

## SHORT COMMUNICATION

## Wing base morphology of Aetalionidae (Hemiptera: Cicadomorpha) and its phylogenetic implications

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Aetalionidae is a small family belonging to the treehopper superfamily Membracoidea (Hemiptera: Cicadomorpha). Although the wing-base morphology of Cicadomorpha was examined in detail recently, the wing base of this family has not been investigated to date. We examined morphology of the wing-base structure of Aetalionidae. Using the characters selected from the wing base, we inferred the phylogenetic placement of this family and confirmed that it belongs to the superfamily Membracoidea and is likely a sister group of the Membracidae.

**Key words:** Aetalioninae, Biturritiinae, Membracoidea, treehoppers.

The family Aetalionidae is a small group of the superfamily Membracoidea (Hemiptera: Cicadomorpha) and includes about 40 species (Deitz & Wallace 2010). This family is composed of two subfamilies, Aetalioninae and Biturritiinae. The Aetalioninae are distributed in South America, southern North America and the Oriental region whereas the Biturritiinae are unique to South America (Deitz & Dietrich 1993). Generally, the family Aetalionidae is regarded as the sister group of Membracidae (Forero 2008), which shows extremely specialized and diversified morphology (e.g. helmet) and behavior (e.g. ant attendance and egg guarding) (Wood 1993). Therefore Aetalionidae is a key taxon for uncovering the origin and diversifications of the treehoppers.

Recently, Yoshizawa and Wagatsuma (2012) inferred phylogenetic relationships among the cicadomorphan families (Cicadidae, Tettigarctidae, Aphrophoridae, Cercopidae, Machaerotidae, Cicadellidae and Membracidae) based on characters selected from the wing-base structure, and obtained a result that is concordant with the results of a recent phylogenetic analysis based on multi-locus DNA sequence data (Cryan & Urban 2012).

In Membracoidea, the wing base structures of Membracidae and Cicadellidae were investigated. However, the wing base of Aetalionidae has not been examined to date.

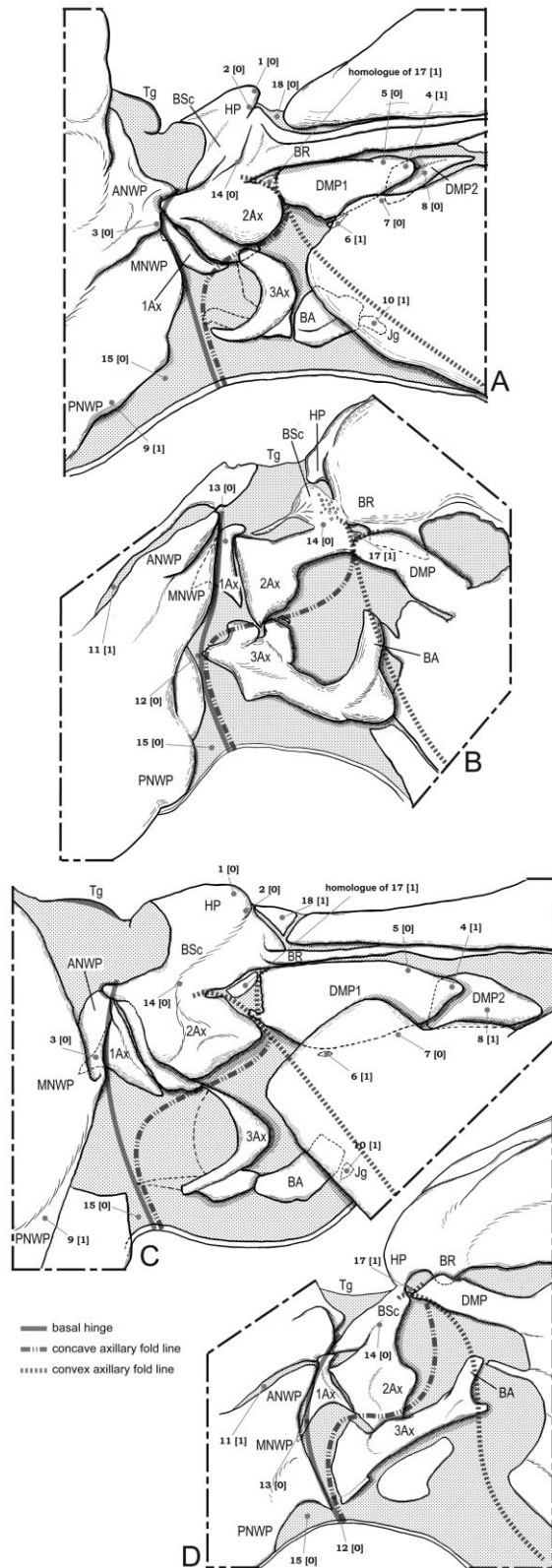
In this study, we examined the fore- and hindwing base structures of *Darthula hardwicki* (Aetalioninae) (China: Niubo Dam, Luchun, Hinghe, Yunnan province, 13.v.2012, M.Maruyama leg.) and *Lophyraspis scutellata* (Biturritiinae) (French Guiana: Roura, Cayenne, 18.iii.2013, N.Ogawa leg.) as representatives of both the subfamilies of Aetalionidae. Dried specimens or specimens stored in 80% ethanol were used. Methods for preparation, observation and illustration followed Yoshizawa (2011). Vouchers are deposited in the Hokkaido University Insect Collection. Terminology followed Yoshizawa and Saigusa (2001) and Yoshizawa and Wagatsuma (2012). The following abbreviations were used in the text and figures: anterior, median, posterior notal wing processes = ANWP, MNWP, PNWP, respectively; pseudo-PNWP = pPNWP; first, second, third axillary sclerites = 1Ax, 2Ax, 3Ax, respectively; proximal, distal median plates = PMP, DMP, respectively; tegula = Tg; humeral plate = HP; Jugum = Jg; basisubcostale = BSc; basiradiale = BR; basanale = BA.

Forewing base (Fig. 1A,C): ANWP and MNWP apparent; PNWP indistinct, but corresponding notal region expanded laterally in Biturritiinae; pPNWP unrecognized. Tg obvious. HP fused with BSc posteriorly, distally fused with additional triangular sclerite in

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**Figure 1** Wing-base structures of Aetalionidae. (A,B) *Darthula hardwicki* (Aetalionidae: Aetalioninae); (C,D) *Lophyraspis scutellata* (Aetalionidae: Biturritiinae). (A,C) Forewing; (B,D) hindwing. Numbers followed by a number in square brackets indicate the character number, and the numbers in the square brackets indicate state of the character (see Supporting Information).

Biturritiinae for which the homology could not be determined. 1Ax triangular, lacking neck and head. 2Ax fused with BSc and BR anteriorly, anterior region strongly convex, distally tightly associated with DMP, with small sclerite between them (recognized as part of 2Ax by Yoshizawa & Saigusa 2001); these sclerites fused with each other in Aetalioninae but separated in Biturritiinae. 3Ax crescent in shape, with three arms; anterior arm tightly articulated with posterodistal corner of 2Ax, proximal arm widely separated from notum when wings are opened; distal arm tightly articulated with BA. DMP1 well developed and bulged strongly, transversally long, posteriorly with tiny sclerite. DMP2 much smaller than (Aetalioninae) or about same size as 2Ax (Biturritiinae). PMP completely membranous. Jg small.

Hindwing base (Fig. 1B,D): Notal region containing ANWP and MNWP partly separated from other notal region; PNWP with (Biturritiinae) or without (Aetalioninae) distinct projection; pPNWP unrecognized. Tg absent. HP separated from BSc. 1Ax triangular with neck and head. 2Ax partly fused with BSc anteriorly, posterior region convex, distally tightly associated with DMP, with small sclerite located between them; these sclerites fused with each other in Aetalioninae but separated in Biturritiinae. 3Ax transversally long, anterior arm less developed, distally fused with BA; PMP completely membranous. Jg absent, but Jg-like sclerite present in Biturritiinae.

Based on the above observations, we coded characters selected from the aetalionid wing base and appended them to the character matrix presented in Yoshizawa and Wagatsuma (2012) with following modifications: Character 11 (hindwing; anterolateral notal region) was re-coded as (0) not separated, (1) partly separated posteriorly, (2) partly separated anteriorly and (3) completely separated. This multistate character was treated as unordered; Character 17 [hindwing; sclerite between 2Ax and DMP1: (0) absent, (1) present] and Character 18 [forewing; sclerite distal to HP: (0) absent, (1) present] were newly added. The maximum parsimony analysis based on the data matrix (Appendix S1; Table S1) using branch-and-bound option of PAUP\* 4.0b10 (Swofford 2002) resulted in two equally

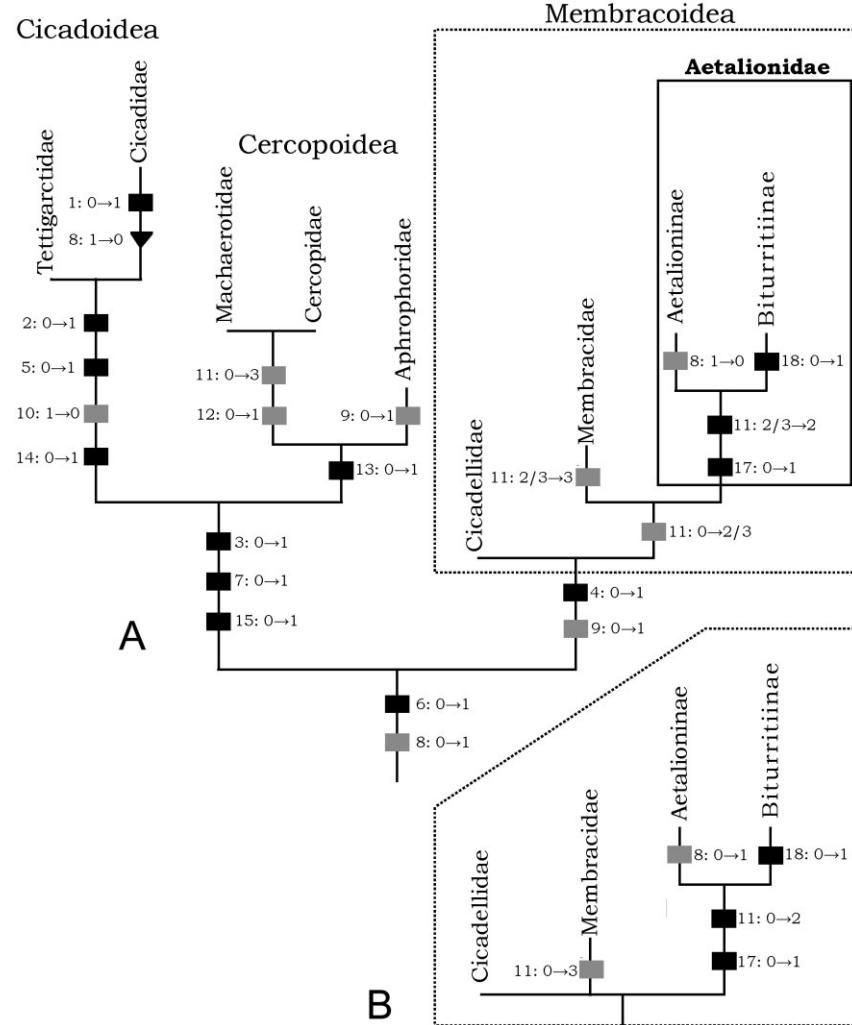


Figure 2 Two most parsimonious trees inferred from the characters selected from the wing-base structure. Outgroups are omitted. Character and character state changes reconstructed on the branches are indicated by black bars (non-homoplasious) and gray bars (homoplasious). See Online Supporting Information for the characters used for the analysis.

parsimonious trees (tree length = 24, consistency index = 0.79, retention index = 0.86). These were completely congruent with those presented in Yoshizawa and Wagatsuma (2012), and two trees differed in the placement of Aetalionidae (Fig. 2). Both trees identified Aetalionidae as monophyletic group, and the family was placed either to the sister of Membracidae (Fig. 2A) or into Membracoidea with unresolved familial relationships (Fig. 2B).

The character states supporting the monophyly of Membracoidea are DMP1 swelling distally and overlapping DMP2 (Character 4-1) and absence of PNWP projection (Character 9-1). These states of DMP1 and PNWP have been identified as autapomorphies of Membracoidea by Yoshizawa and Wagatsuma (2012) and are also observed in Aetalionidae. The placement of Aetalionidae as sister to Membracidae was supported by one of two equally parsimonious trees, with partly or

completely separated anterolateral region of metanotum as their synapomorphy (Character 11-2/3). The state of this character for their common ancestor can not be decided unambiguously because this multistate character was treated as unordered. Homology of the partly and fully separated condition as observed between Aetalionidae and Membracidae is highly plausible morphologically, and a sister group relationship between the families is also likely. A similar state is also observed in some cercopids (Yoshizawa & Wagatsuma 2012), but its independent origin is evident from the tree. The Aetalionidae was identified as a monophyletic group by the following character state: presence of small sclerite between 2Ax and DMP of hindwing (Character 17-1). This sclerite is undoubtedly the serial homologue of the sclerite presented at the corresponding region of the forewing base, which was homologized as a part of 2Ax by Yoshizawa and Saigusa (2001). The sclerite is almost

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consistently observed throughout hemipteran forewing base including Aetalionidae (Yoshizawa & Saigusa 2001) but has never been observed in the hindwing of other hemipteran families (Yoshizawa & Wagatsuma 2012).

The phylogenetic placement of Aetalionidae estimated here is completely concordant with those estimated from other morphological (Dietrich & Deitz 1993) and molecular (Dietrich & Deitz 1993; Dietrich *et al.* 2001; Cryan 2005; Cryan & Urban 2012) data. It is especially notable that aetalionids lack the ability of jumping (M. Maruyama, pers. obs., 2012; N. Ogawa, pers. obs., 2013) whereas Membracidae and Cicadellidae are good jumpers. Although this behavior can potentially affect the mode of their flight and flying apparatus, the phylogeny estimated from the wing base is concordant with that from other data. In addition, the two species examined here also differ significantly in body size (~15 mm in *Darthula hardwicki* vs ~3 mm in *Lophyraspis scutellata*). This can also strongly affect their flight mode, but the two aetalionids retain a unique and non-homoplasious synapomorphy supporting monophyly of the family. The above evidence further corroborates that the wing-base morphology contains a clear phylogenetic signal and is useful in estimating higher level phylogeny. Because some differences between two species were detected in their wing-base morphology, this character system also contains potential for estimating the intra-familial relationships of Aetalionidae.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

- Appendix S1** Characters scored in the wing database.  
**Table S1** Data matrix for phylogenetic analysis.

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