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Wing base morphology supports paraphyly of Papilionoidea s.s. (Lepidoptera)

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Abstract

Traditionally, skippers (Hesperiidae) and moth-like butterflies (Hedylidae) were classified in separate superfamilies outside the butterfly superfamily Papilionoidea *sensu stricto*. However, recent molecular phylogenies including phylogenomics placed the swallowtails (family Papilionidae) as the sister group to all other butterflies, including skippers and moth-like butterflies, making the traditionally recognized Papilionoidea s.s. paraphyletic. However, no morphological studies have supported this relationship to date. Here, we conducted a cladistic analysis based on wing base characters to evaluate these competing systematic hypotheses. Our results support a sister-group relationship between skippers and moth-like butterflies and their inclusion within Papilionoidea *sensu lato*, consistent with molecular-based phylogenies. In contrast, the wing base morphology suggests the monophyly of Pieridae + Riodinidae + Lycaenidae, a result that contradicts both molecular and other morphological evidence, highlighting the potential limitations of this character system. Based on our morphological examinations and ancestral state reconstruction, we propose a hypothetical groundplan for the butterfly wing base.

Key words: Papilionidae, Hedyloidea, Hesperioidea, butterfly, skipper, phylogeny

1 INTRODUCTION

2 Recent advances in phylogenomics have provided the robust framework for insect
3 systematics. In many cases, phylogenomic results agree with the hypotheses proposed
4 morphologically, but significant discordances exist. For example, moss bugs (suborder
5 Coleorrhyncha of Hemiptera) have long been recognized as a sister taxon of true bugs (suborder
6 Heteroptera), but phylogenomic analyses suggest they are closer to leaf and plant hoppers (suborder
7 Auchenorrhyncha) (Misof et al., 2014; Johnson et al., 2018). A subsequent morphological study
8 identified a significant synapomorphy in the wing base between Coleorrhyncha and
9 Auchenorrhyncha (Yoshizawa et al., 2017), corroborating the phylogenomic result. Psocodea
10 (barklice and true lice) are demonstrated that they are the sister group of Holometabola (insects
11 with a pupal stage) (Misof et al., 2014; Johnson et al., 2018), a finding which strongly contradicts
12 the long-recognized Paraneoptera (a superorder including Psocodea, Thysanoptera, and Hemiptera).
13 However, subsequent morphological evaluations recovered no morphological evidence supporting
14 the close relationship between Psocodea and Holometabola (Yoshizawa & Lienhard, 2016; Huang
15 et al., 2016; Kawata et al., 2024), and the monophyly of Paraneoptera remains generally accepted.
16 These examples highlight the importance of morphological datasets in refuting or corroborating
17 phylogenomic results, especially when they contradict generally or traditionally recognized
18 systematic classifications.

19 The phylogeny of butterflies *sensu lato*, previously referred to the Rhopalocera (e.g., Scoble,
20 1986), is one such examples (the word "butterflies" is hereafter used in this broader sense unless
21 specified). Butterflies have traditionally been classified into three superfamilies, Hedyloidea
22 (Neotropical moth-like butterflies), Hesperioidea (skippers), and Papilionoidea *sensu stricto*
23 (butterflies *s.s.*). The traditional separation of skippers from Papilionoidea *s.s.* probably has its roots
24 in their superficial differences, such as their broad heads and characteristic flight (Ackery et al.,
25 1998). The specialized larval prolegs of Hesperioidea were also likely a historical reason for
26 separating skippers from other butterflies, but these are now considered an adaptation related to
27 their sheltered feeding strategy (Scott & Wright, 1990; Heikkilä et al., 2015). The monophyly of
28 Papilionoidea *s.s.* is supported morphologically by the following apomorphies: retinaculo-frenate
29 wing coupling lost in both sexes; antennae with apical clubs (Kristensen, 1976). However, the first
30 apomorphy is also observed in Hesperioidea. Heikkilä et al. (2012) suggested the following
31 additional apomorphies supporting the monophyly of Papilionoidea *s.s.*: mesothoracic anepisternum
32 reduced; membrane attaching tegula to mesonotum at ventral edge; precoxal sulcus not reaching
33 anterior margin of basisternum. However, the validity of these characters is controversial among
34 different workers (see Simonsen et al., 2012). Therefore, despite the long recognition of
35 Papilionoidea *s.s.*, morphological support for this superfamily remains ambiguous.

Recent molecular phylogenies have provided results contradicting this traditional view: i.e., Hedyliidae and Hesperiiidae are embedded within Papilionoidea *s.s.*, making this superfamily paraphyletic, and the family Papilionidae (swallowtails) is recovered as the sister group to all other butterflies (Mutanen et al., 2010; Heikkilä et al., 2012; Kawahara & Breinholt, 2014). This hypothesis has been corroborated by phylogenomic studies (Espeland et al., 2018; Kawahara et al., 2019, 2023), but no morphological studies have ever indicated this relationship (de Jong et al., 1996; Simonsen et al., 2012). These findings challenge the traditional systematics and requires further investigation. Butterflies serve as model organisms in variable biological fields, including ecology, evolutionary biology, biogeography, population genetics, developmental biology, and conservation biology (e.g., Boggs et al., 2003; Wahlberg et al., 2005; Kawahara et al., 2023) so that getting consensus on their phylogenetic relationships is very important. The importance of morphological data in the molecular era was also highlighted by Simonsen et al. (2012).

The present study aims to evaluate the contradicting phylogenetic hypotheses of butterflies using characters selected from the wing base structure. The wing base structure is known to evolve very slowly and thus to be useful for higher-level phylogenetic estimations (e.g., Hörnschemeyer, 2002; Yoshizawa & Saigusa, 2002; Yoshizawa, 2011; Yoshizawa et al., 2017). Despite its potential phylogenetic usefulness, the butterfly wing base has only been examined in a limited number of studies (Sharplin, 1963a,b; de Jong et al., 1996; Ivanov, 1997; Ackery et al., 1998; Heikkilä et al., 2012). Furthermore, their examinations are either limited in taxonomic and character samplings or homology identifications ambiguous. Here, we present the first comprehensive morphological analysis of the butterfly wing base across a wide range of taxa based on a strict landmark-based homology identification.

MATERIALS AND METHODS

Taxon Sampling

The taxa analyzed are listed in Table 1, which include all the currently recognized butterfly families. In general, two species were selected from each butterfly family. However, three species were chosen from Hesperiiidae due to the significant diversity in their wing folding styles (particularly Pyrginae compared to other subfamilies), whereas only one species was selected from Hedyliidae due to its small taxonomic diversity, comprising only 35 species of a single genus. Outgroups included representatives from three moth families, Erebiidae and Saturniidae (both from the sister clade of butterflies) and Tortricidae (a more distantly related lepidopteran outgroup, following Kawahara et al., 2023), as well as Trichoptera, the sister taxon of Lepidoptera.

Morphological Observations and Terminology

The general methods followed Yoshizawa (2011). The present analyses focused only on the forewing base structure, and the right wing was used for observation. The tegula was initially removed and omitted from further examinations and illustrations, as it is strongly developed in Lepidoptera and almost entirely covers the dorsal side of the wing hinge. Observations were conducted using Olympus SZX16 (Tokyo, Japan) and Leica M205C (Wetzlar, Germany) stereoscopic microscopes.

Terminology of the wing base structure followed Yoshizawa (2011), Sharplin (1963a) and Ivanov (1998). The following abbreviations are used throughout the text and figure plates: anterior, antemedian, median, postmedian, posterior notal processes = ANP, AmNP, MNP, pMNP, PNP; first, second, third axillary sclerites = 1, 2, 3Ax; proximal, distal median plates = PMP, DMP; tegula = Tg; humeral plate = HP; basisubcostale = BSc; basiradiale = BR; lateropostnotum = LPN.

Phylogenetic Analyses

Phylogenetic analyses were performed using PAUP* 4.0a build 168 (Swofford, 2002) under the parsimony criterion. Tree searches were conducted using the Branch-and-Bound algorithm, and branch support values were assessed via bootstrap and jackknife resampling (10,000 replicates each). Parsimony-based character mapping was performed using Mesquite 3.81 and MacClade 4 (Maddison & Maddison, 2001, 2023).

RESULTS

Homology Interpretations

The present study revealed that the lepidopteran wing base structure is highly specialized and modified. However, most wing base sclerites could be identified based on their shape, relative position, and articulations (Sharplin, 1963a). Fig. 1 compares the wing base structure of Trichoptera (which retains the neopteran basic condition), the less specialized lepidopteran Tortricidae, and two representative butterflies. Key landmarks for identifying sclerite homologies are indicated. In contrast, the homology of certain structures requires further justification before character coding for phylogenetic analysis. Thus, the interpretations of 2Ax, BR, PMP, and DMP are first addressed here (Sharplin, 1963a).

The homology of 2Ax is particularly debatable due to its significant modification in Lepidoptera. In Trichoptera, this sclerite retains the neopteran groundplan condition, making its identification straightforward. It is divided into two regions, a bare proximal and a setose distal region separated by the vertical ligament ("f" in Fig. 1A). However, in Lepidoptera, the ligament runs almost transversely ("f" in Fig. 1B–D), and the setose (or scaled) sclerite (indicated by gray) is positioned anterior to the bare sclerite. Using the ligament as a landmark, along with the presence

of setae, an anti-clockwise rotation of 2Ax in Lepidoptera is evident.

The boundary between 2Ax and BR is also highly ambiguous. In neopterans, the convex axillary fold line, which contributes to backward wing folding, helps delineate this border ("b" in Fig. 1A, B) (Wootton, 1979). This line is retained in Trichoptera and Tortricidae but is obscure in macrolepidopterans, whose backward wing folding is reduced or absent (Fig. 1C, D). In taxa lacking this fold line, the relationship with PMP and DMP provides an alternative clue for identifying 2Ax and BR. In Trichoptera, the distal tip of 2Ax, the anterior tip of PMP, and the proximal tip of DMP articulate at a point ("h" in Fig. 1A), and DMP narrowly fuses to BR ("i" in Fig. 1A: note that DMP is labeled as 2mPL+Cu2 or Cu2 in Sharplin, 1963a). This articulation pattern is also retained in Tortricidae (Fig. 1B). However, in macrolepidopterans, a membranous region between PMP and DMP disrupts this arrangement. Nevertheless, the sclerite proximal to this membranous region should still be considered a part of 2Ax, as inferred from the narrow BR-DMP fusion ("i" in Fig. 1B–D), which locates the distal end of 2Ax in Trichoptera ("i" in Fig. 1A). Consequently, 2Ax in Lepidoptera appears to consist of three distinct regions: anterior (a2Ax), median (m2Ax), and posterior (p2Ax). The exact boundary between 2Ax and BR remains obscure, though a large portion of Sharplin's radial plate corresponds to a2Ax in the present interpretation (Sharplin, 1963a).

Additionally, a sclerite was observed in the membranous region between PMP and DMP in several taxa ("23" in Figs. 1D, 2, S1–S7). This sclerite could not be homologized to any principal components of the neopteran wing base and is therefore treated here as an unnamed sclerite. It corresponds to the "median plate 2" of de Jong et al. (1996) and Ackery et al. (1998). Similar novel elements have also been documented in cicadomorph insects, located in the membranous region posterior to DMP (Yoshizawa & Wagatsuma, 2012).

Character Coding

Based on the homology interpretations outlined above (Figs. 1, 2, S1–S8), 26 characters were selected from the forewing base for phylogenetic analysis (data matrix available at DOI: 10.6084/m9.figshare.29178344). Uninformative characters were retained if character state changes were detected between Trichoptera and Lepidoptera. However, species-level uninformative characters (e.g., enlarged DMP in *Macrosoma*) were excluded.

1. Between ANP and AmNP: (0) straight or only slightly concave; (1) deeply concave. State 1 was observed in Pieridae, Lycaenidae, and Nymphalidae, as well as *Lymantria* (outgroup).
2. AmNP, posterior margin: (0) almost straight; (1) apparently concave. State 1 was observed in Lycaenidae and *Ochlodes* (Hesperiidae).

- 1 3. MNP and pMNP: (0) located closely; (1) widely separated. State 1 was observed in all
2 lepidopterans examined here.
- 3 4. pMNP: (0) not protruding distally; (1) strongly protruding distally. State 1 was observed in all
4 macrolepidopterans.
- 5 5. Base of PNP: (0) almost on same plane with pMNP; (1) placed over pMNP. State 1 was observed
6 in all lepidopterans examined here.
- 7 6. Bases of PNP and LPN: (0) widely separated; (1) closely located; (2) no membranous separation
8 between them. State 1 was observed in Hedyliidae and Nymphalidae, and 2 was observed in
9 Hesperidae.
- 10 7. Head of 1Ax: (0) with no dorsal upheave; (1) with plateau-like dorsal upheave. State 1 was
11 observed in all butterflies except for Papilionidae and *Aporia* (Pieridae).
- 12 8. Head of 1Ax, shape of plateau: (0) square to oval; (1) triangular to crescent. State 1 was observed
13 in Pieridae, Lycaenidae, Riodinidae and Nymphalidae. Taxa without plateau was coded as ?.
- 14 9. Head of 1Ax, distal corner: (0) not articulated with 2Ax; (1) articulated with 2Ax. State 1 was
15 observed in all butterflies.
- 16 10. Neck of 1Ax: (0) almost flat; (1) proximal margin with dorsal flip-over. State 1 was observed all
17 butterflies except for Papilionidae and *Aporia* (Pieridae).
- 18 11. Neck and body of 1Ax: (0) fused; (1) separated by membrane. State 1 was observed in Pieridae
19 (but not in *Aporia*), Lycaenidae, Riodinidae and Nymphalidae.
- 20 12. Body of 1Ax, anterior region: (0) flat or with weak swellings; (1) with strongly developed
21 transversal ridge. State 1 was observed in Pieridae, Lycaenidae, Riodinidae and
22 Nymphalidae.
- 23 13. Body of 1Ax, posteroproximal corner: (0) not elongated; (1) elongated. State 1 was observed in
24 all lepidopterans observed here.
- 25 14. Body of 1Ax, shape of the posteroproximal corner: (0) narrow; (1) broad; (2) broad with
26 proximal expansion apically; (3) broad and bifurcated apically. State 1 is widely observed in
27 macrolepidopterans, but (0) is observed in Papilionidae. State 2 was observed in Pieridae,
28 Lycaenidae, Riodinidae, and Nymphalidae, and 3 was observed in Hedyliidae.
- 29 15. Body of 1Ax, anterodistal corner: (0) extended anteriorly toward tip of BR; (1) not extended.
30 State 1 was observed in Papilionidae.
- 31 16. Body of 1Ax, posterodistal corner: (0) directed distally; (1) directed anterodistally; (2)
32 anteriorly with flap extending over posteroproximal part of 2Ax. State 1 was observed in
33 Papilionidae, and state 2 was observed in Nymphalidae and Riodinidae.
- 34 17. 2Ax, posteroproximal corner: (0) without well-developed lobe; (1) with well-developed lobe.
35 State 1 was observed in Papilionidae.

- 1 18. 2Ax, median region: (0) strongly sclerotized; (1) weakly sclerotized and separated by
2 membrane. State 1 was observed in HesperIIDae.
- 3 19. 2Ax, median and posterior regions: (0) fused; (1) separated by narrow notch; (2) separated by
4 triangular membranous invagination. State of this character is even variable within a family.
- 5 20. 2Ax, anterior and median regions: (0) no membranous invagination; (1) with membranous
6 notch between them. State 1 was observed in *Luehdorfia* (Papilionidae), Hedylidae,
7 HesperIIDae, *Aporia* (Pieridae), Riodinidae and *Favonius* (Lycaenidae).
- 8 21. 3Ax, distal margin: (0) smooth; (1) with small conical projection; (2) large triangular lobe. State
9 1 was observed in Pieridae (tooth of 3Ax of Ivanov, 1998) and Riodinidae (but the condition
10 in *Lyropteryx* was somewhat intermediate between 0 and 1), and state 2 was observed in
11 HesperIIDae and Hedylidae, as well as *Lymantria* (outgroup).
- 12 22. 3Ax and PMP: (0) separated; (1) fused. State 1 was observed in Pieridae, Lycaenidae,
13 Riodinidae, as well as Tortricidae and Trichoptera in the outgroups.
- 14 23. Un-named sclerite between PMP and DMP: (0) absent; (1) present proximally; (2) present
15 distally. State 1 was observed in *Lymantria* (outgroup), and state 2 was observed in all
16 butterflies except for Papilionidae. This sclerite corresponds to "median plate 2" of de Jong
17 et al. (1996) and Ackery et al. (1998).
- 18 24. BSc, apex: (0) tapering; (1) with projection directed posterodistally; (2) truncated. State 1 was
19 observed in Papilionidae, Pieridae, Riodinidae, and Lycaenidae, but the condition of
20 Lycaenidae was somewhat intermediate between 0 and 1. State 2 was observed in *Caligula*
21 (outgroup).
- 22 25. BSc, distal part: (0) broad; (1) narrow. State 1 was observed in Lycaenidae, Riodinidae and
23 Trichoptera.
- 24 26. BR: (0) apically reached to anteroproximal corner of 2Ax; (1) membrane invaginated, and its
25 apex distant from anteroproximal corner of 2Ax. State 1 was observed in all
26 macrolepidopterans.

28 **Phylogenetic Analysis**

29 Preliminary analyses indicated that the dataset lacked a clear phylogenetic signal supporting the
30 outgroup relationships, which also disrupted butterfly monophyly. However, regarding butterfly
31 monophyly, there is no conflict between morphological and phylogenomic evidence (e.g.,
32 Simonsen et al., 2012; Kawahara et al., 2019, 2023). Therefore, to identify phylogenetic signals
33 contained in the wing base structure in butterflies, the outgroup relationships including butterfly
34 monophyly were constrained according to Kawahara et al. (2023).

35 The constrained analysis yielded three equally parsimonious and well-resolved trees, with

length = 54, consistency index = 0.63, and retention index = 0.81. Three trees differed only in the relationship within the clade composed of Pieridae, Lycaenidae and Riodinidae (see below for detail). Within butterflies, Papilionidae was recovered as the sister to all other families, whose monophyly received strong support (bootstrap/jackknife values = 94/90%). The remaining families formed two clades, Hesperidae + Hedyliidae (84/77) and Pieridae + Riodinidae + Lycaenidae + Nymphalidae (68/66). Within the latter clade, the monophyly of Pieridae + Riodinidae + Lycaenidae was weakly supported (60/55), but the relationships within this clade were highly unstable. The monophyly of all families was moderately to strongly supported, except for Pieridae, for which monophyly was not supported. Apomorphies supporting each clade are shown in Fig. 3.

DISCUSSION

Hypothetical Groundplan Condition of the Butterfly Wing Base

According to the present morphological examinations and the ancestral state reconstruction, the groundplan condition of the butterfly's wing base structure can be summarized as follows. Many of the specializations mentioned below are not unique to butterflies and probably evolved in much older lepidopteran ancestors (Sharplin, 1963a).

Notal margin. Anterior notal projection broadened, apically with two articular points with 1Ax (ANP with neck; AmNP with anteroproximal tip of body). Median notal projection strongly developed and located posteriorly, very close to PNP, distally broadened with two articular points with body of 1Ax (MNP with proximal margin, MNP placed above; pMNP with posteroproximal tip, 1Ax placed above), MNP strongly extended anteriorly. PNP long, region around base of PNP and LPN strongly swelling anterodorsally, causing placement of PNP over pMNP.

Hinge sclerites. 1Ax: enlarged, articulated with BSc by head, ANP by neck, and AmNP by anteroproximal tip of body; proximal margin of body expanded proximally under MNP-pMNP margin; posteroproximal tip of body extended posteriorly and articulated with pMNP; distal part of body extended distally, articulated with posteroproximal corner of 2Ax by its anterior end (2Ax placed above) and posterodistal corner of 2Ax by distal tip (1Ax placed above). 2Ax: rotated anti-clockwise, causing scaled (or setose in Trichoptera) region located anterior to bare region, and ligament toward ventral 2Ax running transversally; posterior margin concave to accept posterodistal arm of body of 1Ax; anteriorly connected with BR and distally articulated with PMP; with narrow membranous invagination between median and posterior 2Ax regions. 3Ax: strongly twisted medially and its proximal arm flipped; anterior arm articulated with PMP; distal arm reduced, articulated with base of A vein along distal margin. PMP: concave, separated from 3Ax; articulated with 2Ax with its anteroproximal and posteroproximal corners; with broad membranous region between PMP and DMP. DMP: strongly convex and transversally elongated, densely scaled

1 but its proximal tip, connecting to BR, with small bare region. Tg enlarged, densely scaled, strongly
2 expanded posteriorly over wing hinge.

3 *Vein bases.* HP only weakly sclerotized or mostly membranous. Distal region of BSc broadly
4 expanded anteriorly. BR broad, connected with BSc anteriorly and with 2Ax posteriorly; its distal
5 end strongly squeezed.

7 **Phylogeny**

8 The present phylogenetic analysis supports a basal split between the family Papilionidae and
9 the remaining butterfly families, including Hesperidae and Hedyidae, which agrees with recent
10 phylogenomic studies (Kawahara et al., 2019, 2023) and earlier Sanger-based molecular
11 phylogenies (Mutanen et al., 2010; Heikkilä et al., 2012). These findings conflict with the
12 traditional definition of Papilionoidea s.s. (butterflies excluding skippers and moth-like butterflies).
13 The widespread acceptance of Papilionoidea s.s. may be influenced by the significant external
14 morphological differences between skippers and other butterflies, but previous morphological
15 studies have failed to provide strong apomorphies supporting this traditional classification.

16 Our analysis identified several wing base synapomorphies supporting the exclusion of
17 Papilionidae from the remaining butterflies. These characters include a plateau-like dorsal upheave
18 of the head of 1Ax (Cha.7[1]), a dorsally flipped-over neck of 1Ax (Cha.10[1]), a triangular
19 membranous invagination separating the median and posterior regions of 2Ax (Cha.19[2]), and
20 presence an unnamed sclerite on the membranous region anterior to PMP (Cha.23[2]), with the
21 latter being non-homoplasious. Previous morphology-based phylogenetic studies have not
22 supported this relationship (Simonsen et al., 2012), but there are a few additional morphological
23 characters supporting this result. Kristensen (1976) identified two potential synapomorphies uniting
24 all butterflies except Papilionidae: the presence of a mesothoracic aorta with a horizontal chamber
25 and the absence of the CuP vein. Heikkilä et al. (2012) also noted a reduced median arm in the
26 hindwing as a potential apomorphy for this clade. Heikkilä et al. (2012) proposed another potential
27 apomorphy, an outer margin of 3Ax with a blunt tooth articulating with the base of A veins, but this
28 could not be confirmed in the present study (Figs. 1, 2, S1–S8).

29 The wing base characters recovered additional higher-level relationships that are largely
30 concordant with molecular studies. The sister-group relationship between Hesperidae and
31 Hedyidae is supported by two prominent synapomorphies: a membranous notch between the
32 anterior and median plates of 2Ax (Cha.20[1]) and a triangular lobe on the distal margin of 3Ax
33 (Cha.21[2]). Although both characters exhibit homoplasy, the condition of Cha.21[2] differs
34 significantly between Hesperidae + Hedyidae and the outgroup *Lymantria*: in the former, the lobe
35 arises from the upper side of the lateral margin of 3Ax (Fig. 2AB), whereas in *Lymantria*, it

1 originates from the lower side near the anterodistal end (Fig. S1). Thus the condition in Hesperidae
2 and Hedyidae is unique to this clade. Molecular data also strongly support this relationship
3 (Mutanen et al., 2010; Heikkilä et al., 2012). However, historically, the placement of Hedyidae has
4 been controversial. Scoble (1986, 1988) initially placed Hedyidae within butterflies and suggested
5 a possible sister-group relationship with Papilionoidea *s.s.* Later morphology-based studies (de Jong
6 et al., 1996; Ackery et al., 1998) placed Hedyidae as the sister group to (Hesperioidea +
7 Papilionoidea *s.s.*), a view later supported by combined morphological and molecular analyses
8 (Wahlberg et al., 2005). The close relationship between Hedyidae and Hesperidae was previously
9 proposed by Heikkilä et al. (2012) based on the presence of a triangular 3Ax (corresponding to
10 Cha.21[2] in our study) and mesophragma with flat ridges.

11 The monophyly of a clade composed of Pieridae, Lycaenidae, Riodinidae and Nymphalidae is
12 supported by four apomorphies, two of which are non-homoplasious (Fig. 3): a deeply concave
13 margin between ANP and AmNP (Cha.1[1]), a membrane separating the neck and body of 1Ax
14 (Cha.11[1]), a strongly developed transversal ridge on the body of 1Ax (Cha.12[1]), and the
15 elongated posteroproximal corner of 1Ax with proximal expansion apically (Cha. 14[2]). This clade
16 is consistent with both phylogenomic studies (Kawahara & Breinholt, 2014; Kawahara et al., 2019,
17 2023) and traditional morphology-based hypotheses (Kristensen, 1976; de Jong et al., 1996; Ackery
18 et al., 1998; Simonsen et al., 2012).

19 Some relationships recovered in the present analysis are inconsistent with generally accepted
20 hypotheses. As mentioned above, the wing base characters failed to unambiguously support the
21 monophyly of butterflies, although non-homoplastic apomorphy supporting the butterfly
22 monophyly was identified by parsimonious character mapping (Fig. 3: Cha. 9[1]). In addition, the
23 present analysis suggests a close relationship between Pieridae, Riodinidae and Lycaenidae,
24 whereas previous morphological and molecular studies consistently support a closer relationship
25 between Lycaenidae + Riodinidae and Nymphalidae (Simonsen et al., 2012; Kawahara & Breinholt,
26 2014; Kawahara et al., 2019, 2023). Support values for this clade were low (Fig. 3), suggesting that
27 the wing base characters alone do not strongly resolve this phylogenetic question.

28 The wing base characters also provide additional support for the monophyly of all butterfly
29 families, except for Pieridae. The instability of Pieridae's monophyly appears to be influenced by
30 numerous homoplasies within *Aporia*. This taxon exhibits homoplastic similarities with *Favonius*
31 (Lycaenidae) (e.g., Cha.20[1]: Figs. 1D, 2C), resulting in some bootstrap and jackknife resampling
32 recovering their sister-group relationship. Additional reversals to the ancestral condition were also
33 observed in *Aporia* (Fig. 3), suggesting that taxa with unusual evolutionary trends can significantly
34 affect phylogenetic stability.

35 In conclusion, the present study demonstrates that wing base morphology contains strong

phylogenetic signals that align with molecular-based phylogenies, particularly in supporting the sister-group relationship between Hedyliidae and Hesperiiidae within Papilionoidea *s.l.* However, certain limitations of wing base characters in phylogenetic estimation are also evident, as they fail to consistently recover butterfly monophyly and suggest a less-likely relationship between Pieridae and Lycaenidae + Riodinidae. The wing base structure in Lepidoptera is highly specialized and modified, with many of the observed modifications in butterflies likely originating in deeper evolutionary ancestors (Sharplin, 1963a). Therefore, wing base morphology may prove valuable for resolving higher-level relationships within Lepidoptera. Future studies should explore the functional aspects driving these modifications, as they may provide further insights into the evolutionary history of butterfly wing morphology (Sharplin, 1963; Emoto, 1988).

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AUTHOR CONTRIBUTIONS

KY conceived the project. KS and KY designed study, examined morphology, and performed phylogenetic analyses. KS, KY and MY analyzed morphology and interpreted the results. All authors wrote and approved the final manuscript.

REFERENCES

- Ackery PR, de Jong R, Vane-Wright RI (1998) The butterflies: Hedyloidea, Hesperioidea and Papilionoidea. In: Kristensen NP (ed.) *Lepidoptera, Moths and Butterflies. Evolution, Systematics and Biogeography*, pp 399–435. Handbook of Zoology, vol. 1. Walter de Gruyter, Berlin.
- Boggs CL, Watt WB, Ehrlich PR (2003) *Butterflies: Evolution and Ecology Taking Flight*. Rockymountain Biological Lab Symposium Series. University of Chicago Press, IL.
- de Jong R, Vane-Wright RI, Ackery PR (1996) The higher classification of butterflies (Lepidoptera): problems and prospects. *Entomologica Scandinavica* **27**, 65–101.
- Emoto J (1988) The morphological peculiarity and evolution of the mesothorax in butterflies from a

- view point of relationships between structure and function. *Special Bulletin of Lepidopterous Society of Japan* **6**, 101–122. (In Japanese with English summary)
- Espeland M, Breinholt J, Willmott KR et al. (2018) A comprehensive and dated phylogenomic analysis of butterflies. *Current Biology* **28**, 770–778.
- Heikkilä M, Kaila L, Mutanen M, Peña C, Wahlberg N (2012) Cretaceous origin and repeated tertiary diversification of the redefined butterflies. *Proceedings of the Royal Society B* **279**, 1093–1099.
- Heikkilä M, Mutanen M, Wahlberg N, Sihvonen P, Kaila L (2015) Elusive ditrysian phylogeny: an account of combining systematized morphology with molecular data (Lepidoptera). *BMC Evolutionary Biology* **15**, 260.
- Hörschemeyer T (2002) Phylogenetic significance of the wing-base of the Holometabola (Insecta). *Zoologica Scripta* **31**, 19–27.
- Huang DY, Bechly G, Nel P et al. (2016) New fossil insect order Permopsocida elucidates major radiation and evolution of suction feeding in hemimetabolous insects. *Scientific Reports* **6**, 23004.
- Ivanov VD (1998) Wing articulation in whites (Lepidoptera, Peridae). *Entomological Review* **78**, 667–681.
- Johnson KP, Dietrich CH, Friedrich F et al. (2018) Phylogenomics and the evolution of hemipteroid insects. *PNAS* **115**, 12775–12780.
- Kawahara AY, Breinholt JW (2014) Phylogenomics provides strong evidence for relationships of butterflies and moths. *Proceedings of the Royal Society B* **281**, 20140970.
- Kawahara AY, Plotkin D, Espeland M et al. (2019) Phylogenomics reveals the evolutionary timing and pattern of butterflies and moths. *PNAS* **116**, 22657–22663.
- Kawahara AY, Storer C, Carvalho APS et al. (2023) A global phylogeny of butterflies reveals their evolutionary history, ancestral hosts and biogeographic origins. *Nature Ecology & Evolution* **7**, 903–913.
- Kawata A, Ogawa N, Yoshizawa K (2024) Morphology of the pterothoracic musculature in Paraneoptera and its phylogenetic implication (Insecta: Neoptera). *Journal of Morphology* **285**, e21712.
- Kristensen NP (1976) Remarks on the family-level phylogeny of butterflies, (Insecta, Lepidoptera, Rhopalocera). *Zeitschrift für Zoologische Systematik und Evolutionsforschung* **14**, 25–33.
- Maddison DR, Maddison WP (2001) *MacClade 4: Analysis of Phylogeny and Character Evolution*. Sinauer, Sunderland, MA.
- Maddison WP, Maddison DR (2023) *Mesquite: A Modular System for Evolutionary Analysis*, Version 3.81. <https://www.mesquiteproject.org>.

- 1 Misof B, Liu S, Meusemann K et al. (2014) Phylogenomics resolves the timing and pattern of
2 insect evolution. *Science* **346**, 763–767.
- 3 Mutanen M, Wahlberg N, Kaila L (2010) Comprehensive gene and taxon coverage elucidates
4 radiation patterns in moths and butterflies. *Proceedings of the Royal Society B* **277**, 2839–
5 2848.
- 6 Scoble MJ (1986) The structure and affinities of the Hedyloidea: a new concept of the butterflies.
7 *Bulletin of British Museum of Natural History (Entomology)* **53**, 251–286.
- 8 Scott JA, Wright DM (1990) Butterfly phylogeny and fossils. In: Kudrna O (ed.) *Butterflies of*
9 *Europe, vol. 2: Introduction to lepidopterology*, pp 152–208. Aula-Verlag, Wiesbaden.
- 10 Sharplin J (1963a) Wing base structure in Lepidoptera I. Fore wing base. *The Canadian*
11 *Entomologist* **95**, 1024–1050.
- 12 Sharplin J (1963b) Wing base structure in Lepidoptera II. Hind wing base. *The Canadian*
13 *Entomologist* **95**, 1121–1145.
- 14 Simonsen TJ, de Jong R, Heikkilä M, Kaila L (2012) Butterfly morphology in a molecular age –
15 Does it still matter in butterfly systematics? *Arthropod Structure & Development* **41**, 307–
16 322.
- 17 Swofford DL (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods)*,
18 Version 4. Sinauer, Sunderland, MA.
- 19 Wahlberg N, Braby MF, Brower AVZ et al. (2005) Synergistic efforts of combining morphological
20 and molecular data in resolving the phylogeny of butterflies and skippers. *Proceedings of*
21 *the Royal Society B* **272**, 1577–1586.
- 22 Wootton RJ (1979) Function, homology and terminology in insect wings. *Systematic Entomology*
23 **11**, 389–400.
- 24 Yoshizawa K (2011) Monophyletic Polyneoptera recovered by wing base structure. *Systematic*
25 *Entomology* **36**, 377–394.
- 26 Yoshizawa K, Lienhard C (2016) Bridging the gap between chewing and sucking in the hemipteroid
27 insects: new insights from Cretaceous amber. *Zootaxa* **4079**, 229–245.
- 28 Yoshizawa K, Ogawa N, Dietrich CH (2017) Wing base structure supports Coleorrhyncha +
29 Auchenorrhyncha (Insecta: Hemiptera). *Journal of Zoological Systematics and*
30 *Evolutionary Research* **55**, 199–207.
- 31 Yoshizawa K, Saigusa T (2001) Phylogenetic analysis of paraneopteran orders (Insecta: Neoptera)
32 based on forewing base structure, with comments on monophyly of Auchenorrhyncha
33 (Hemiptera). *Systematic Entomology* **26**, 1–13.
- 34 Yoshizawa K, Wagatsuma M (2012) Phylogenetic relationships among superfamilies of
35 Cicadomorpha (Hemiptera: Auchenorrhyncha) inferred from the wing base structure.

1 *Entomological Science* **15**, 408–421.

2

Legends

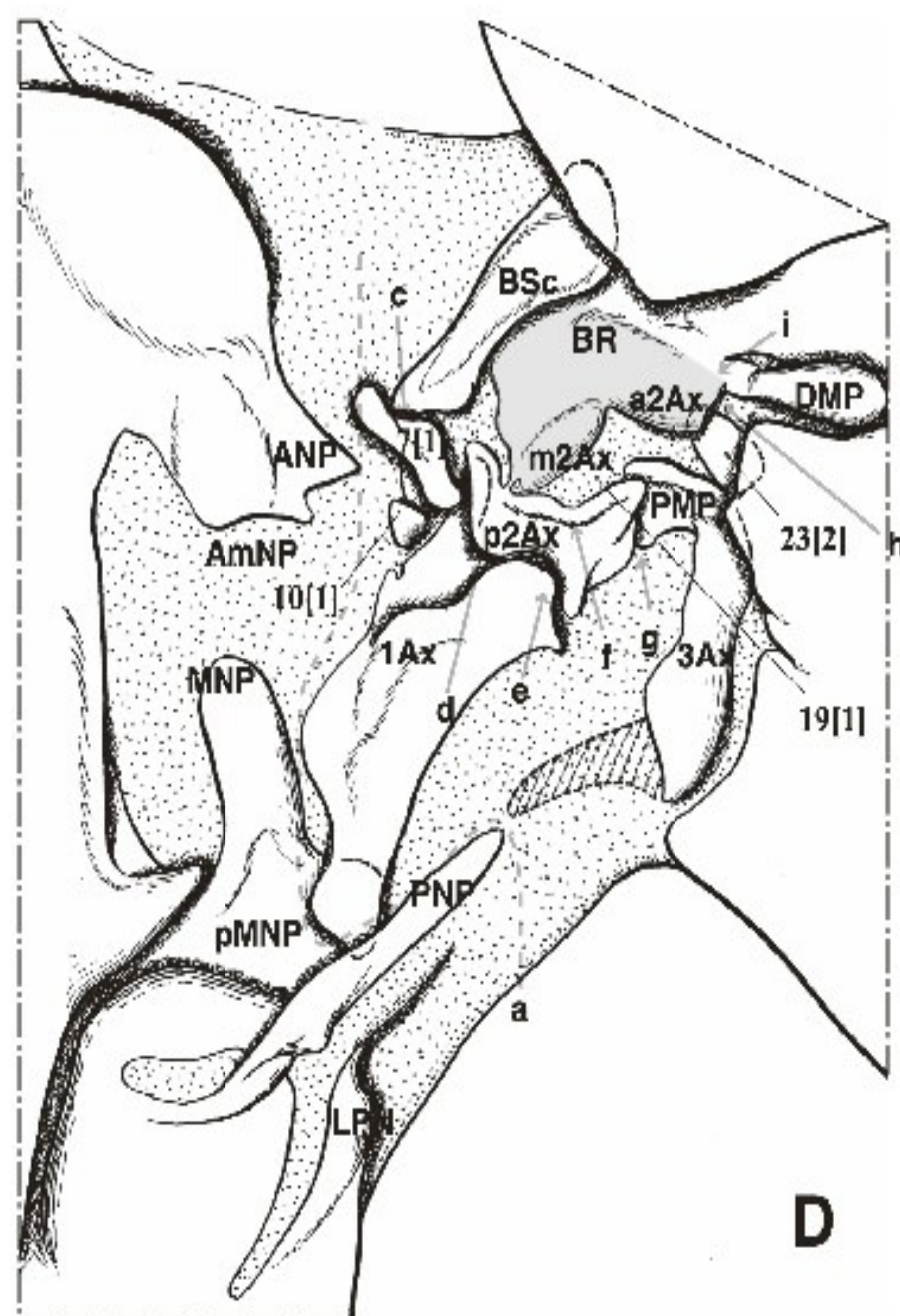
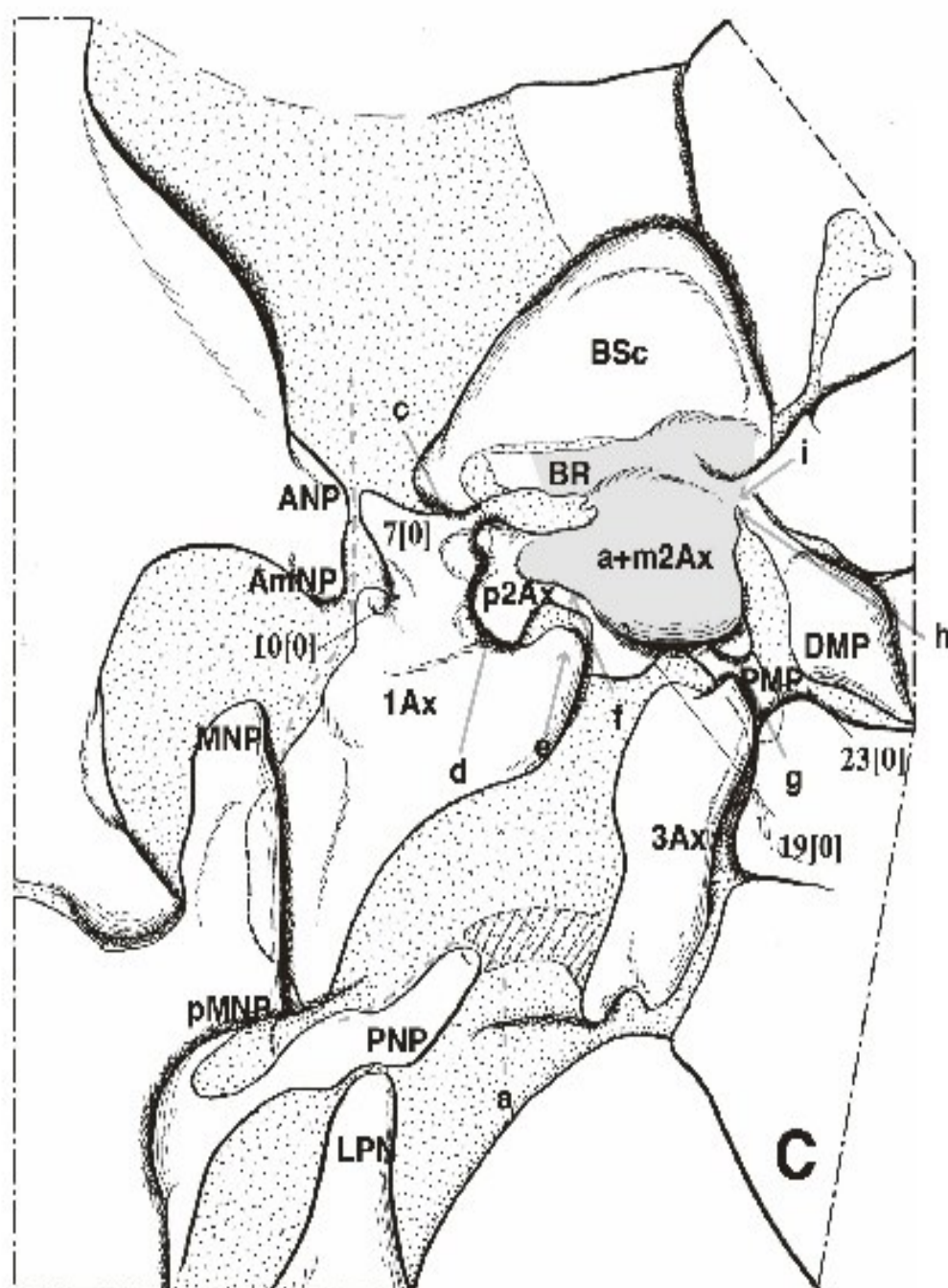
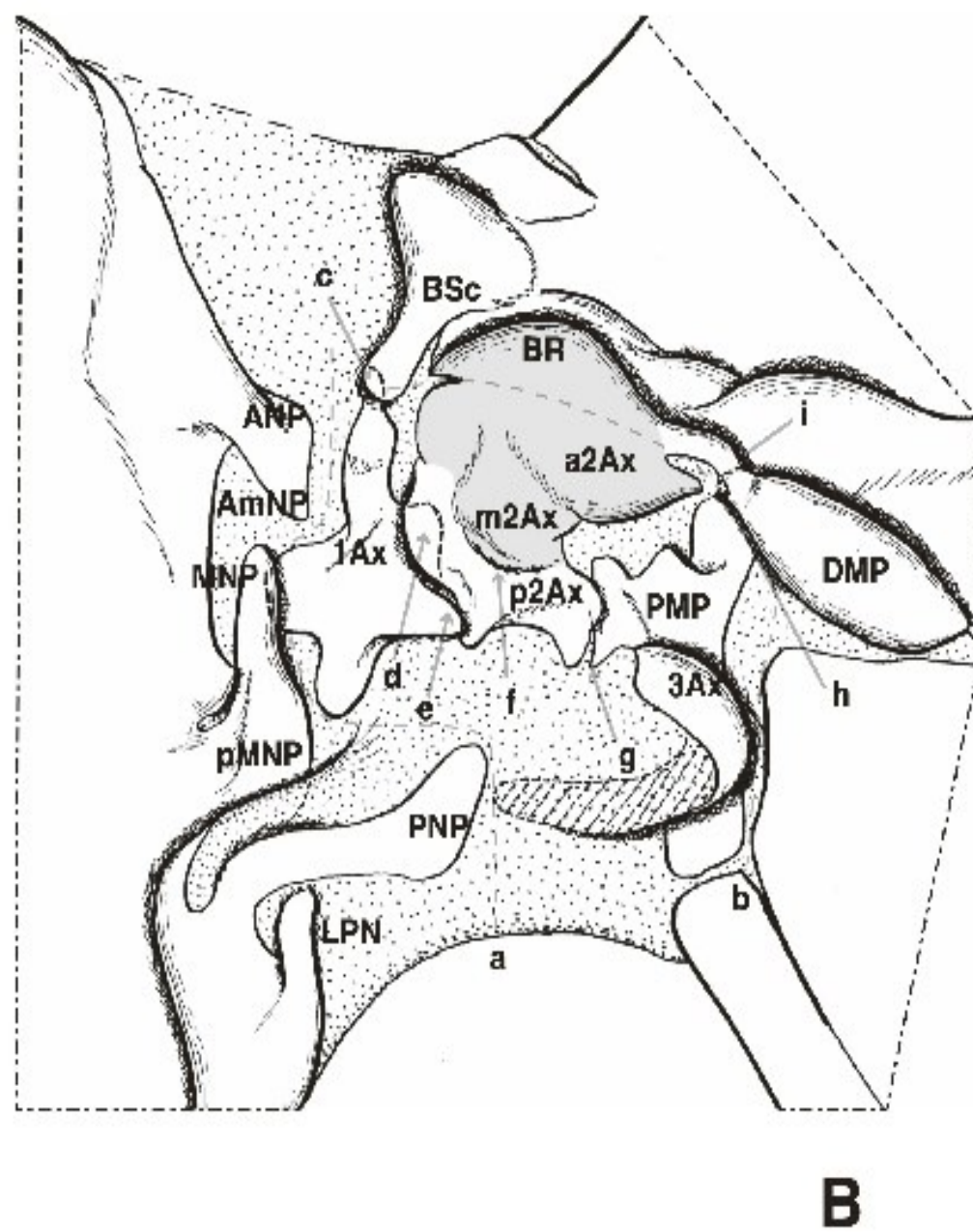
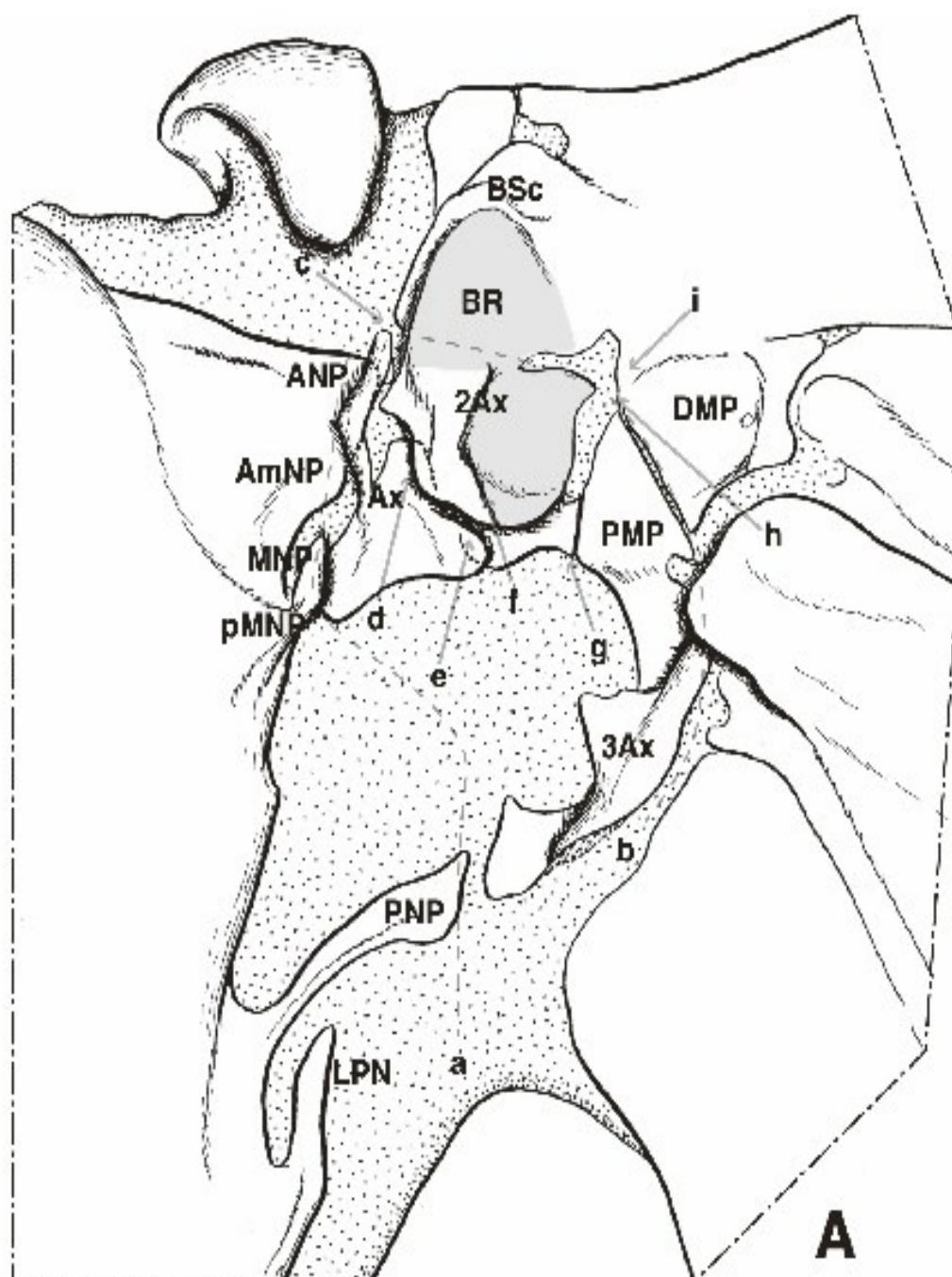
Figure 1 Forewing base structure of (A) *Stenopsyche marmorata* (Trichoptera), (B) *Archips ingentana* (Tortricidae), (C) *Byasa alcinous* (Papilionidae) and (D) *Favonius jezoensis* (Lycaenidae). Gray indicates setose (or scaled) area of 2Ax and BR. See text for abbreviations. Broken lines and arrows indicate the following key landmarks to distinguish associated structures: (a) basal hinge and articular points along this line (articulations between ANP and AmNP–1Ax neck, MNP and pMNP–1Ax body, PNP–3Ax); (b) convex axillary folding line (articulations between BR–2Ax, PMP–DMP, 3Ax–A veins); (c) articulation between 1Ax head–BSc; (d, e) articulation between 1Ax body–2Ax; (f) 2Ax ligament; (g) articulation between 2Ax–PMP; (h) articulation between 2Ax–PMP–DMP; (i) connection between DMP–BR. For C and D, characters supporting basal split of Papilionidae from the rest of butterflies are also indicated by the following format: character#[state].

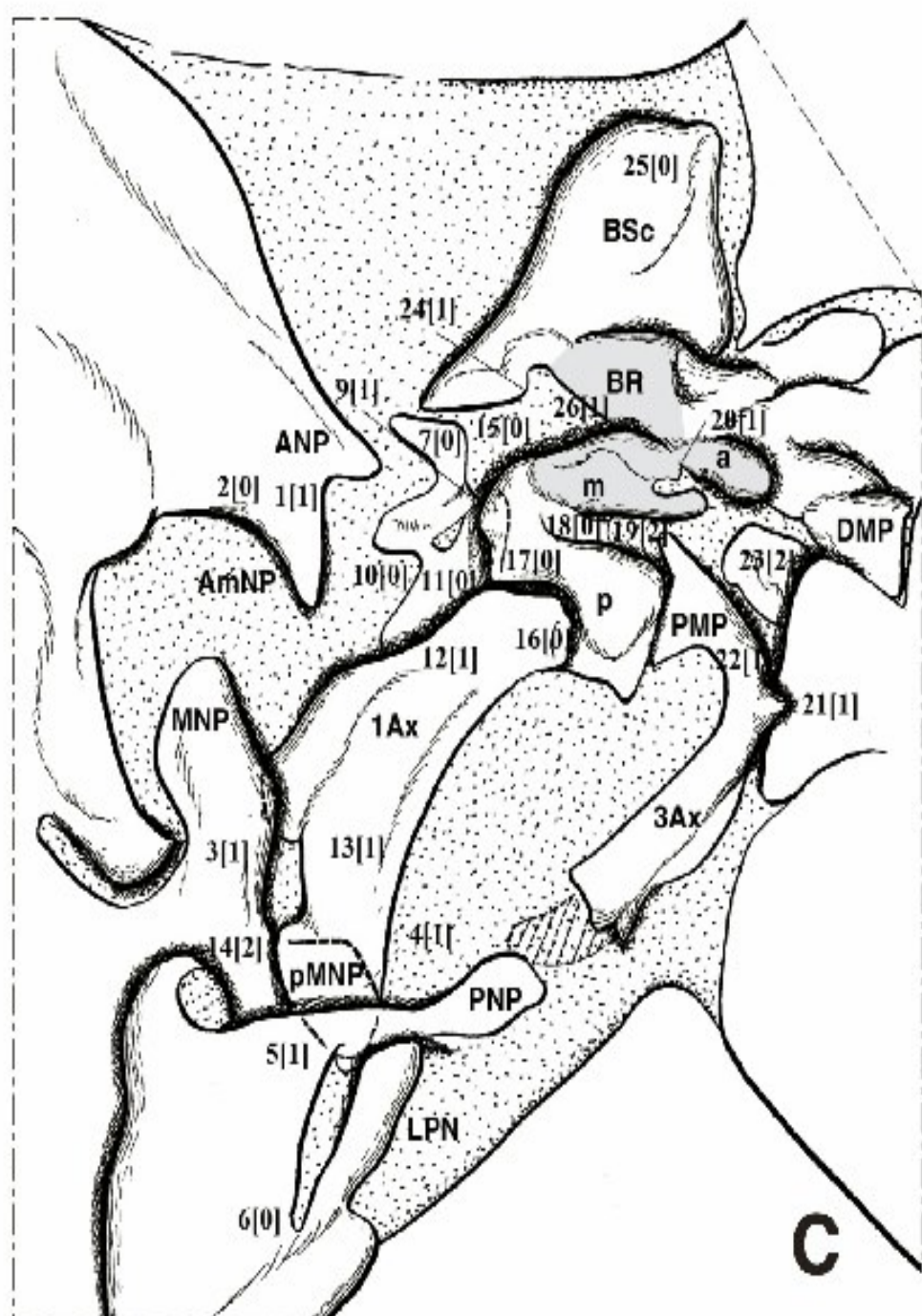
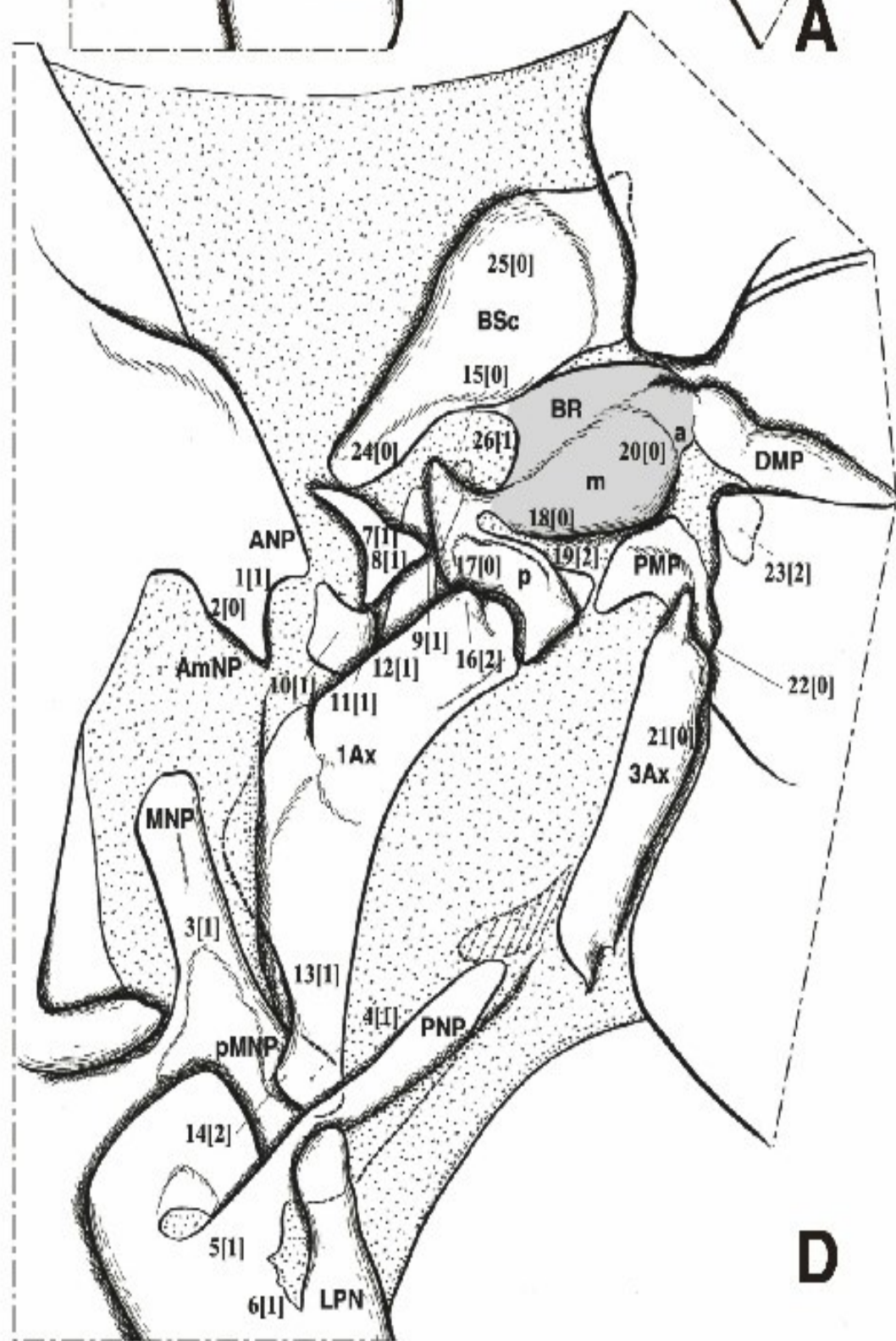
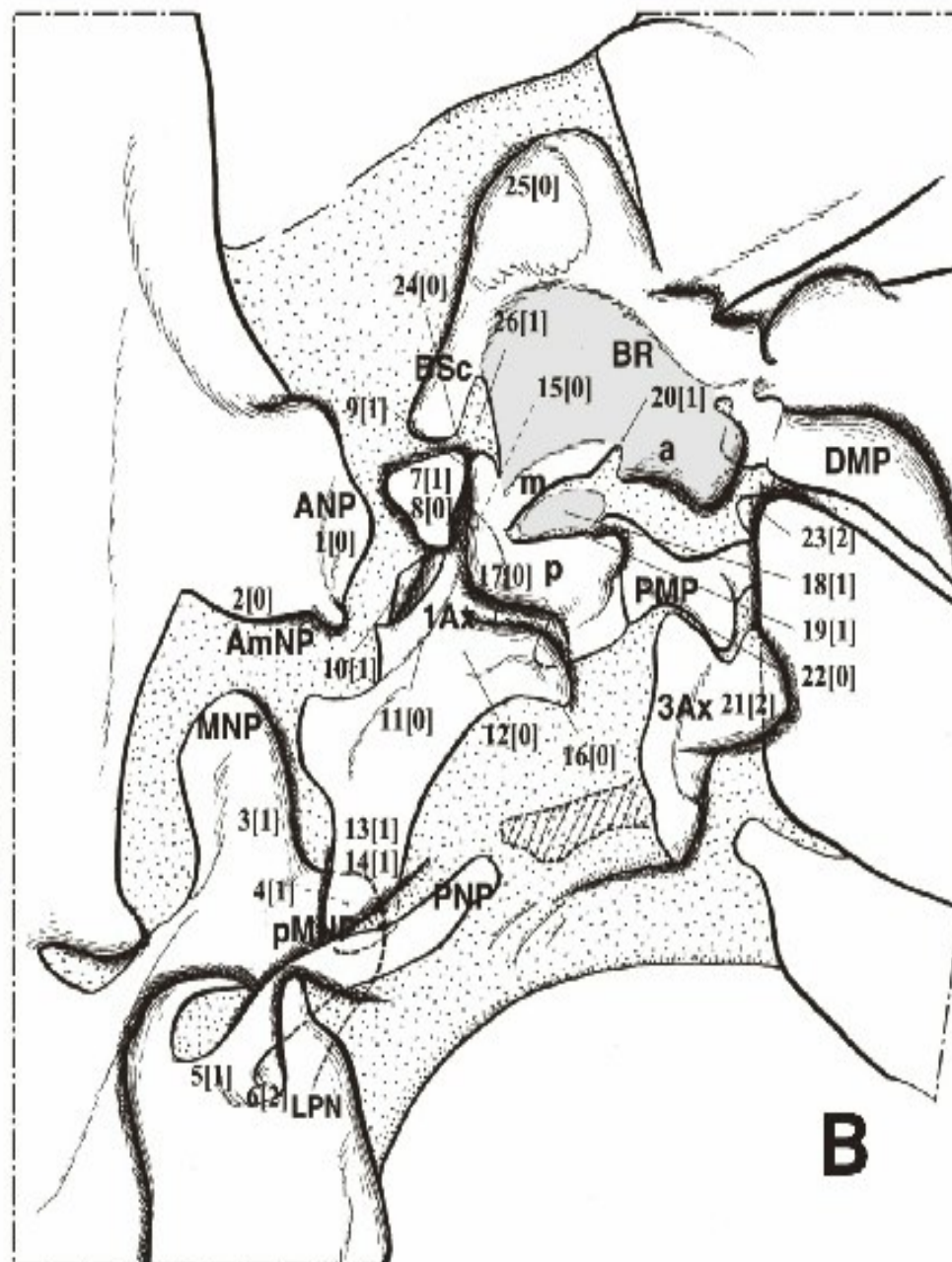
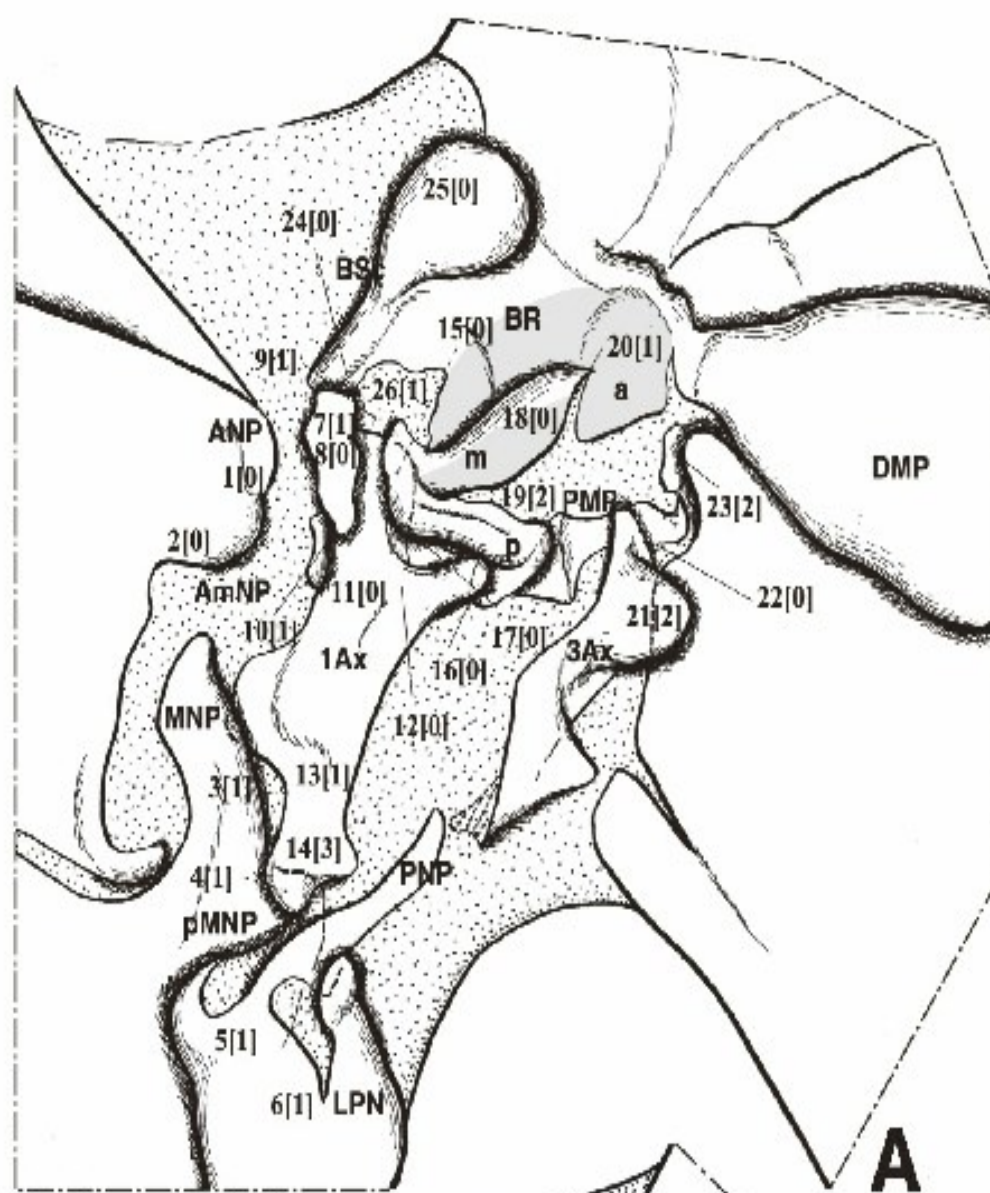
Figure 2 Forewing base structure of (A) *Macrosoma hedyllaria* (Hedylidae), (B) *Burara aquilina* (Hesperiidae), (C) *Aporia crataegi* (Pieridae) and (D) *Argynnis paphia* (Nymphalidae). Abbreviations: a, m, p = anterior-, median-, posterior-2Ax. Numbers indicate character#[state].

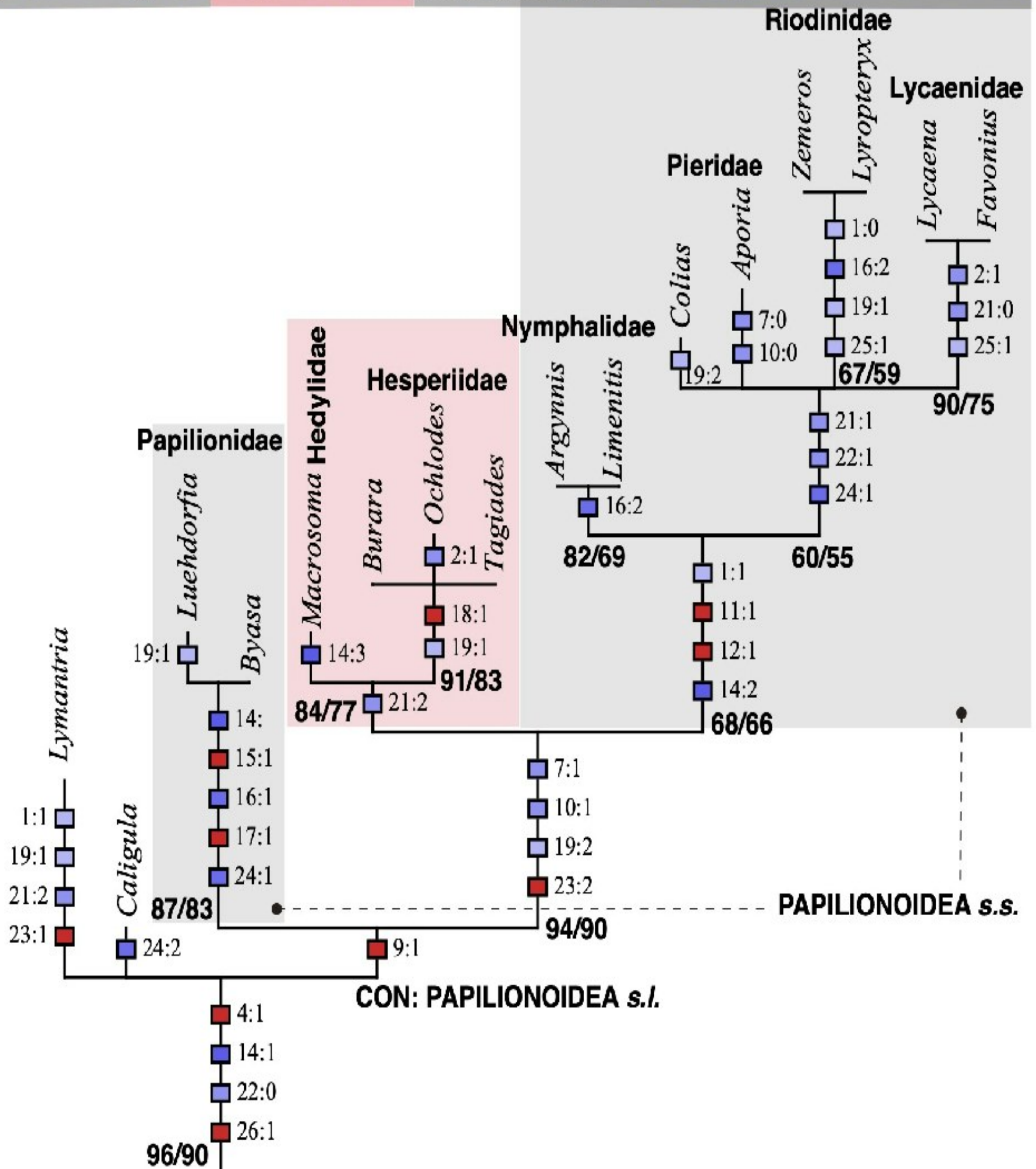
Figure 3 Strict consensus of three equally parsimonious trees estimated from wing base structure. Red squares indicate non-homoplasious apomorphies, and blue squares indicate homoplastic ones. Numbers next to squares indicate characters and their state. Numbers associated with nodes indicate bootstrap/jackknife support values (values lower than 50% are not shown). The monophyly of butterflies *s.l.* was constrained (indicated by CON). Two distant outgroups (Trichoptera and Tortricidae) are omitted from the tree. Photos of butterflies in panel above tree are by Toshiyuki Oshiki.

Table 1 List of taxa examined.

Supplementary Figures S1–S8 (DOI: 10.6084/m9.figshare.29178344) Forewing base structure of (S1) *Lymantria dispar* (Erebidae), (S2) *Caligula japonica* (Saturniidae), (S3) *Luehdorfia puziloi* (Papilionidae), (S4) *Ochlodes ochraceus* (4-1) and *Tagiades tethys* (4-2) (Hesperiidae), (S5) *Colias erate* (Pieridae), (S6) *Lycaena phlaeas* (Lycaenidae), (S7) *Limenitis camilla* (Nymphalidae), (S8) *Lyropteryx apollonia* (8-1) and *Zemeros flegyas* (8-2) (Ridiniidae). Data matrix is also available at same DOI.







Order	Family	Species
(outgroups)		
Trichoptera	Stenopsychidae	<i>Stenopsyche marmorata</i>
Lepidoptera	Tortricidae	<i>Archips ingentana</i>
	Erebidae	<i>Lymantria dispar</i>
	Saturniidae	<i>Caligula japonica</i>
(ingroups)		
Lepidoptera	Papilionidae	<i>Luehdorfia puziloi</i>
		<i>Byasa alcinous</i>
	Hedylidae	<i>Macrosoma hedylaria</i>
	Hesperiidae	<i>Burara aquilina</i>
		<i>Ochlodes ochraceus</i>
		<i>Tagiades tethys</i>
	Pieridae	<i>Aporia crataegi</i>
		<i>Colias erate</i>
	Lycaenidae	<i>Lycaena phlaeas</i>
		<i>Favonius jezoensis</i>
	Riodinidae	<i>Zemeros flegyas</i>
		<i>Lyropteryx apollonia</i>
	Nymphalidae	<i>Argynnis paphia</i>
		<i>Limenitis camilla</i>