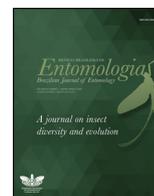




SOCIEDADE BRASILEIRA
DE ENTOMOLOGIA
FUNDADA EM 1937

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution



Another step towards understanding phylogenetic relationships in Asphondyliini: revisiting two hypotheses to *Bruggmanniella s.l.* (Diptera, Cecidomyiidae)

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ARTICLE INFO

Article history:

Received 25 November 2021

Accepted 07 March 2022

Available online 11 April 2022

Associate Editor: Marcia Couri

Keywords:

Asphondyliina

Cladistics analysis

Gall inducer

Morphology

Neotropical

Phylogeny

ABSTRACT

An update of the delimitation of the genus *Bruggmanniella* based on phylogenetic analysis using morphological data is presented. In this work, we reinforced the results of the previous phylogenetic analysis of the closely related genera *Bruggmanniella*, *Pseudasphondylia*, *Illiciomyia* and *Odontokeros*, assigned here as *Bruggmanniella s.l.* after the controversial molecular approach of Lin et al. (2020). We also included the species described under *Bruggmanniella* between 2019 and 2020 and discuss some aspects of the evolutionary changes of pupal morphology related with niche occupation of *Bruggmanniella* species. The results confirm our previous delimitation of the *Bruggmanniella s.l.* arranged into three branches: one branch composed exclusively with the Neotropical species of *Bruggmanniella*; another branch containing the species of *Pseudasphondylia*, found only in Japan; and the last branch with species of *Odontokeros* with predominant distribution in Taiwan. Our results also support the revalidation of the genus *Odontokeros*, and *Illiciomyia* as synonym of *Pseudasphondylia*.

Introduction

The close relationship among the species of *Bruggmanniella* Tavares, 1909, *Pseudasphondylia* Monzen, 1955, and *Illiciomyia* Tokuda, 2004 has been widely discussed in the literature grounded mainly by the presence of the two separate teeth of the gonostylus and transverse rows of strong spines on the anterior half of the pupal tergites (Kovalev, 1964; Yukawa, 1971; Tokuda, 2004; Tokuda and Yukawa, 2005, 2006; Tokuda et al., 2008; Garcia et al., 2020; Lin et al., 2020). *Pseudasphondylia* and the monotypic genus *Illiciomyia* are only known to Japan, whereas *Bruggmanniella s.l.* is widely distributed. Of the 21 species of *Bruggmanniella*, 12 occur in the Neotropical region, one species occurs in the Southern Nearctic, two species in the Australasian, and six species in the Oriental/Palaearctic regions (Gagné and Jaschhof, 2021).

In our previous study (Garcia et al., 2020), we conducted a comprehensive morphology-based analysis of the *Bruggmanniella* genus, including six species of *Pseudasphondylia* and *Illiciomyia yukawai* Tokuda, 2004. Our phylogenetic hypothesis strongly supported the transference of the Oriental species of *Bruggmanniella* to *Pseudasphondylia* and the

erection of a new genus, *Odontokeros* Garcia et al., 2020 to house *Bruggmanniella brevipes* Lin, Yang & Tokuda, 2019, the only known species of the group recorded from Taiwan at time. Accordingly, *Bruggmanniella* was stated as an endemic genus of the neotropics, while *Pseudasphondylia*, *Illiciomyia*, and *Odontokeros* would be restricted to Japan (Oriental region).

Lin et al. (2020) used molecular approach based on COI as a single marker to refute our phylogenetic hypothesis to *Bruggmanniella s.l.* In addition, their study also contains a considerable gap of taxon sampling. Only the Oriental/Palaearctic species were included in their analysis.

In order to corroborate the results presented in Garcia et al. (2020), we revisited our previous analysis and we included seven species described under *Bruggmanniella* between 2019 and 2020, *B. miconiae* Rodrigues & Maia, *B. notatae* Rodrigues & Maia, and *B. sideroxyli* Rodrigues & Maia from Brazil; *B. litseae* Lin, Tokuda & Yang, *B. sanlianensis* Lin, Yang & Tokuda, *B. shianguei* Lin, Yang & Tokuda; and *B. turoguei* Lin, Yang & Tokuda from Taiwan. We also discuss some aspects of the evolutionary changes of pupal morphology related with niche occupation of the species under *Bruggmanniella*.

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Material and methods

Taxa sampling and phylogenetic analysis

In this study, we included in the matrix of Garcia et al. (2020) the species of *Bruggmanniella* described between 2019 and 2020: *B. miconiae*, *B. notatae*, *B. sideroxyli*, *B. litseae*, *B. sanlianensis*, *B. shianguei*, and *B. turoguei*. These species were studied only through literature data.

Two Australian species, *B. bursaria* (Felt) and *B. orientalis* (Felt) were tentatively transferred to *Bruggmanniella* in Kolesik and Gagné (2020) based on the two separate teeth of the gonostylus. These species have unknown immature stages and one of them is only known from the male. We decided not to include them in our analysis, since an excessive amount of missing data decreases the robustness of the phylogeny.

The cladistic analysis was performed under the parsimony criterion with implicit weighting in TNT v1.5 (Willi Hennig Society Edition) (Goloboff and Catalano, 2016), following the parameters described in Garcia et al. (2020). The resulting tree was displayed in Adobe Illustrator CC software (17.0). The character list follows Garcia et al. (2020), with information about each character and performance values (CI, RI, *fit*) updated (Appendix S1). No character has been added or removed from the list. See Supplementary Information Table S1 for normalized data, Table S2 for matrix, and Appendix S2 for input matrix script.

Results and Discussion

The cladistic analysis under equal weighting results in 9 parsimonious trees with 172 steps. The analysis under implied weight for *k* = 3 results in a single and most stable topology (length = 179 steps, CI = 0.38, RI

= 0.57, *fit* = 29.55,) that will be discussed here. The MPT for *k* = 3 is shown in Figure 1.

Our new phylogenetic analysis shows *Bruggmanniella s.str.*, *Pseudasphondylia*, and *Odontokeros* as a monophyletic group (Fig. 1) supported by four synapomorphies: one pair of inner lateral papillae and terminal papillae of the larva indistinct or absent, spiniform shape of abdominal spiracles in pupa, and gonostyli bi-toothed in males.

The phylogenetic reconstruction also supports the monophyly of (*Pseudasphondylia* + *Bruggmanniella*) which is consistent with our prior results (Garcia et al., 2020). The clade is strongly supported by the larval characters: outer apical teeth of spatula larger than inner ones (or inner completely reduced) and reduction of outer lateral papillae to one pair.

The *Pseudasphondylia* species are grouped by the fusion of the first and second female flagellomeres. In this analysis, *Illiciomyia* appeared nested inside *Pseudasphondylia* clade. This monotypic genus is only distributed in Japan and induces galls on leaves, as most species of *Pseudasphondylia*, indicating that this position is morphologically, ecologically, and biogeographically consistent. This result supports *Illiciomyia* as the new junior synonym of *Pseudasphondylia* and the new combination of *Illiciomyia yukawai* as *Pseudasphondylia yukawai* (Tokuda, 2004) new. comb.

In the previous study of Garcia et al. (2020) *B. actinodaphnes* and *B. cinnamomi* were placed among *Pseudasphondylia*. However, with the inclusion of the new species from the Oriental region, our current results show that *B. actinodaphnes* and *B. cinnamomi* do not fit under either *Bruggmanniella* or *Pseudasphondylia*. The new topology reinforces the validity of the genus *Odontokeros* to house not only *B. brevipes*, but all species occurring in the Oriental/Palaearctic regions, contrasting

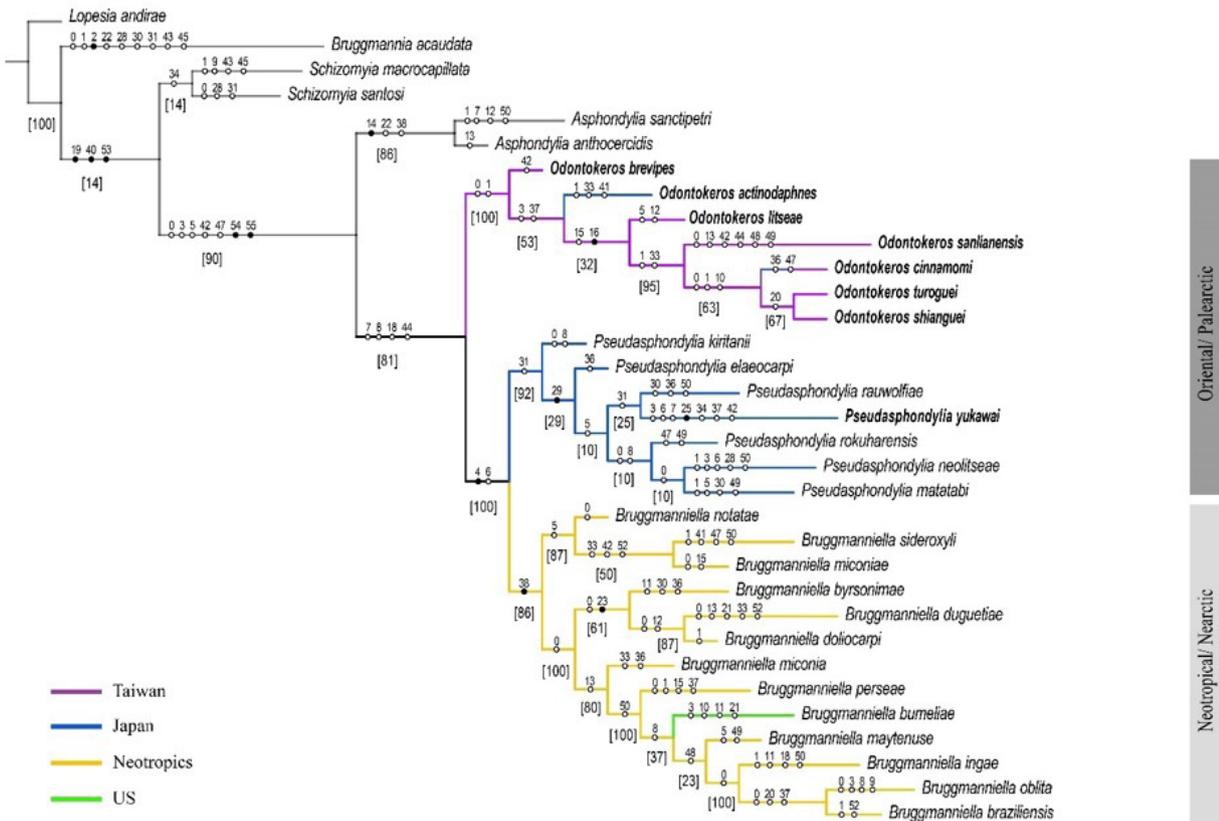


Figure 1 Most parsimonious tree under implied weight (*k* = 3) of the genus *Bruggmanniella* Tavares, 1909. Values in brackets show the relative Bremer support (in percentage). Purple clades symbolize the species distributed in Taiwan. Blue symbolizes the species distributed in Japan. Yellow clades represent the species with distribution in the Neotropics, mostly Brazil. Green clade represents the species with occurrence in the US.

the arguments pointed by Lin et al. (2020) that those species should not be treated as a distinct genus. This clade is highly supported by the main synapomorphies: the increase in length of pupal antennal horn, the decrease in length of pupal cephalic setae, and the molecular similarities indicated in Lin et al. (2020).

We have a different interpretation regarding the close relationship between the non-Neotropical *Bruggmanniella* (= *Odontokerus*) and *Pseudasphondylia*. The conflicting arguments stated in Lin et al. (2020) are due to incomplete taxon sampling once *Illiciomyia* and the Neotropical species of *Bruggmanniella* were not included in their analysis, despite the availability of COI sequences of *I. yukawai* and *B. miconia* in the GenBank.

Lin et al. (2020) also used two other arguments to disqualify the analysis of Garcia et al. (2020): the branch support index and the monophyly of the Asian *Bruggmanniella** indicated by their molecular sequences (using COI as a single marker). The authors stated that the branches in Garcia et al. (2020) topology were supported by low values of *bootstrap* when the measure used in our previous study was not the *Bootstrap* but the *relative Bremer support*. Despite the misunderstanding about the kind of support index used, we agree that they were not high values. However in the current analysis, after including more Palaearctic and Neotropical species the clades previously obtained are stable: *Odontokeros* and (*Pseudasphondylia* + *Bruggmanniella*) with high values of *relative Bremer index* – 100 for both clades. The genera *Pseudasphondylia* and *Bruggmanniella* also are now presenting higher support values, above 80.

Geographical distribution and Niche occupation

The *Odontokeros* species assigned are mostly distributed in Taiwan (except for *B. actinodaphnes*, only found in Japan so far) and induces galls exclusively on leaves or stems in Lauraceae species, chiefly of the genus *Cinnamomum* Schaeff. We agree with Tokuda and Yukawa (2005, 2006) and Lin et al. (2020) that these Lauraceae-associated species are closely related (they occupy very similar positions in the phylogenetic trees of both studies), showing that the phylogenetic relationships among host species drives this association. *Bruggmanniella* and *Pseudasphondylia* are associated with 11 different plant families each. *Pseudasphondylia* induces galls on leaves, fruits, and flower buds while the *Bruggmanniella* species induce gall on stems, fruits, and flower buds. The niche tissue type seems to be associated with the host plant in these two groups while phylogenetic identity of the host is less important.

The progressive decrease from four to two teeth of the spatula and in the size of the antennal horn in *Pseudasphondylia* suggest that the females lay their eggs in soft tissues (as parenchyma in leaves, fruits, and flower buds). The Neotropical *Bruggmanniella* shows an intermediate condition (the majority in stems, but some species are gall inducers in soft tissues) reflected by the decrease from four to three teeth of the spatula in some species (the type-species *B. braziliensis* and *B. oblita*) and the less developed antennal horns.

Bruggmanniella is confirmed here as a monophyletic and sister to the clade with *Pseudasphondylia* species, based on the loss of the parameres. This result also allows us to understand the presence of parameres as a plesiomorphic state to the Asphondyliina subtribe. A study with a total evidence approach (molecular data and morphology) and a complete taxon sampling of the group including Oriental/Palaearctic and Neotropical species, will greatly contribute to understanding this interesting evolutionary history.

Taxonomy

For all the arguments presented here, we re-established the validity of the genus *Odontokeros* and the combination *Odontokeros brevipes*

comb. rev. We also transfer the species *B. actinodaphnes*, *B. cinnamomi*, *B. brevipes*, *B. litseae*, *B. sanlianensis*, *B. shianguei*, and *B. turoguei* to the same genus. The diagnoses of *Bruggmanniella*, *Odontokeros*, and *Pseudasphondylia* are updated, with the new diagnostic characters underlined.

Order Diptera Linnaeus, 1758

Family Cecidomyiidae Newman, 1834

Tribe Asphondyliini Gagné, 1994

Genus *Odontokeros* Garcia, Lamas and Urso-Guimarães, 2020

Odontokeros Garcia, Lamas and Urso-Guimarães, 2020: 11.

Type species. *Odontokeros brevipes* (Lin, Yang and Tokuda, 2019) **comb. rev.**

Bruggmanniella brevipes Lin, Yang and Tokuda, 2019: 205–206, Figs 1B–1E, 2A–2F.

Diagnosis. Prothoracic spatula with 2 or 4-teeth, if four, inner teeth shorter than outer ones; pupa with deeply toothed antennal horns and prothoracic spiracles well developed, upper pupal antennal horn elongated and pupal cephalic setae reduced, upper and frontal horns absent, presence of thickening on pupal cephalic margin; antenna with 12 flagellomeres, male genitalia with two well-developed gonostylar teeth, hypoproct deeply bilobed, parameres membranous, cerci-like lobes, female with shortened legs; and molecular similarities indicated in Lin et al. (2020) (Lin et al., 2019; Garcia et al., 2020).

Odontokeros actinodaphnes (Tokuda and Yukawa, 2006) New combination.

Bruggmanniella actinodaphnes Tokuda and Yukawa, 2006: 633–635, Figs 2A, 2B, 3A and 3B.

Pseudasphondylia actinodaphnes (Tokuda and Yukawa, 2006) **comb. nov.** in Garcia, Lamas & Urso-Guimarães 2020: 11

Odontokeros cinnamomi (Tokuda and Yukawa, 2006) New combination.

Bruggmanniella cinnamomi Tokuda and Yukawa, 2006: 630–633, Figs 2C, 3D, 3C, and 3D.

Pseudasphondylia actinodaphnes (Tokuda and Yukawa, 2006) **comb. nov.** in Garcia, Lamas & Urso-Guimarães 2020: 11.

Odontokeros litseae (Lin, Yang and Tokuda, 2020) New combination.

Bruggmanniella litseae Lin, Yang and Tokuda, 2020: 13–15, Figs 4–5.

Odontokeros sanlianensis (Lin, Yang and Tokuda, 2020) New combination.

Bruggmanniella sanlianensis Lin, Yang and Tokuda, 2020: 7–9, Figs. 4, 5C, 6C, F, and Table 4.

Odontokeros shianguei (Lin, Yang and Tokuda, 2020) New combination.

Bruggmanniella shianguei Lin, Yang and Tokuda, 2020: 6–8, Figs. 3 and 5B, 6B, E, and Table 3.

Odontokeros turoguei (Lin, Yang and Tokuda, 2020) New combination.

Bruggmanniella turoguei Lin, Yang and Tokuda, 2020: 4–6, Figs. 2, 5A, 6A, D, and Table 2.

Genus *Pseudasphondylia* Monzen, 1955

Pseudasphondylia Monzen, 1955: 41

Type species. *Pseudasphondylia rokuharensis* Monzen, 1955

Philadelphella Kovalev, 1964: 440

Type species. *Philadelphella philadelphi* Kovalev, 1964

Illiciomyia Tokuda, 2004: 4 **syn. nov.**

Type species. *Illiciomyia yukawai* Tokuda, 2004

Diagnosis. Prothoracic spatula with 2 or 4-teeth, if four, inner teeth larger than outer ones; antenna with 12 flagellomeres, first and second female flagellomeres fused, gonostylus suboval with two sclerotized teeth, gonocoxite slightly extending beyond the insertion of gonostylus,

parameres membranous, cerci-like lobes, ovipositor protractile, conical, slender, and aciculate (Monzen 1955; Tokuda & Yukawa, 2005).

Pseudasphondylia yukawai (Tokuda, 2004) New combination.

Illiciomyia yukawai Tokuda 2004: 1–11. Fig. 2 and Table 2.

Genus *Bruggmanniella* Tavares, 1909

Bruggmanniella Tavares, 1909: 19.

Type species. *Bruggmanniella braziliensis* Tavares, 1909.

Hemibruggmanniella Möhn, 1961b: 6.

Type species. *Bruggmanniella oblita* Tavares, 1920.

Diagnosis. Prothoracic larval spatula with 3 or 4-teeth, inner teeth (or tooth) larger than outer ones; pupa with antennal horns and well-developed prothoracic spiracles, upper and frontal horns absent, pupal cephalic margin thickened; male genitalia with two-toothed gonostyli, parameres absent; cerci-like lobes on female abdominal segment VIII (Tavares, 1909; Garcia et al., 2020).

Acknowledgments

The authors acknowledge Dr. John Wenzel for helpful comments on the draft version of the manuscript.

Funding

This study was funded by “Fundação de Amparo à Pesquisa do Estado de São Paulo” (FAPESP – 2016/19010-4) and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil” (CAPES – Finance Code 001). CJEL was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Proc. 302751/2019-0).

Conflicts of interest

The authors have no competing interests to declare that are relevant to the content of this article.

Author contribution statement

All authors contributed to the manuscript conceptualization. CAG performed the material preparation, data collection, and analysis. CAG wrote the first draft of the manuscript. CJEL and MVUG supervised the study, reviewed and edited the manuscript. All authors read and approved the final version.

Supplementary material

The following online material is available for this article:

Appendix S1 - List Characters.

Appendix S2 - Input Matrix Script.

Table S1 - Normalized Data.

Table S2 - Matrix.

This material is available as part of the online article from <https://www.scielo.br/j/rbent>

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