Molecular Phylogenetics and Evolution 81 (2014) 232–241

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

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

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ARTICLE INFO

Article history:
Received 24 March 2014
Revised 2 July 2014
Accepted 25 July 2014
Available online 16 September 2014

Keywords:
Ecotype
Historical biogeography
Adaptive radiation
Hawaiian Islands
Divergence dating
Dolichopodidae

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, EF1α) 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.

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; Kambysellis 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 *Eurynogaster* 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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http://dx.doi.org/10.1016/j.ympev.2014.07.015
1055-7903/

records from Sri Lanka and Nepal (N. Evenhuis, unpubl.), nine described species from central and southern Africa and Afrotropical 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, EF1α) 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 μL final volumes, with slight modifications for the various loci. Thermal cycling involved a simple protocol for EF1α, 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