563 ecological diversification in the Hawaiian *Nesosydne* planthoppers. *Evolution*. **66**, 2798–
564 2813.
- 565 Goodman, K.R. & O'Grady, P.M. (2013) Molecular phylogeny and biogeography of the
566 Hawaiian craneflies *Dicranomyia* (Diptera: Limoniidae). *PLoS One*, **8(9)**:e73019.
- 567 Goodman, K.R., Evenhuis, N.L., Bartosoya-Sojkova, P. & O'Grady, P.M. (2014) Diversification
568 in Hawaiian long-legged flies (Diptera: Dolichopodidae: *Campsicnemus*): Biogeographic
569 isolation and ecological adaptation. *Molecular Phylogenetics and Evolution*, **81**, 232-241.
- 570 Goodman, K.R., Kelley, J.P., Welter, S.C., Roderick, G.K. & Elias, D.O. (2015). Rapid
571 diversification of sexual signals in Hawaiian *Nesosydne* planthoppers (Hemiptera:
572 Delphacidae): the relative role of neutral and selective forces. *Journal of Evolutionary*
573 *Biology*. **28(2)**, 415-427.

- 574 Grace, J. L. and Shaw, K. L. (2011) Coevolution of male signal and female preference during
575 early lineage divergence of the Hawaiian cricket, *Laupala cerasina*. *Evolution*. **65**, 2184-
576 2196.
- 577 Haines, W.P. & D. Rubinoff (2012) Molecular phylogenetics of the moth genus *Omiodes*
578 Guenee (Crambidae: Spilomelinae), and the origins of the Hawaiian lineage. *Molecular*
579 *Phylogenetics and Evolution*, **65**, 305-316.
- 580 Haines, W.P., Schmitz, P., Rubinoff, D. (2014) Ancient diversification of Hyposmocoma moths
581 in Hawaii. *Naure Communications*, **5**, 3502, doi: 10.1038/ncomms4502.
- 582 Harbaugh, D. T. & Baldwin, B. G. (2007) Phylogeny and biogeography of the sandalwoods
583 (*Santalum*, Santalaceae): repeated dispersals throughout the Pacific. *American Journal*
584 *of Botany*, **94**, 1028–1040.
- 585 Hardin, J. (1960) The Competitive Exclusion Principle. *Science*. **131**, 1292-1297.
- 586 Hardy, D.E. & Kohn, M.A. (1964) Dolichopodidae. *Insects of Hawaii*. University of
587 Hawaii Press, Honolulu, HI, **vol 11**, 1-256.
- 588 Heads, M. (2005) Dating nodes on molecular phylogenies: a critique of molecular biogeography.
589 *Cladistics*, **21**, 62-78.
- 590 Heany, L.R. (2007) Is a new paradigm emerging for oceanic island biogeography? *Journal of*
591 *Biogeography*, **34**, 753-757.
- 592 Hembry D.H., Kawakita A., Gurr N.E., Schmaedick M.A., Baldwin B.G., Gillespie R.G. (2013)
593 Non-congruent colonizations and diversification in a coevolving pollination mutualism
594 on oceanic islands. *Proceedings of the Royal Society B*, **280**, 20130361.
- 595 Hennig, W. (1966) *Phylogenetic Systematics*. University of Illinois Press, Urbana, Illinois.
- 596 Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogenetic trees. **17**,
597 754-5.
- 598 Jordan, S., Simon, C. & Polhemus, D. (2003) Molecular Systematics and Adaptive Radiation of
599 Hawaii's Endemic Damselfly Genus *Megalagrion* (Odonata: Coenagrionidae). *Systematic*
600 *Biology*. **52(1)**, 89-109.
- 601 Kaneshiro, K.Y. (1976) Ethological Isolation and Phylogeny in the Planitibia Subgroup of
602 Hawaiian *Drosophila*. *Evolution*. **30(4)**, 740-745.
- 603 Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. (2012) PartitionFinder: combined selection
604 of partitioning schemes and substitution models for phylogenetic analyses. *Molecular*
605 *Biology and Evolution*, **29**, 1695-1701.
- 606 Lapoint, R.T., Gidaya, A., & O'Grady, P.M. (2011) Phylogenetic relationships in the spoon
607 tarsus subgroup of Hawaiian *Drosophila*; Conflict and concordance between gene trees.
608 *Molecular Phylogenetics and Evolution*, **58**, 492-501.
- 609 Lapoint, R.T., O'Grady, P.M. & Whiteman, N.K., (2013) Diversification and Dispersal of the
610 Hawaiian Drosophilidae: the evolution of *Scaptomyza*. *Molecular Phylogenetics and*
611 *Evolution*, **69(1)**, 95-108.
- 612 Lapoint, R.T., Magnacca, K.N., and O'Grady, P.M. (2014) Phylogenetics of the antopocerus-
613 modified tarsus clade of Hawaiian *Drosophila*: Diversification across the Hawaiian
614 Islands. *PLoS ONE*, **9(11)**, e113227.
- 615 Liebherr, J.K. (2015) The *Mecyclothorax* beetles (Coleoptera, Carabidae, Moriomorphini) of
616 Haleakala, Maui: Keystone of a hyperdiverse Hawaiian radiation. *Zookeys*. **544**, 1-407.
- 617 Lim, G.S., Hwang, W.S., Kutty, S.N., Meier, R. & Grootaert, P. (2010) Mitochondrial and
618 nuclear markers support the monophyly of Dolichopodidae and suggest a rapid origin of
619 the subfamilies (Diptera: Empidoidea). *Systematic Entomology*, **35**, 59-70.

- 620 Lerner, H.R.L., Meyer, M., James, H.F., Hofreiter, M. & Fleischer, R.C. (2011) Multilocus
621 Resolution of Phylogeny and Timescale in the Extant Adaptive Radiation of Hawaiian
622 Honeycreepers. *Current Biology*, **21**, 1-7.
- 623 MacArthur, R.H. & Wilson, E.O. (1967). *The Theory of Island Biogeography*. pp. 203. Princeton
624 University Press, Princeton.
- 625 Maddison, W.P. & Maddison, D.R. (2009) *Mesquite: a modular system for evolutionary*
626 *analysis*. Version 2.72. <http://mesquiteproject.org>.
- 627 Medeiros, M.J., Davis, D., Howarth, F.G. & Gillespie, R.G. (2009) Evolution of cave living in
628 Hawaiian *Schrankia* (Lepidoptera: Noctuidae) with description of a remarkable new cave
629 species. *Zoological Journal of the Linnean Society*. **156**, 114-139.
- 630 Medeiros, M.J. & Gillespie, R.G. (2011) Biogeography and the evolution of flightlessness in a
631 radiation of Hawaiian moths (Xyloryctidae: *Thyrocopa*). *Journal of Biogeography*. **38**,
632 101-111.
- 633 Mendelson, T.C. & Shaw, K.L. (2005). Sexual Behavior: rapid speciation in an arthropod.
634 *Nature*, **433**, 375-376.
- 635 Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) "Creating the CIPRES Science Gateway for
636 inference of large phylogenetic trees" *Proceedings of the Gateway Computing*
637 *Environments Workshop (GCE), 14 Nov. 2010* (ed by, pp. 1-8. New Orleans, LA.
- 638 Moulton, J.K. & Weigmann, B.M. (2004) Evolution and phylogenetic utility of cad
639 (rudimentary) among Mesozoic-aged Eremoneuran Diptera (Insecta). *Molecular*
640 *Phylogenetics and Evolution*, **31**, 363-378.
- 641 Nishida, G.N. (2002) Hawaiian terrestrial arthropod checklist. Fourth edition. *Bishop Museum*
642 *Technical Report* **22**, 1-313.
- 643 O'Grady, P.M. and DeSalle, R. (2008) Out of Hawaii: The biogeographic history of the genus
644 *Scaptomyza* (Diptera: Drosophilidae). *Biology Letters* **4(2)**, 195-199.
- 645 O'Grady, P.M., Magnacca, K.N. & Lapoint, R.T. (2009) *Drosophila*. *Encyclopedia of Islands*
646 (ed. by R.G. Gillespie and D.A. Clague), pp. 232-235. University of California Press,
647 Berkeley, CA.
- 648 O'Grady, P.M., Lapoint, R.T., Bonacum, J., Lasola, J., Owen, E., Wu, Y., & DeSalle, R. (2011)
649 Phylogenetic and ecological relationships of the Hawaiian *Drosophila* inferred by
650 mitochondrial DNA analysis. *Molecular Phylogenetics and Evolution*. **58**, 244-256.
- 651 Pape, T. & Thompson, F.C. (editors). (2013) *Systema Dipteroorum*. Version 1.5. Available at:
652 <http://www.diptera.org/>. [Last accessed 16 October 2014].
- 653 Plunkett, G. M., Soltis, D. E. & Soltis, P. S. (1997) Clarification of the relationship between
654 Apiaceae and Araliaceae based on matK and rbcL sequence data. *American Journal of*
655 *Botany*. **84**, 565-580.
- 656 Price, D. K. & Boake, C. R. B. (1995). Behavioral reproductive isolation in *Drosophila*
657 *silvestris*, *D. heteroneura* and their F1 hybrids (Diptera: Drosophilidae). *Journal of Insect*
658 *Behaviour* **8**, 595-616.
- 659 Price, J.P. & Clague, D.A. (2002) How old is the Hawaiian biota? Geology and phylogeny
660 suggest recent divergence. *Proceedings of the Royal Society of London, B*, **269**, 2429-
661 2435.
- 662 Price, J.P. & Wagner, W.L. (2004) Speciation in Hawaiian angiosperm lineages: cause,
663 consequence, and mode. *Evolution*. **58**, 2185-2200.

- 664 Rambaut, A. & Drummond, A.J. (2012) *Tracer v1.7.2*, obtained from the “Workshop on
665 Molecular Evolution”, August 2011. *Tracer v1.5* is available from
666 <http://beastbioedacuk/Tracer>.
- 667 Rubinoff, D. & Schmitz, P. (2010) Multiple aquatic invasions by an endemic, terrestrial hawaiian
668 moth radiation. *Proceedings of the National Academy of Sciences*, **107**, 5903-5906.
- 669 Shapiro, L.H., Strazanac, J.S. & Roderick, G.K. (2006) Molecular phylogeny of Banza
670 (Orthoptera: Tettigoniidae), the endemic katydids of the Hawaiian Archipelago.
671 *Molecular Phylogenetics and Evolution*. **41**, 53-63.
- 672 Sharp, W.D. & Clague, D.A. (2006) 50-Ma Initiation of Hawaiian-Emperor Bend Records Major
673 Change in Pacific Plate Motion. *Science*, **313**, 1281-1284.
- 674 Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution,
675 weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of
676 conserved polymerase chain reaction primers. *Annals of the Entomological Society of*
677 *America*, **86**, 651-701.
- 678 Stamatakis, A. (2006) RAxML-VI-HPC: Maximum Likelihood-based Phylogenetic Analyses
679 with Thousands of Taxa and Mixed Models. *Bioinformatics*, **22**, 2688-2690.
- 680 Sunose, T. & Sato, M. (1994) Morphological and Ecological Studies on a Marine Shore
681 Dolichopodid Fly, *Conchopus borealis* Takagi (Diptera, Dolichopodidae). *Japanese*
682 *Journal of Entomology*. **62(4)**, 651-660.
- 683 Takagi, S. (1965) A contribution to the knowledge of the marine shore dolichopodidae of Japan
684 (Diptera). *Insecta Matsumaurana*. **27(2)**, 49-84.
- 685 Tenorio, J.M., 1969. Supplement, Diptera: Dolichopodidae, Appendix (Phoridae), Volume 11.
686 *Insects of Hawaii*, Honolulu, v + 73 pp.
- 687 Ulrich, H. (2005) Predation by adult Dolichopodidae (Diptera): a review of literature with an
688 annotated prey-predator list. *Studia Dipterologica*, **11**, 369-403.
- 689 Williams, F.X. (1938) Biological studies in Hawaiian water-loving insects part III, Diptera or
690 flies B. Asteiidae, Syrphidae and Dolichopodidae. *Proceedings of the Hawaiian*
691 *Entomological Society*, **X**, 281-315.
- 692 Wagner, W.L. & Funk, V.A. (editors) (1995) *Hawaiian biogeography: evolution on a hot spot*
693 *archipelago*. Smithsonian Institution Press, Washington and London, 467.
- 694 Wilson, J.T. (1963) A Possible Origin of the Hawaiian Islands. *Canadian Journal of Physics*, **41**,
695 863-870.
- 696 Yang, D., Zhu, Y., Wang, M. & Shang, L. (2006) *World Catalog of Dolichopodidae (Insecta:*
697 *Diptera)*. China Agricultural University Press, Beijing.
- 698 Zimmer, M., Diestelhorst, O. & Lunau, K. (2003) Courtship in long-legged flies (Diptera:
699 Dolichopodidae): function and evolution of signals. *Behavioral Ecology*. **14**, 526-530.
- 700 Zimmerman, E.C. (2001) Volume 1 Reissue - Introduction with a new preface and dedication.
701 *Insects of Hawaii*, Honolulu, 206 pp.
702

704 **FIGURES**

705

706 **Figure Legends**

707

708 **Figure 1.** Maximum clade credibility tree summarizing BEAST analysis of the *Eurynogaster*
709 complex with geologic history of the archipelago. Node bars are the 95% highest posterior
710 density intervals of the divergence time estimate – bars that are offset are not to scale. Islands
711 that each specimen was collected from are shown next to each tip.

712 **Figure 2.** Majority rule consensus tree summarizing Bayesian analysis of the endemic
713 Dolichopodidae, with the large radiations, *Eurynogaster* complex and *Campsicnemus* collapsed.
714 Bayesian posterior probabilities (Mr. Bayes) and bootstrap supports from the maximum
715 likelihood analysis (RAxML) are displayed as ovals.

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Table 1 (on next page)

Table 1

Composition and status of Dolichopodidae fauna of Hawaii. Genera with endemic species are **boldface**.

1 **Table 1.** Composition and status of Dolichopodidae fauna of Hawaii. Genera with endemic
 2 species are **boldface**.

Genus	Total spp. in Hawaii	Number of endemic spp.	Number of non-endemic spp.	Number of described spp, included in this study (undescribed spp.) [included from outside Hawaii]
<i>Achradocera</i>	2	0	2	0
<i>Amblypsilopus</i>	1	0	1	0
<i>Asyndetus</i>	1	1	0	0
<i>Austrosciapus</i>	1	0	1	0
<i>Campsicnemus</i>	183	183	0	70[14]
<i>Chrysosoma</i>	2	0	2	0
<i>Chrysotus</i>	1	0	1	1(1)[1]
<i>Conchopus</i>	6	6	0	3
<i>Condylostylus</i>	1	0	1	1
<i>Dactylomyia</i>	1	0	1	0
<i>Diaphorus</i>	1	0	1	0
<i>Dolichopus</i>	1	0	1	1
<i>Eurynogaster</i> complex				
<i>Adachia</i>	6	6	0	2 (1)
<i>Arciellia</i>	3	3	0	2
<i>Elmoia</i>	8	8	0	2
<i>Eurynogaster</i>	23	23	0	6 (7)
<i>Major</i>	1	1	0	1
<i>Sigmatineurum</i>	11	11	0	1
<i>Sweziella</i>	7	7	0	1
<i>Uropachys</i>	7	7	0	3
<i>Hydrophorus</i>	2	2	0	0
<i>Krakatauia</i>	1	0	1	0
<i>Medetera</i>	1	0	1	0
<i>Paraliancalus</i>	2	2	0	0
<i>Pelastoneurus</i>	1	0	1	0
<i>Sympycnus</i>	1	0	1	1[5]
<i>Syntormon</i>	1	0	1	1[5]
<i>Tachytrechus</i>	1	0	1	1
<i>Thinophilus</i>	1	1	0	1

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Table 2 (on next page)

Table 2

Primer names and references. Mitochondrial primer numbers correspond to the location in the *Drosophila yakuba* mitochondrial genome (Clary & Wolstenholme, 1985) . Sequences with no reference were designed as a part of this study.

1 **Table 2.** Primer names and references. Mitochondrial primer numbers correspond to the location
 2 in the *Drosophila yakuba* mitochondrial genome (Clary & Wolstenholme, 1985). Sequences with
 3 no reference were designed as a part of this study.

Primer name	Length	Genome	Reference or Sequence
Cytochrome Oxidase I (COI): 2183 or 2640 and 3041	829	mitochondrial	(Bonacum <i>et al.</i> , 2001)
Cytochrome Oxidase II (COII): 3037 and 3771	681	mitochondrial	(Bonacum <i>et al.</i> , 2001)
NADH Dehydrogenase 2 (ND2): 192 and 732	527	mitochondrial	(Bonacum <i>et al.</i> , 2001)
16S	530	mitochondrial	(DeSalle, 1992)
12S	559	mitochondrial	F14233, R14922 (Simon <i>et al.</i> , 1994) 12S_exF: 5'-TCC AGT ACA TCT ACT ATG TTA CG-3' 12S_inF: 5'-ATG TGT RCA TAT TTT AGA GC-3' 12S_inR: 5'-TAT TRG CTA AAT TTG TGC CAG C-3'
rudimentary (CAD), nested reaction: 320F and 843R, 338F and 680R	896	nuclear	(Moulton & Weigmann, 2004)
EF1 α A	1036	nuclear	EF4 and EF5 (Collins & Wiegmann, 2002) EFF: 5'-CNC CTG GCC ATC GTG ATT TC-3' EFR: 5'-CAG CAT CTC CYG ATT TGA TGG C-3'
EF1 α B	858	nuclear	EFF_B: 5'-GAT TAC TGG TAC ATC TCA AGC-3' EFR_B: 5'-TAG CAG CAT CYC CYG ATT-3'

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Figure 1

Figure 1

Maximum clade credibility tree summarizing BEAST analysis of the *Eurynogaster* complex with geologic history of the archipelago. Node bars are the 95% highest posterior density intervals of the divergence time estimate - bars that are offset are not to scale. Islands that each specimen was collected from are shown next to each tip.

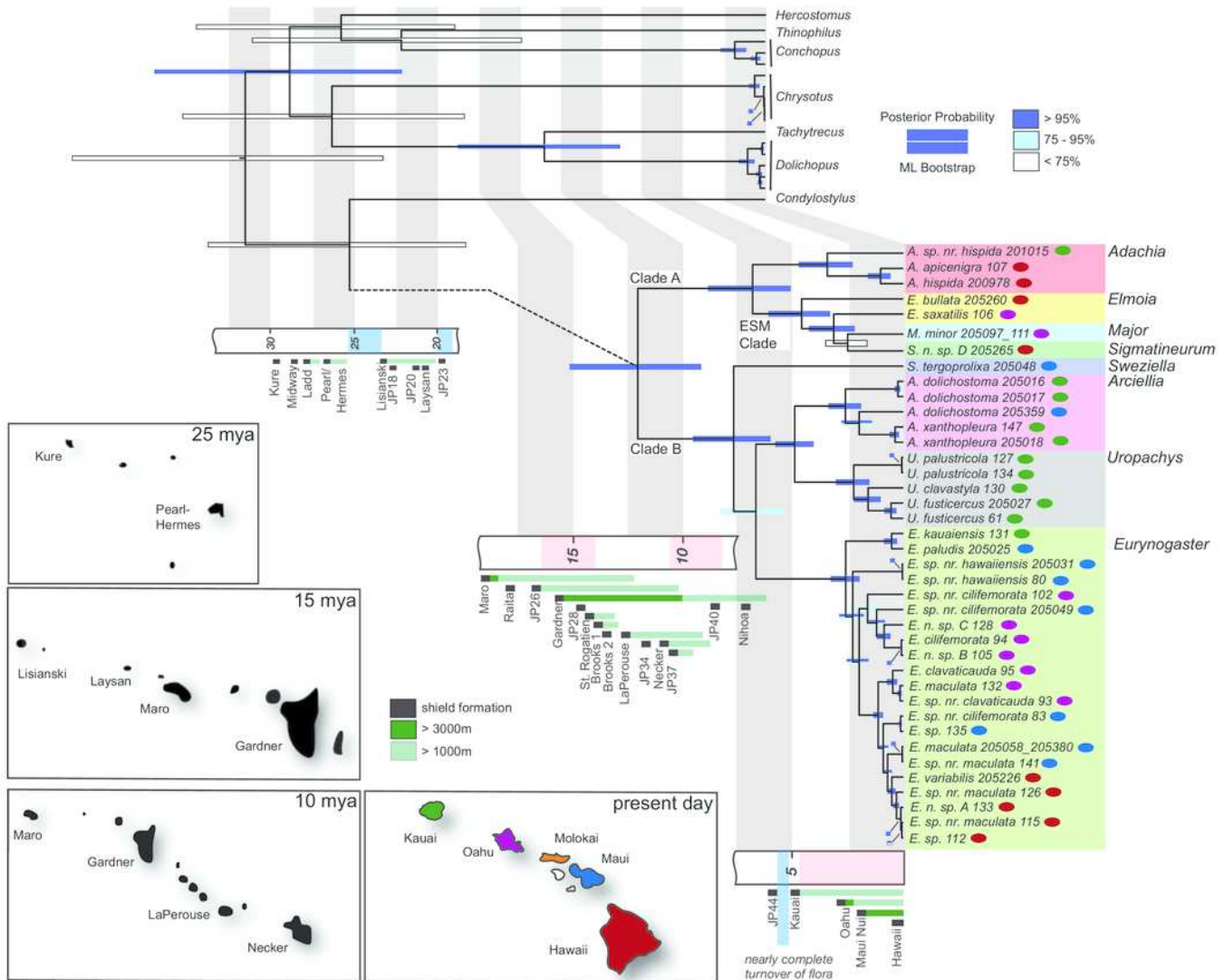


Figure 2

Figure 2

Majority rule consensus tree summarizing Bayesian analysis of the endemic Dolichopodidae, with the large radiations, *Eurynogaster* complex and *Campsicnemus* collapsed. Bayesian posterior probabilities (Mr. Bayes) and bootstrap supports from the maximum likelihood analysis (RAxML) are displayed as ovals.

