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# Diversification in Hawaiian long-legged flies (Diptera: Dolichopodidae: *Campsicnemus*): Biogeographic isolation and ecological adaptation

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## ABSTRACT

Flies in the genus *Campsicnemus* have diversified into the second-largest adaptive radiation of Diptera in the Hawaiian Islands, with 179 Hawaiian endemic species currently described. Here we present the first phylogenetic analysis of *Campsicnemus*, with a focus on the Hawaiian fauna. We analyzed a combination of two nuclear (CAD, EF1 $\alpha$ ) and five mitochondrial (COI, COII, 12S, 16S, ND2) loci using Bayesian and maximum likelihood approaches to generate a phylogenetic hypothesis for the genus *Campsicnemus*. Our sampling included a total of 84 species (6 species from Europe, 1 from North America, 7 species from French Polynesia and 70 species from the Hawaiian Islands). The phylogenies were used to estimate divergence times, reconstruct biogeographic history, and infer ancestral ecological associations within this large genus. We found strong support for a South Pacific + Hawaiian clade, as well as for a monophyletic Hawaiian lineage. Divergence time estimates suggest that Hawaiian Islands were colonized approximately 4.6 million years ago, suggesting that most of the diversity within *Campsicnemus* evolved since the current high islands began forming ~5 million years ago. We also observe a novel ecotype within the Pacific *Campsicnemus*; a widespread obligate water-skating form that has arisen multiple times across the Pacific Islands. Together, these analyses suggest that a combination of ecological, biogeographic and temporal factors have led to the impressive diversity of long-legged flies in Hawaii and elsewhere in the Pacific.

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## 1. Introduction

Extreme isolation combined with the tempo and mode of island formation in the Hawaiian Islands have provided conditions for dramatic diversification in many of the lineages of arthropods and plants that managed to colonize its rugged volcanic landscapes (Bennett and O'Grady, 2012; Gillespie, 2004; Magnacca and Danforth, 2006; Rubinoff, 2008; Wagner and Funk, 1995). Movement of the Pacific plate over a more or less stationary hotspot has generated a volcanic archipelago whose contemporary high islands (Kauai, Oahu, Molokai, Maui, and Hawaii Island) began forming approximately five million years ago (Clague, 1996). One of the largest radiations in Hawaii (Hardy, 1964), with 179 currently described species and an additional 60 species awaiting

description, is the predatory long-legged fly genus *Campsicnemus* Haliday (Diptera: Dolichopodidae) – second within the Diptera only to the well-known Hawaiian Drosophilidae, which has approximately 1000 species (Carson and Kaneshiro, 1976; Kambyseilis et al., 1995; O'Grady et al., 2011).

The family Dolichopodidae includes more than 6800 described species worldwide (Yang et al., 2007). There are two endemic dolichopodid lineages in Hawaii, *Campsicnemus* and a related clade of genera referred to as the *Euryrogaster* group (Sympycninae or Hydrophorinae: Evenhuis, 2005). These lineages are quite distinct morphologically (Evenhuis pers. comm.) and likely represent a separate colonization events. *Campsicnemus* currently contains approximately 350 described species and is found in most of the world's biotic regions. This genus was erected based on Holarctic material and roughly 100 species are known from the northern hemisphere. Interestingly, this genus does not seem to be diverse in continental tropical habitats; there are no species known from the Neotropics or Australia and only a few taxa known from Africa and Asia. Known tropical species include some unpublished

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records from Sri Lanka and Nepal (N. Evenhuis, unpubl.), nine described species from central and southern Africa and Afro-tropical Atlantic islands (Grichanov, 1998, 2009, 2012), one species from southern China, and six more species described from Taiwan and the Philippines (but these Taiwanese and Philippine species are likely misidentifications of *Sympycnus* spp.; D.J. Bickel and N.L. Evenhuis, unpubl.). The bulk of species-level diversity is found in the Pacific region, with more than 200 described species occurring in Hawaii and French Polynesia alone (e.g.: Evenhuis, 2009, 2011, 2012a,b). In addition, approximately 100 new species from these two island groups and one each from Tonga and Fiji currently await description (Evenhuis pers. obs.). Comparison in this study of morphological characters among *Campsicnemus* from the Pacific, North America, Europe, Africa, and Asia, suggests that the Pacific species are distinct from *Campsicnemus* in the rest of the world and may constitute a distinct genus. However, no phylogenetic study to date has addressed the monophyly of the genus *Campsicnemus* or the Pacific taxa relative to the other members of this genus.

#### Ecological adaptations in the genus *Campsicnemus*

Pacific *Campsicnemus* are quite distinct relative to other members of this genus with respect to their co-occurrence, morphology, and microhabitat preference. For example, in the Holarctic region it is uncommon to find more than two species of *Campsicnemus* in broad sympatry. In contrast, in the Hawaiian Islands it is routine to collect between three and six species at the same locality. An extreme example is a single locality on Hawaii Island that yielded 11 species in the same collecting event (Evenhuis, unpubl.). Such a large number of species existing in sympatry within the Hawaiian rainforest suggests they must vary in sexual signaling and/or how they partition niche space.

There are also significant morphological and microhabitat differences between Pacific and non-Pacific *Campsicnemus*. For example, *Campsicnemus* outside of the Pacific region tend to be dull colored flies collected almost exclusively on leaf litter and low vegetation. These non-Pacific species exhibit little variation in their dark gray to black body coloration, although there is some variation in leg color that may correspond to differences in habit (M. Pollet, J. Runyon, and A. Stark, pers. comm.). Adults and immatures are known to be generalist predators on other invertebrate groups (Ulrich, 2005; Evenhuis, pers. obs.) and nondescript coloration may allow these species to better hide from their prey when hunting in low vegetation and leaf litter. Some European species are known to opportunistically skate on pools of water during seasons when there are fewer leaves, but there are no known obligate water skating species (M. Pollet, pers. comm.).

In contrast, Pacific island *Campsicnemus* are diverse with respect to microhabitat type and coloration. They are found as obligate water skaters on pools of water and surfaces of slow-moving streams, as well as on leaf litter and low vegetation. They also have correspondingly diverse morphology, ranging from small pale yellow flies to brown-bodied species to more sclerotized, larger black forms (Evenhuis, 2003). The obligate water-skating species are darker and more sclerotized than the others and have only been documented in the Pacific Islands where water striders (Gerridae) are absent in the terrestrial fauna (Marquesas and Hawaii: Evenhuis, pers. obs.).

Here we present the first molecular phylogeny of *Campsicnemus* based on a combination of five mitochondrial and two nuclear loci. We focus primarily on the Hawaiian fauna to address (1) whether the Pacific and Hawaiian species form a monophyletic group, (2) estimate divergence times and historical biogeography of the Hawaiian taxa, and (3) study the evolution of obligate water skating within the genus *Campsicnemus*.

## 2. Materials and methods

### 2.1. Taxonomic sampling

*Campsicnemus* specimens were collected from 2004 to 2012 from sites across the Hawaiian Islands and French Polynesia (Appendix A) in mesic forests and riparian habitats by general sweeping of vegetation and leaf litter, pan and Malaise trapping, and hand collecting. All specimens were preserved in 95% ethanol. The Hawaiian radiation is very large and, unlike Hawaiian *Drosophila*, does not currently have defined clades below the genus level. However, this group does have marked sexual dimorphism, with females looking extremely similar in most species, and with males possessing a wide array of modification to fore and mid legs. Male secondary sexual characters (MSSC) have been used to define species groups in the French Polynesian *Campsicnemus* (Evenhuis, 2008, 2009). The constancy of these species groupings through discoveries of additional species prompted an attempt to divide the Hawaiian species into species groups also based on MSSC; and 24 more-or-less distinct groups emerged after much study. Only one of the 24 proposed groups analyzed in this study, the *fumipennis* group, has been published thus far (Evenhuis, 2012b). The remaining Hawaiian species groups are provisional and were used to measure our sampling effort using MSSC as a proxy for the morphological diversity present within *Campsicnemus*. We sampled taxa from 23 of the 24 different leg types (Evenhuis unpublished).

All Pacific material was identified using keys in Tenorio (1969) and Evenhuis (2003, 2008, 2009). New descriptions were published (Evenhuis, 2011, 2012a,b, 2013) and others were written and are in preparation to accommodate the remaining 90 undescribed species discovered as a result of this project (Evenhuis, unpubl.). Some newly discovered, yet undescribed, species were included in this study. These taxa were given provisional names and are indicated here as unpublished by placing them in quotes (sensu Grimaldi, 1987). Whenever possible, a series of conspecifics from the same site were also preserved in 95% ethanol. Voucher material has been deposited in the Bernice Pauahi Bishop Museum (Honolulu). European specimens were collected and identified by Marc Pollet, and the North American species was collected and identified by Scott Brooks. In addition, sequences were downloaded from GenBank to include as outgroups: seven species of *Teuchophorus* Loew, five species of *Syntormon* Loew, two species of *Sympycnus* Loew (all from the Sympycninae, the same subfamily as *Campsicnemus*) and two species of *Scotiomyia* Meuffels & Grootaert from the related subfamily Peloropeodinae. Finally, new sequences were generated for three species of Australian *Sympycnus* and one species of *Yumbera* Bickel to use as outgroup specimens (Tables A.1 and A.2).

#### 2.1.1. DNA extraction, amplification and sequencing

Genomic DNA was extracted from individuals using a Qiagen DNeasy® (Qiagen Inc.) DNA extraction kit, following the manufacturer's protocol. Five mitochondrial (COI, COII, 12S, 16S, ND2) and two nuclear loci (CAD, EF1 $\alpha$ ) were then amplified and sequenced to estimate phylogenetic relationships within this group (Table C.1). PCR reactions were performed using standard master mixes in 25  $\mu$ L final volumes, with slight modifications for the various loci. Thermal cycling involved a simple protocol for EF1 $\alpha$ , a touchdown protocol for the mitochondrial genes and a nested reaction for CAD (described in Moulton and Weigmann (2004)). The simple protocol began with an initial denaturing step at 95 °C for 4 min, 30 cycles of 90 °C for 30 s, 54–58 °C for 30 s, 72 °C for 60 s and a final extension for 5–10 min 72 °C. The touchdown protocol began with an initial activation cycle at 96 °C for 2.5 min followed by 25 cycles of 30 s denaturing at 96 °C, 30 s annealing

through a touchdown series starting from 55 °C and stepping down 0.4 °C per cycle, with 45 s extension at 72 °C. This was followed by 15 cycles of 30 s denaturing at 96 °C, 30 s annealing at 45 °C and 45 s extension at 72 °C. The touchdown protocol was completed by a final extension for 7 min at 72 °C. PCR products were purified using *ExoSAP-IT* (USB Corporation, Cleveland, OH) following standard protocols, and the products were sent to the UC Berkeley DNA Sequencing Center for sequencing in both directions on an ABI 3730 capillary sequencer.

### 2.1.2. Sequence editing and alignment

Contigs were assembled and edited using Geneious Pro 5.4.6 (Biomatters), then were aligned to each other using the ClustalW Alignment plugin in Geneious. The alignments for each protein coding gene were imported into MacClade 4.08 (Maddison and Maddison, 2000) in order to calculate codon positions, using the conceptual translation and comparison to a *Drosophila yakuba* or *Drosophila melanogaster* reference sequence. There were insertions in CAD, ND2 and EF1 $\alpha$ . The 12S and 16S alignment and intron regions were inspected and adjusted manually when necessary. The alignment is provided in [Appendix E](#).

## 2.2. Phylogenetic analysis

Eighty-four species of *Campsicnemus* were included in the phylogenetic analysis (6 species from Europe, 1 species from North America, 7 species from French Polynesia and 70 species from the Hawaiian Islands), including species from 23 of the 24 proposed morphological species groups (Evenhuis, unpublished). GenBank accession numbers are available in [Supplementary Appendix Table A.1](#). Phylogenetic analyses were performed on a data set consisting of 122 individuals ([Appendix A](#)) and seven loci containing a total of 5204 base pairs. Analyses were conducted on each gene individually using maximum likelihood (ML) and on the combined dataset using ML and Bayesian inference (BI) optimality criteria. For both the ML and the BI analyses of the concatenated data set, the optimum partitioning scheme was calculated in PartitionFinder (Lanfear et al., 2012) from 20 original data partitions (16S, 12S and 1st, 2nd, and 3rd codon positions for COI, COII, ND2, CAD and EF1 $\alpha$ , intron regions for CAD, EF1 $\alpha$  and ND2) and selected using Bayesian Information Criterion ([Table C.2](#)). For the BI analysis, the best-fit model of sequence evolution for each data partition was also selected using PartitionFinder ([Table C.2](#)). An additional analysis was run on a data matrix containing a single individual per species to examine character evolution (Character Tree, [Fig. B.9](#)), selection of models and partitions proceeded as described above and these are reported in [Table C.2](#). The ML analysis was performed on individual genes and on the concatenated data set in RAxML 3.7.2 (Stamatakis, 2006) on CIPRES (Miller et al., 2010) with each partition selected as described above run under the GTR GAMMA model with 1000 bootstrap replicates and a final search for the best tree. An additional ML analysis was performed on the concatenated data, partitioned and fit to models as described in [Table C.2](#) using GARLI 2.0 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl, 2006). We performed 100 replicate searches for the best ML tree and 2000 bootstrap replicates on the GARLI web service hosted at [molecularevolution.org](#) (Bazin et al. and Zwickl, 2014).

The BI analysis was performed on the concatenated data set using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) on CIPRES (Miller et al., 2010), with the analysis run for 30,000,000 generations with 2 independent runs each.

### 2.2.1. MCMC convergence diagnostics

For the BI analysis, stationarity was assessed within and convergence among each of the runs using several complimentary

approaches: (1) convergence metrics provided by MrBayes 3.1.2 were checked (Huelsenbeck and Ronquist, 2001) to ensure that the maximum standard deviation of split frequencies of any of the runs was under 0.05 and that the potential scale reduction factor for all parameters approached 1.0, and (2) the log-likelihood values for each run were plotted, the effective sample sizes were checked to ensure there were an adequate number of independent samples, and the posterior distributions of all parameters were examined using Tracer v.1.72 (Rambaut and Drummond, 2012). Tracer v.1.72 was also used to determine the burn-in phase by assessing each run's plot of log-likelihood values over generations – stationarity was assumed to have been reached when the log likelihood values reached a stable plateau. Finally, a 50% majority rule consensus tree was created from the resulting post burn-in trees.

## 2.3. Ancestral range reconstruction in Hawaii

The historical biogeographic ranges of the Hawaiian *Campsicnemus* were reconstructed using BioGeoBEARS (Matzke, 2013a) in R (R Core Team, 2013). The ranges were estimated under two different unconstrained models (1) Dispersal–Extinction–Cladogenesis (DEC) (first implemented in Ree and Smith, 2008) and (2) Dispersal–Extinction–Cladogenesis–Jump (DEC + J) using maximum likelihood and model performance was assessed using a likelihood ratio test. Comparison of these two models allowed an assessment of the relative roles of range expansion, range extinction and founder events (defined in this model as the acquisition of a new range without the parent lineage having already expanded into it) in the evolution of ranges in this group (Matzke, 2013b). Reconstructions were conditioned in absolute time with the dated phylogeny from BEAST (see below). An eight-area model was used for both analyses, including Singapore (for the root taxa), Europe, French Polynesia, Kauai, Oahu, Molokai, Maui and Hawaii Island.

## 2.4. Divergence time estimation

Divergence time estimation was performed using a Bayesian relaxed-clock method implemented in BEAST 1.7.5 (Drummond et al., 2012) on CIPRES ([www.phylo.org](#); Miller et al., 2010). The genus *Campsicnemus* is of unknown age; the only fossil that has been described (*Campsicnemus gracilis* Meunier, 1907 from Baltic amber, ~40 million years old) was incorrectly classified and is actually a member of the subfamily Diaphorinae and not the Sympycninae (D.J. Bickel, pers. comm. 2012). Therefore, it was inappropriate to apply the fossil as a calibration in this analysis (Ho et al., 2008). In this study we sought to be conservative and thorough and because each approach has its own set of limitations, we performed a series of analyses using island calibrations and divergence rates ([Appendix D](#)) in order to fully explore dating parameter space.

The application of island calibrations rests on the assumption that a taxon colonized a new island from an older island after it

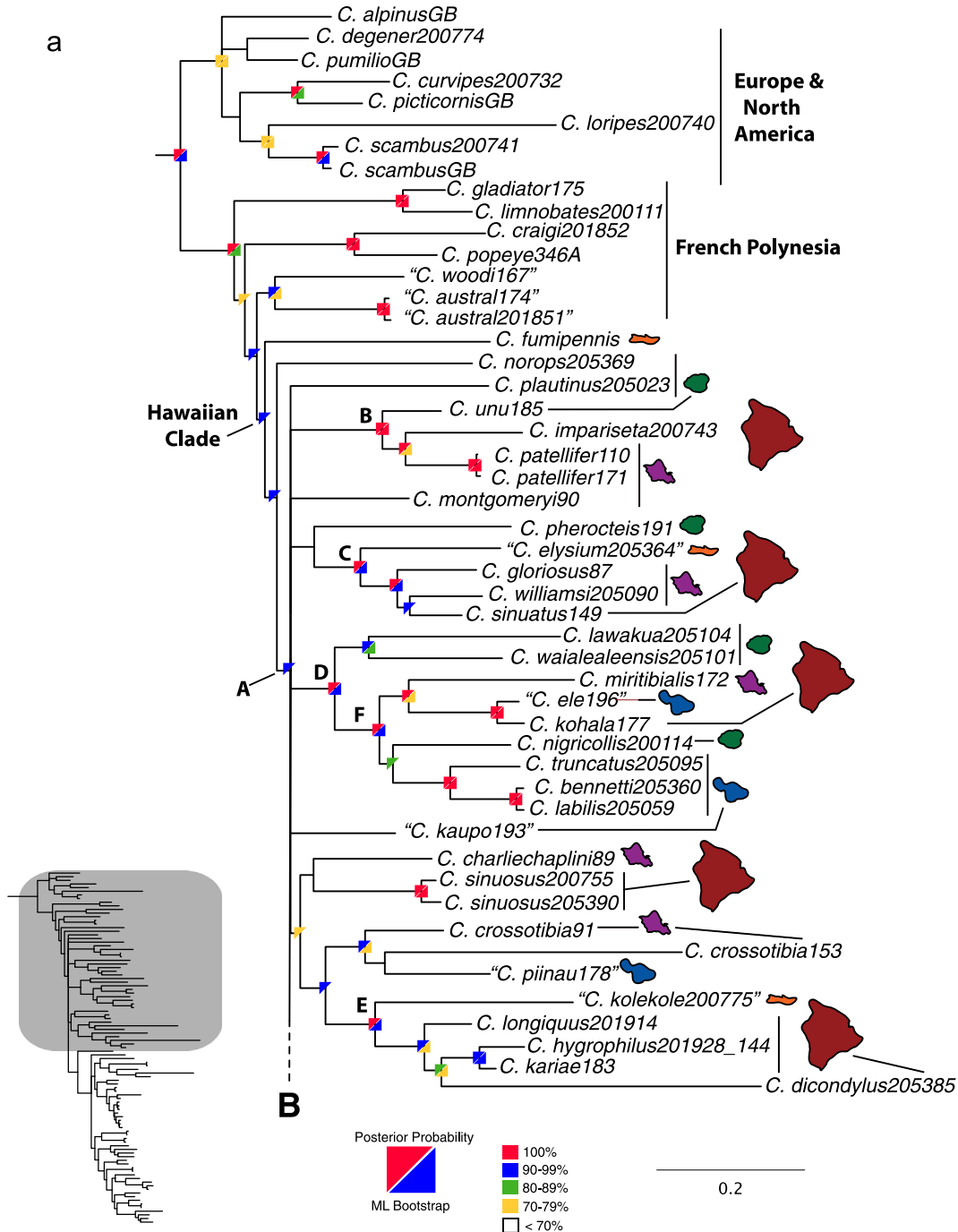
**Table 1**  
Nodes used for calibrating divergence time estimation in BEAST: support, ancestral range reconstructions and calibration used.

Node calibrated (see <a href="#">Fig. 2</a> )	Support: MrBayes	LaGrange ML	Mesquite ML	Calibration: mean (SD), (reference)
1. Maui 1	0.99	Maui	Maui	1.3 (0.15), <a href="#">Carson and Clague (1995)</a>
2. Maui 2	0.99	Maui	Maui	1.3 (0.15), <a href="#">Carson and Clague (1995)</a>
3. Oahu	0.90	Oahu	Oahu	3.86 (0.089), <a href="#">Sherrod et al. (2007)</a>

emerged and thus the age of that biogeographic event can then be used to date the most recent common ancestor (MRCA) of that group. Thus, in order to apply calibrations, pairs of taxa must be used in which one group is restricted to an older island (or islands) and the other group is restricted to a younger island (or islands) (Fleischer et al., 1998). The biogeography of *Campsicnemus* is strongly suggestive that substantial diversification did occur within the present high islands in that there are several groups of multiple species that are distributed within single islands.

In order to calibrate nodes, we performed ancestral state reconstruction in Lagrange (Ree and Smith, 2008). We ran an unconstrained analysis that restricted the number of ancestral states to a maximum of two, which is reasonable given that all taxa

represented here are single-island endemics and compared these results to a maximum likelihood analysis of ancestral range reconstruction performed in Mesquite (Maddison and Maddison, 2009) – for all nodes calibrated in this analysis, the reconstructed range was the same (Table 1). We selected well-supported nodes with clear ancestral range reconstructions for the application of the calibrations (Table 1). All three nodes were calibrated with island dates from Carson and Clague (1995) and Sherrod et al. (2007). While island calibrations have been widely used in Hawaiian lineages (e.g., (Lerner et al., 2011; Rubinoff and Schmitz, 2010), it is plausible that divergence among populations occurred prior to island emergence and was thus unrelated, or that it occurred well after the emergence of the younger island (Heads, 2005). Standard



**Fig. 1.** Majority rule consensus tree summarizing Bayesian analysis of *Campsicnemus*. Bayesian posterior probabilities (MrBayes) and bootstrap supports from the maximum likelihood analysis (RAXML) are displayed as colored boxes. Islands that each specimen was collected from are shown next to each tip.



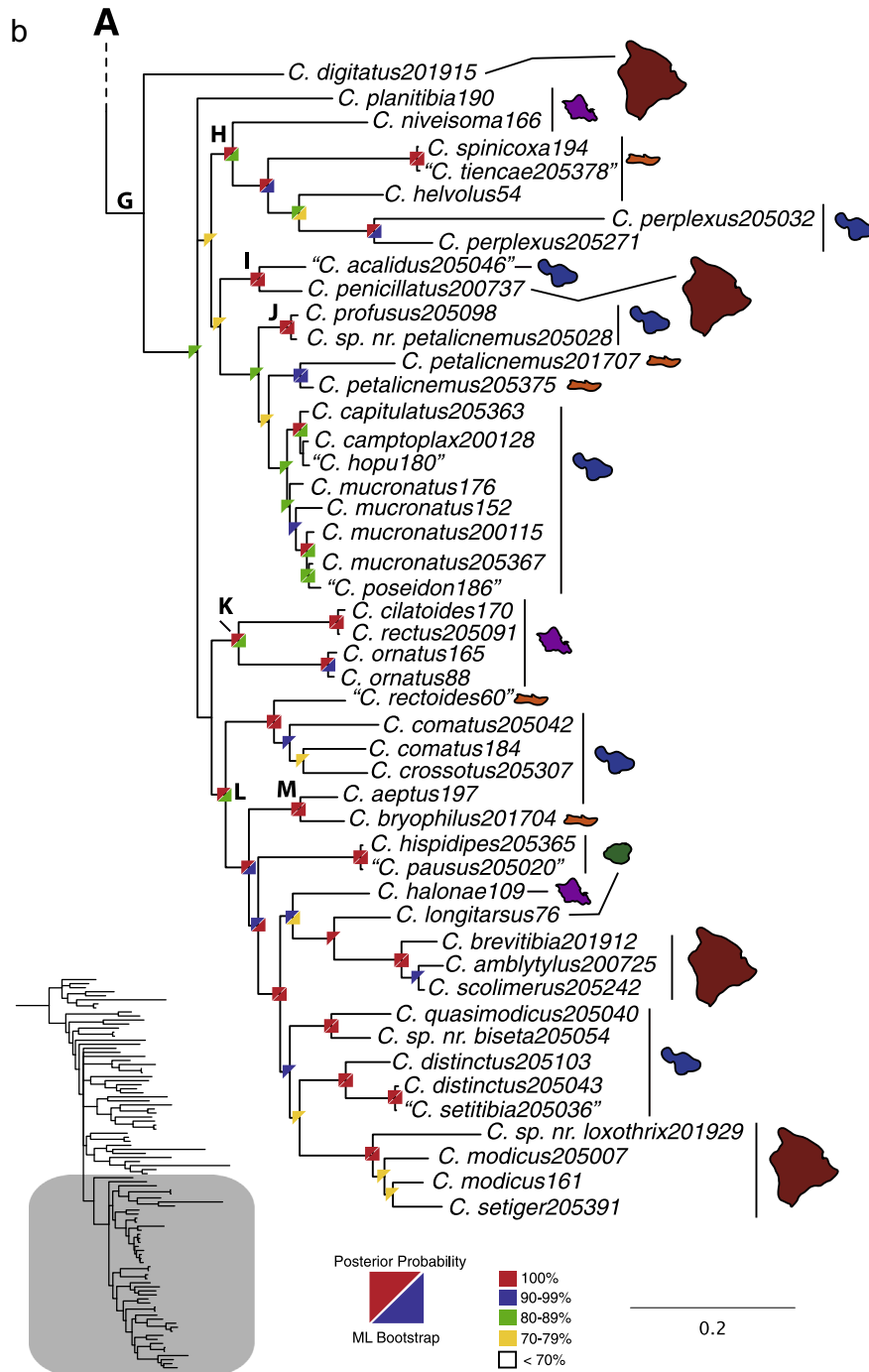


Fig. 1 (continued)

deviations were set to accommodate uncertainty as to when the taxa colonized the islands (Table 1 and Fig. 2).

Divergence time estimation was performed on a data matrix composed of one individual per species of *Campsicnemus* (excluding *C. rhenocrenus* and three other terminals that only had two loci), and the ingroup was rooted by *Scotiomyia*, which is clearly outside of the ingroup. The same seven gene concatenated data set (COI, COII, ND2, 12S, 16S, EF1 $\alpha$  and CAD) was analyzed in each of the analyses described here and in Appendix D. Partitions and the best fit models of evolution for each partition were selected using BIC in PartitionFinder (Lanfear et al., 2012). Initial analyses indicated that these models overparameterized the data, so all GTR models were changed to HKY for the final runs (Table C.2),

and base frequencies were estimated from the data. The partitioning scheme in the divergence rate analyses differed only slightly from the island calibration analyses in that COI was assigned its own partition (Table C.2). Site and clock models were unlinked and all partitions were analyzed using an uncorrelated lognormal relaxed clock except for the partition comprised of CAD (positions 1 & 2) and the EF1 $\alpha$  intron, for which a strict clock could not be rejected and was thus applied. The tree-shape prior was linked across partitions and the tree-shape prior was specified as a Yule Process. The xml file was hand edited to include a starting tree, generated using maximum likelihood in RAxML 3.7.2 (Stamatakis, 2006). Two independent MCMC searches were conducted, each running for 30 million generations and sampled every

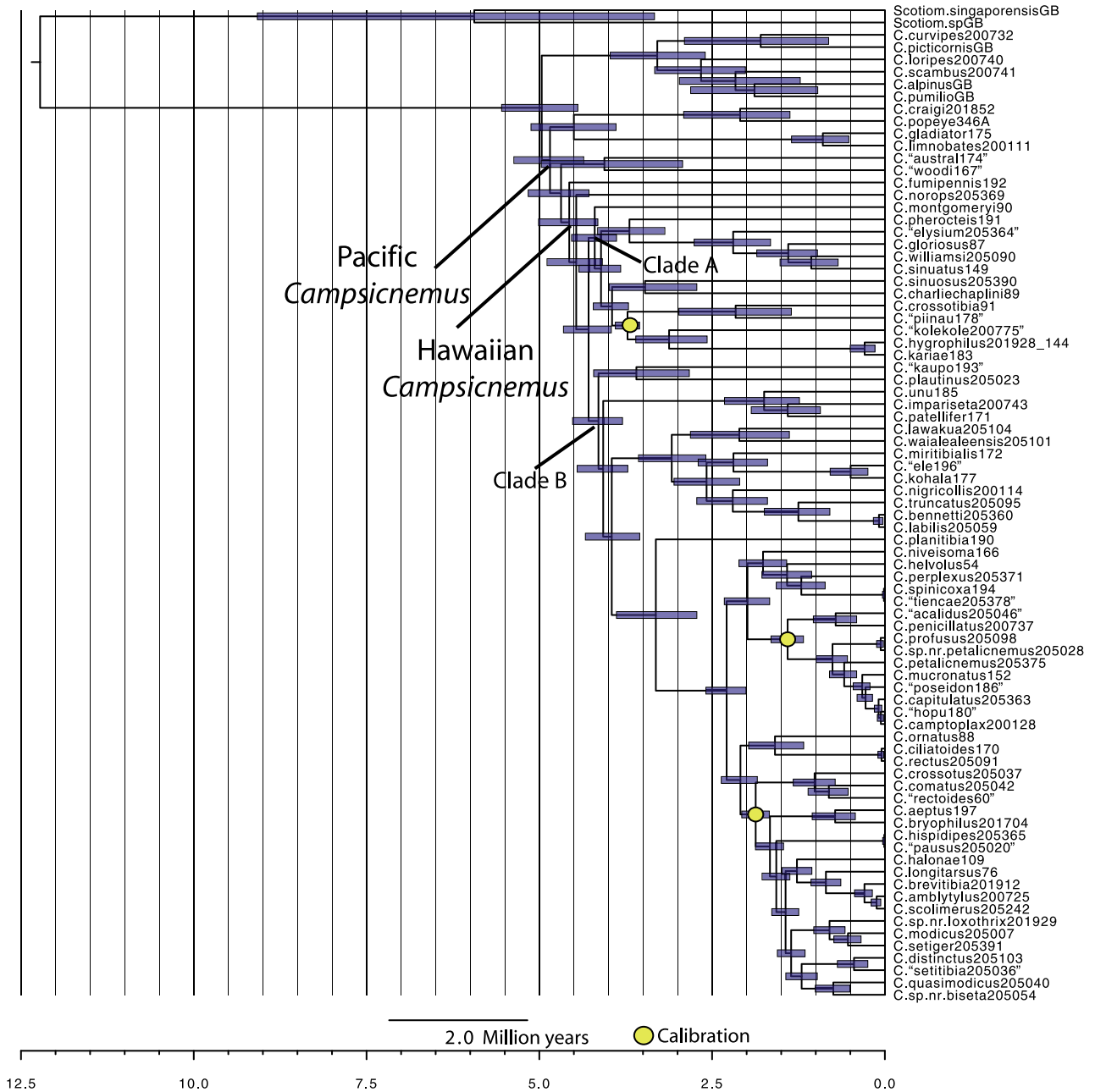


Fig. 2. Time tree from the BEAST (Drummond et al., 2012) analysis. Purple bars indicate 95% highest posterior density intervals.

1000 generations. The number of generations was selected to generate Effective Sample Sizes (ESS) values greater than 200 for each of the parameters (Drummond et al., 2007). Convergence was assessed using Tracer v. 1.7.5 and trees were summarized to one Maximum Clade Credibility (MCC) tree using Tree Annotator v. 1.7.5 after removing a burn-in phase.

### 2.5. Ancestral state reconstruction of ecotypes

To examine the evolution of ecotypes and body color, the ancestral states for “ecotype” were reconstructed using maximum likelihood on the Character Tree in Mesquite 2.72 (Maddison and Maddison, 2009). Ecotype (low vegetation, water skater, cryptogenic) was noted at the time of collection. The cryptogenic ecotype is suspected to live in the canopy because it has not been collected from water, leaf litter, or low vegetation and they have been observed flying downward towards traps (Evenhuis pers. obs).

Directed fogging efforts will be needed to confirm this ecological association. The ecotype character was coded as a categorical variable with three states; unknown states were coded with “-”. At each node, proportional likelihoods were calculated using the MK1 model of equal transition rates.

## 3. Results and discussion

### 3.1. Species and gene sampling, partition results

Collecting for this project yielded 113 previously unknown species from French Polynesia and Hawaii (23 have been published; the remainder are in various stages of manuscript preparation). Phylogenetic results from the analyses of individual genes are presented in Figs. B.1–7. Concatenated phylogenies generated using ML and BI approaches yielded similar topologies and, at most well-supported nodes are identical. The BI tree is used as the

reference tree for further analyses (Fig. 1a, b). The RAXML and GARLI ML trees are presented in Figs. B.8a and B.8b; topologies and support values are very similar between the two methods.

### 3.2. Phylogenetic results and taxonomic implications

#### 3.2.1. Monophyly of *Campsicnemus*

We found evidence that *Campsicnemus*, as currently defined, was not monophyletic with respect to a single taxon, *Campsicnemus rheocrenus*. *C. rheocrenus*, a member of the *lobatus* species group (Evenhuis, 2008), did not group with the rest of the genus *Campsicnemus*. Instead, this species was most closely grouped with *Sympycnus turbidus*, an outgroup taxon. In addition to the molecular evidence placing these taxa together, *S. turbidus* and *C. rheocrenus* also share a distinctive morphological character: each have large yellow areas on the lateral margin of the first two abdominal tergites. These tergites are normally not contrastingly colored in other species of *Sympycninae* genera. However, being a much larger-sized species, *C. rheocrenus* may actually be a member of *Sympycnus*. Based on our review of the morphology of the available *C. rheocrenus* specimens a re-classification is necessary, and we do not consider it as part of the *Campsicnemus* in this paper. Aside from the dubious placement of *C. rheocrenus*, there is strong support for the monophyly of the remaining *Campsicnemus* species examined in this study (PP = 1, BS = 0.98: Fig. 1a).

#### 3.2.2. Basal relationships in *Campsicnemus*

Within *Campsicnemus*, there is strong support for the sister relationship between a European/North American clade of species and the Pacific taxa (PP = 1, BS = 98: Fig. 1a). There is not strong support for the monophyly of the European/North American clade (PP = 0.73, BS = 71: Fig. 1a), either owing to the reduced sampling in this lineage or because the Holarctic taxa may represent a basal paraphyly in *Campsicnemus*. There is good support for a Pacific clade uniting the French Polynesian and Hawaiian lineages (PP = 1, BS = 89: Fig. 1a). While there is strong support (PP = 0.98: Fig. 1a) for a Hawaiian lineage in the Bayesian analyses, this relationship is not strongly supported in the ML phylogeny (BS = 62: Fig. 1a). The basal portion of the Pacific clade is a paraphyletic grade, with species pairs from the Marquesas (PP = 1, BS = 100: Fig. 1a) and Tahiti (PP = 1, BS = 100: Fig. 1a) grouping by island leading to the Austral group + the Hawaiian taxa (PP = 0.98, BS = 43: Fig. 1a). The backbone of the basal portion of the tree is a large unresolved polytomy containing several well-supported species groupings within it, while the more derived portion of the tree is better resolved (Fig. 1b).

#### 3.2.3. Phylogeny and biogeography in Hawaiian *Campsicnemus*

The base of the Hawaiian *Campsicnemus* is a paraphyletic grade with two species, *C. fumipennis* and *C. norops* branching sequentially. Support for these basal nodes is mixed, with PP values between 0.90 and 0.99 and BS < 50%. There is a large polytomy (node A, Fig. 1a) at the base of the Hawaiian *Campsicnemus* with eight distinct lineages branching from a single point. Three individual species, *C. plautinus*, *C. montgomeryi* and *C. "kaupo"* originate from this large polytomy. There are also several other nodes of two or more species (e.g., *C. charliechaplani* and *C. sinuosus*) that are not strongly supported in terms of bootstrap proportion or posterior probability values branching from node A. These may represent distinct lineages for which we do not have adequate character or taxon sampling to resolve with strong support. This lack of support within the basal nodes is a common characteristic of many Hawaiian insect groups (e.g., O'Grady et al., 2011; Bennett and O'Grady, 2012) and may be a byproduct of the rapid adaptive diversification process. While node A is poorly supported and

may not form a monophyletic group, several of the descendant lineages are well supported and monophyletic (nodes B–E, Fig. 1a).

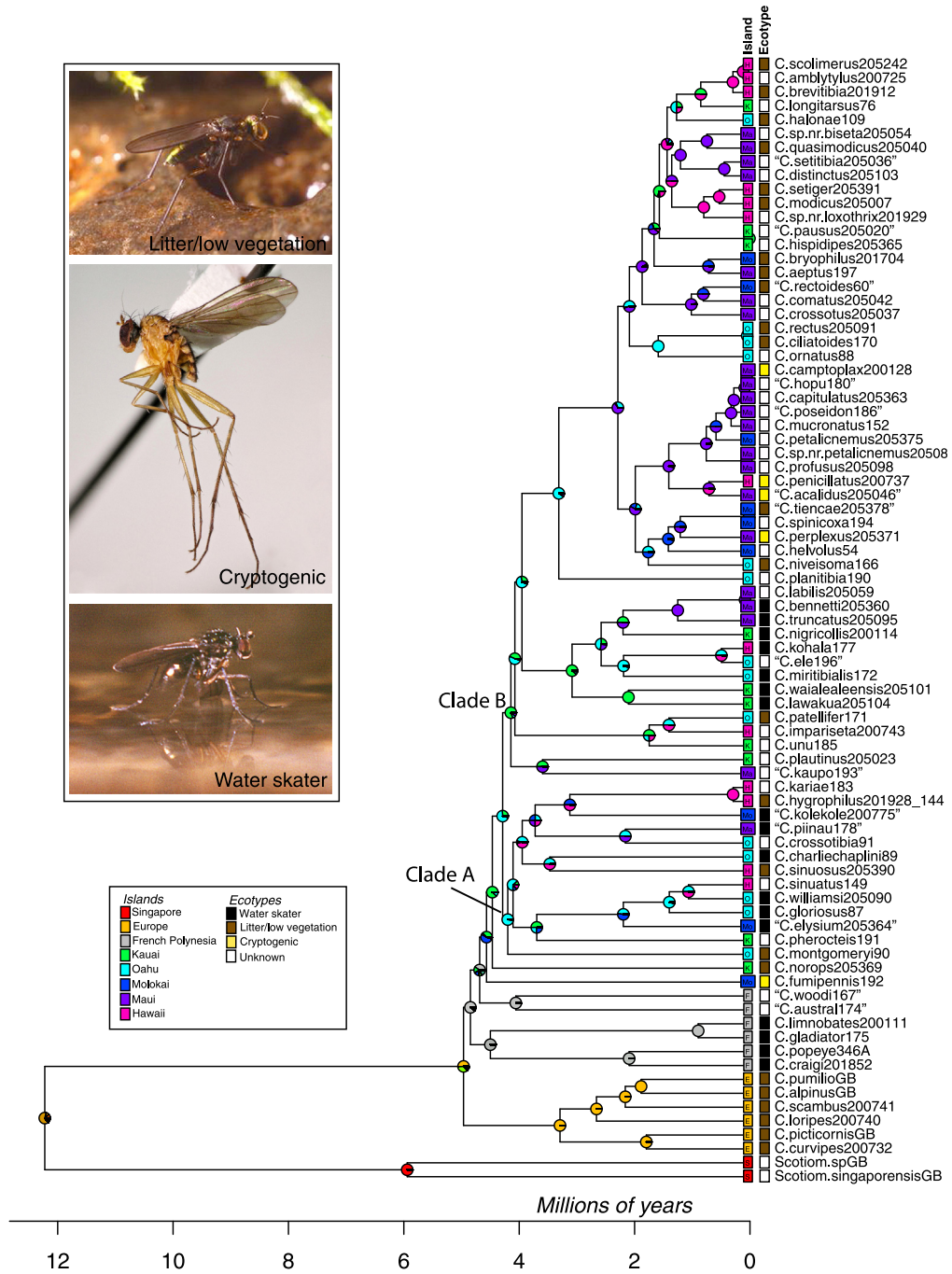
Node B is a well-supported clade (PP = 1.0, BS = 100: Fig. 1a) comprised of three species, *C. unu*, *C. impariseta* and *C. patellifer*. These species conform to a progression rule pattern with the basally branching taxon, *C. unu*, found on Kauai and the more apically branching species found on Oahu and Hawaii. Likewise, node C is also well-supported clade (PP = 1.0, BS = 97: Fig. 1a), and a progression rule pattern, while seen in the *C. gloriosus* – *C. williamsi* – *C. sinuatus* clade (PP = 1.0, BS = 98, Fig. 1a), is not supported in all node C species. Node D is another well-supported monophyletic group (PP = 1.0, BS = 98: Fig. 1a). Two species from Kauai, *C. lawakua* and *C. waialealeensis*, are sister to the remaining species (node F, PP = 1.0, BS = 84; Fig. 1a). Progression rule patterns are evident in the two lineages descended from node F, but support values are not strongly supported. Interestingly, the species in node F are all water skaters and share a suite of morphological characters, including black body color and mid tibiae with a basomesal "comb" of long peg-like setae with truncate apices. Node E is a well-supported clade (PP = 1.0, BS = 99; Fig. 1a) that shows progression rule patterns between the basal taxon, *C. "kolekole"* from Molokai, and the remaining species in this lineage, all of which are from Hawaii.

Node G (Fig. 1b) represents the largest lineage derived from this basal polytomy (node A, Fig. 1a) and comprises the bulk of the species in the Hawaiian *Campsicnemus* clade. The basal relationships within node G are not well supported with either posterior probability or bootstrap proportions. Two species, *C. digitatus* and *C. planitibia*, form a paraphyletic grade that is sister to the remaining species, although there is little statistical support for these relationships. There are, however, several descendant lineages are very well supported (nodes H–L: Fig. 1b). Node H consists of five species that show a clear progression rule from Oahu (*C. niveisoma*) to Molokai (*C. spinicoxa*, *C. "tienca"*, *C. helvolus*) to Maui (*C. perplexus*). There is strong support for the sister group relationship between *C. "acalidus"* and *C. penicillatus* (PP = 1.0, BS = 100: Fig. 1b, node I), although there is little support for the placement of these two species. Likewise, node J shows support (PP = 1.0, BS = 100: Fig. 1b) for the sister relationship between *C. profusus* and a new species closely related to *C. petalincnemus*, but without much statistical support for the exact placement of these two taxa. Six other taxa are sister to node J, although there is little consistent support for this group or for the relationships among these species. Interestingly, five of the six species in this poorly supported lineage are endemic to Oahu, suggesting a number of diversification events within this island.

Nodes K and L are sister taxa, although there is little statistical support for this relationship. Node K (PP = 1.0, BS = 80: Fig. 1b) consists of four species, all of which are from Oahu, suggesting a number of in-situ diversification events occurring on that island since it formed ~3 million years ago. This group is united by lack of conspicuous MSSC (all mid tibia of species in this group have just short hairs) and is tentatively named the "simplex" species group. Node L is a large clade of 19 species. Like node K, this lineage also has support for within island diversification events, as opposed to progression rule patterns that occur due to allopatric diversification events between islands.

#### 3.2.4. Evolution of flightlessness

Flightlessness has evolved multiple times in the Diptera and constitutes a complex suite of character modifications and losses. There are a number of flightless taxa in the Hawaiian *Campsicnemus* clade. These have had a complicated taxonomic history with some species being described in a separate genus, *Emperoptera*, and others placed within *Campsicnemus*, *sensu stricto*. *Emperoptera* was first erected by Grimshaw (Grimshaw in Grimshaw and



**Fig. 3.** Time tree from the BEAST (Drummond et al., 2012) analysis with distribution, body color and ecotype information mapped onto the tips. Pie charts on nodes show ancestral range reconstructions from BioGeoBEARS (Mutzke, in revision-a) analysis. Photo credits: Cryptogenic (*C. aniani*) by Neal Evenhuis, Litter/low vegetation (*C. brevipes*) and Water skater (*C. uncleremus*) by Ron Englund.

Speiser, 1902) and several authors added taxa to this genus (Zimmerman, 1938; Adachi, 1954). When Hardy and Kohn (1964) revised the Hawaiian Dolichopodidae, they synonymized *Emperoptera* with *Campsicnemus*. Evenhuis (1997) reversed this placement and resurrected *Emperoptera*. Recent molecular and morphological work (Evenhuis and O’Grady, 2010) have further revised this placement and *Emperoptera* is now considered a junior synonym of *Campsicnemus*. Interestingly, the flightless species included in this study fall out in two distinct lineages. *Campsicnemus montgomeryi*, a flightless species lacking wings and halteres, is one of the singleton species that branches off node A. This taxon was previously placed in *Emperoptera*. Two other flightless taxa, *C. aeptus* and *C.*

*bryophilus*, are a strongly supported lineage (node M, PP = 1.0, BS = 100) nested within clade L (Fig. 1b). The latter two species have reduced wings and fully developed halteres.

### 3.3. Biogeographic model testing

Overall likelihood scores, *d*, *e* and *j* parameters for the two biogeographic models tested were as follows: (1) DEC = LnL = -158.9, *d* = 0.043, *e* = 0.116, *j* = 0 and (2) DEC + *j* = LnL = -104.4, *d* = 0, *e* = 0, *j* = 0.094. The DEC + *j* model performed significantly better than the DEC (LRT *p*val = 1.6E-25). The difference between the two biogeographic models is that in addition to allowing range expansions



and range extinctions (*d* & *e*), the DEC + *j* model also allows for founding events (*j*). The results are striking in that the DEC + *j* model assigns exactly no weight to the processes of range expansion and extinction, instead explaining everything with the *j* parameter. This is likely due to the very high level of single-island endemism in the Hawaiian *Campsicnemus*. This result is on the extreme end of the spectrum, but consistent with Matzke's (2013b, in revision-b) survey of other island biota, for which range evolution was overwhelmingly explained by the *j* parameter. It is important to note that here, the biogeographic units are islands, and in the Hawaiian *Campsicnemus*, there is a substantial amount of within-island speciation. The models regard within-island speciation as sympatric, but there could be a variety of processes involved, including: founding events at the within-island scale, vicariance or sympatric speciation. The micro-biogeography of this group will need further study.

### 3.4. Divergence time estimation and biogeography

The overall topology of the time tree (Fig. 2) is similar to the trees generated under ML and BI in MrBayes (Figs. 1 and B.8). The TMRCA of the genus *Campsicnemus* is estimated at 4.98 (95% HPD: 4.44–5.55) million years ago, with colonization of the Pacific occurring shortly thereafter, 4.89 (95% HPD: 4.36–5.37) million years ago (Fig. 2). Branch lengths are very short and not well supported within the Pacific radiation; as a result, the sequence and timing of colonization within the Pacific is unclear. However, it does appear that once *Campsicnemus* reached the Pacific, it spread quickly. The age estimate of the genus may get pushed back with the addition of more widespread sampling from outside of the Pacific.

Only one colonization to the Hawaiian Islands is needed to explain the Hawaiian diversity, which is estimated to have occurred 4.58 (95% HPD: 4.15–5.01) million years ago (Fig. 2). After *Campsicnemus* reached the Hawaiian Islands, there was substantial early diversification on Kauai and Oahu (Fig. 3). The basal relationships within the Hawaiian radiation are much better resolved within the time tree than the BI and ML analyses, and suggest that the Hawaiian fauna are split into two main clades that each began diversifying 3.8–4.5 million years ago: a smaller clade (Clade A) that originated on Oahu and a larger clade (Clade B) that originated on Kauai (Figs. 2 and 3). Most early diversification in both clades appears to have occurred on these two islands. In the more derived portion of the phylogeny, substantial diversification appears to have occurred in the younger islands and there are several examples of within-island radiation and also shifts back to the older islands (Fig. 3). Estimates for rates of evolution at each partition are reported in Table D.1.

### 3.5. Evolution of obligate water skating and cryptogenic species

Our results suggest that the ancestral *Campsicnemus* that first colonized the Pacific was most likely a generalist-type species that could be found on low vegetation and litter. The ancestral ecotype is shown for the major clades in Figs. 3 and B.9 and is reconstructed as Litter/Veg for the Pacific *Campsicnemus* (prob = 0.99), and also for the Hawaiian *Campsicnemus* (Litter/Veg, prob = 0.95; Fig. B.9).

Obligate water skaters have been collected from each of the Hawaiian Islands and from the Marquesas, and this analysis reveals that there have been multiple shifts between this colonizing form and the darker obligate water skating form (Figs. 3, B.9). The cryptogenic species have a much more restricted distribution, they are known only from Maui Nui and Hawaii Island. There are multiple shifts between the colonizing low vegetation types and the cryptogenic types. Interestingly, water skaters are not present in clades with the cryptogenic species and vice versa (Figs. 3, B.9). The evolution of novel ecotypes is strongly suggestive of natural

selection acting to promote diversification (Gillespie, 2004; Grant and Grant, 2008; Losos, 2009; Schluter, 2000). The appearance of obligate water skaters on multiple islands and what we hypothesize to be the invasion of the canopy on Maui Nui suggest that a lack of predators in these niches provided the ecological opportunity necessary for *Campsicnemus* to diversify within the Pacific.

## 4. Conclusions

Here we present the first phylogenetic analysis of *Campsicnemus*, with a focus on the Pacific fauna, which will allow future studies to test classifications at the species level in this genus. Collecting for this project yielded 113 previously unknown species. The results suggest that the South Pacific + Hawaiian species form a monophyletic group and there is also support for a monophyletic Hawaiian group that reached the Hawaiian Islands approximately 4.6 million years ago. We document that flightlessness has evolved twice in the Hawaiian *Campsicnemus* and describe the appearance of a widespread obligate water skating form has arisen multiple times across the Pacific. Furthermore, we also note a light-bodied cryptogenic form that is known only from Maui Nui, which we hypothesize live in the canopy. Together, these analyses suggest that a combination of ecological, biogeographic and temporal factors have led to the impressive diversity of long-legged flies in Hawaii and elsewhere in the Pacific.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2014.07.015>.

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