

# Phylogenetic evidence for loss of sound production and a shift in sexual recognition signals in *Hamadryas* butterflies (Nymphalidae: Biblidinae)

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**Abstract.** The neotropical butterfly genus *Hamadryas* Hübner comprises 20 species that exhibit an intriguing variation in their natural history traits. Although revised in 1983, no phylogenetic hypothesis was presented: the first phylogenetic hypothesis is estimated here based on 93 characters and including species from the three other genera in the tribe Ageroniini. The phylogeny is used to test the monophyly of the genus, establish the sister group of *Hamadryas* and identify its apomorphies. The tree allows the inference of patterns of character change in sound production and sexual dimorphism. Implied weights show that *Hamadryas* is monophyletic and corroborate *Ectima* Doubleday as a sister genus. Previously suggested subgenera for *Hamadryas* were non-monophyletic, with the exception of the *laodamia* clade, supported by the presence of a complete sterigma. Sound production is inferred to be a derived condition in *Hamadryas* that has been lost in the *laodamia* clade. This, plus the presence of androconial organs and sexual dimorphism in the *laodamia* clade, suggests a shift in sexual recognition signalling. Furthermore, the phylogeny indicates that the colour pattern of males in the *laodamia* clade is novel, supporting a Darwinian origin of sexual dimorphism.

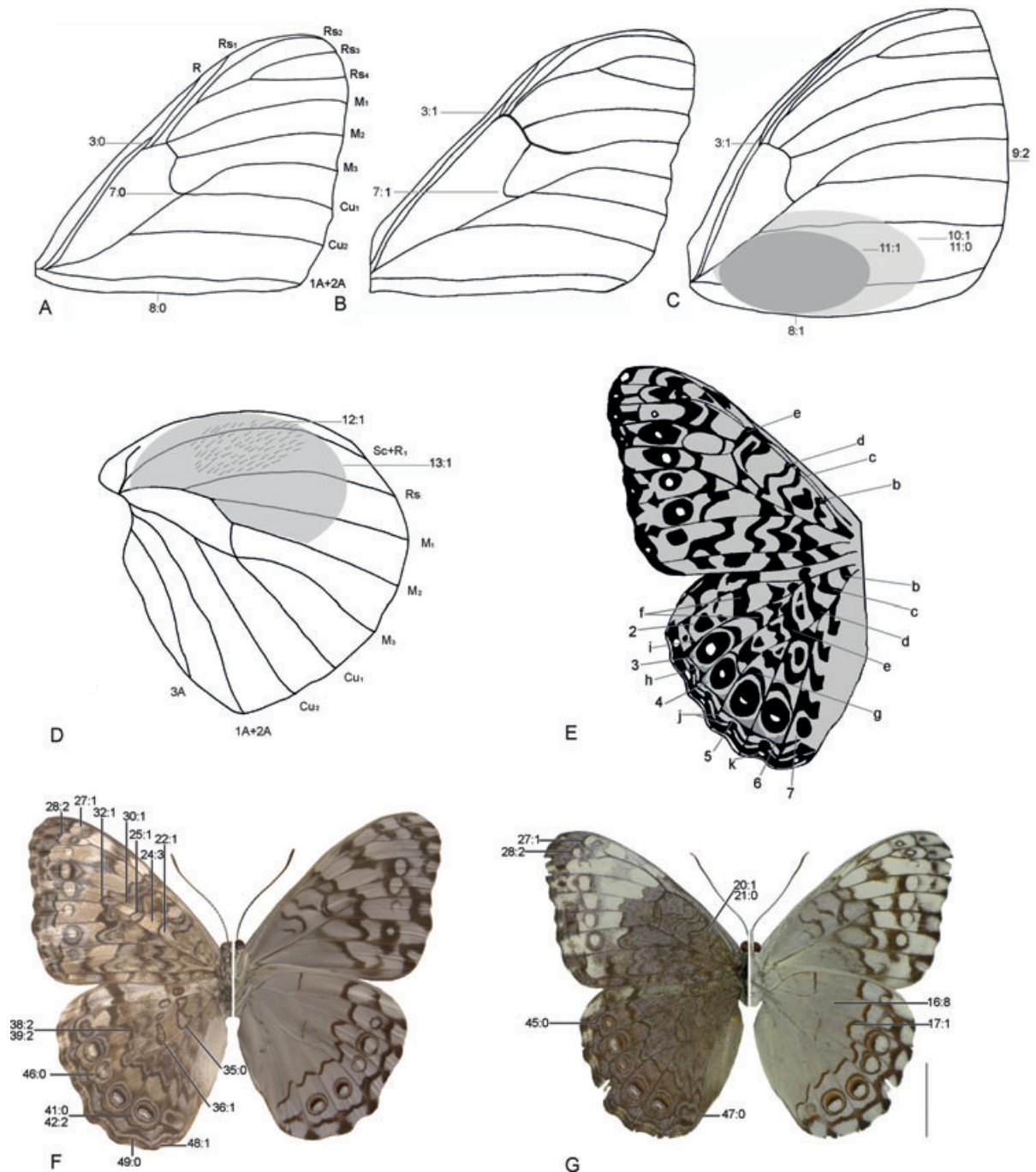
## Introduction

The species of *Hamadryas* Hübner are medium-sized Neotropical nymphalids belonging to the subfamily Biblidinae and the tribe Ageroniini. These butterflies are recognized by their distinctive spotted ‘calico’ dorsal wing pattern (Figs 1, 2) and erratic flight (e.g. Young & Borkin, 1985). As adults they feed on rotten fruits, and typically rest head-down on tree trunks with their wings spread out (Fruhstorfer, 1916). *Hamadryas* are known for the audible clicking sound made by the males during flight (Godman & Salvin, 1883; Otero, 1988): hence their common names of ‘crackers’ in English, ‘matracas’ or ‘rechinadoras’ in Spanish and ‘estaladeiras’ in Portuguese.

Although species of *Hamadryas* are relatively homogenous in their morphology (Jenkins, 1983), they vary in natural history traits such as sound production and sexual dimorphism. Males of some species can produce sound: in the field,

individuals perform aerial interactions that are usually accompanied by an audible clicking. The production of sound by these butterflies has mesmerized many naturalists, who were intrigued by the mechanism and location of the sound production organ (Darwin, 1871; Swinton, 1877; Godman & Salvin, 1883; Hampson, 1892; Fruhstorfer, 1916). Sound production involves thickened veins at the distal portion of the forewing discal cell (Fig. 3), with sound produced in two ways: by these veins striking one another at the end of the upstroke, and also by the deformation of the wing membranes (from straight to concave) during flight, which enables the production of sound by individual wings (Otero, 1990; Yack *et al.*, 2000). Sexual dimorphism is a common condition in Biblidinae: for example, all species of *Epiphile* Doubleday and many species of *Catonephele* Hübner are sexually dimorphic. Although most species of *Hamadryas* are monomorphic, marked sexual dimorphism occurs in a few species (e.g., *Hamadryas laodamia*: Cramer; fig. 2E, F). The variation found in these natural history traits within *Hamadryas* begs the question about their origin and their modifications. A robust and resolved phylogenetic hypothesis will improve our understanding of character

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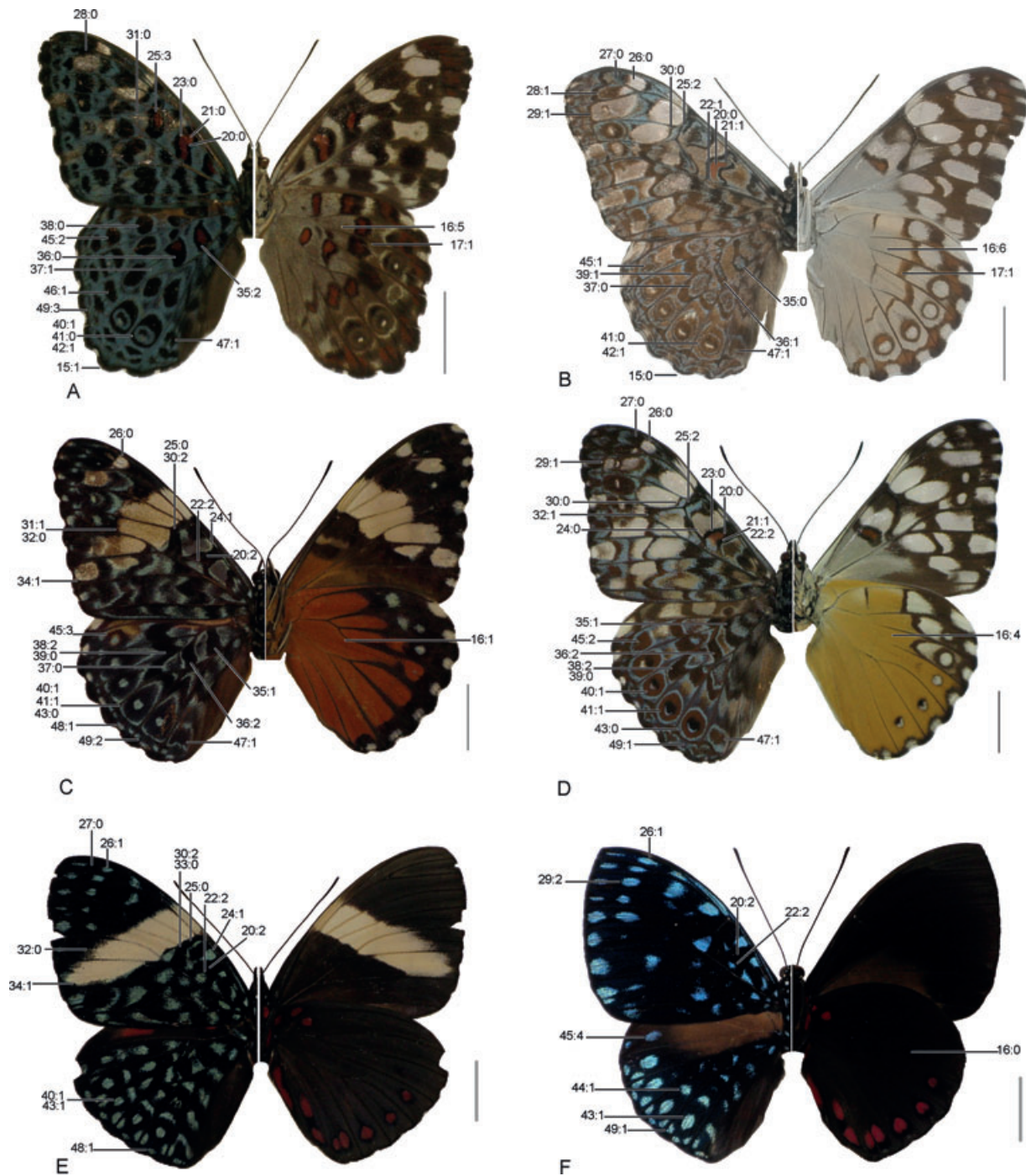


**Fig. 1.** Wing venation characters and wing colour characters used in this study: A–C, the three patterns of forewing venation; C and D, location of scent organs in the fore- and hindwing, respectively; E, map of the pattern elements used in this study (following Nijhout, 1991); F and G, the female and male of *Hamadryas glauconome*, respectively, with wing colour characters labelled; dorsal side on the left, ventral side on the right. Scale bar: 1 cm.

evolution, and allow the evolutionary biology of *Hamadryas* to be disentangled.

Four generic names have been used for species placed currently in *Hamadryas*. Hübner (1806) described *Hamadryas* for *Papilio amphinome* Linnaeus, and *Ageronia* for *Papilio*

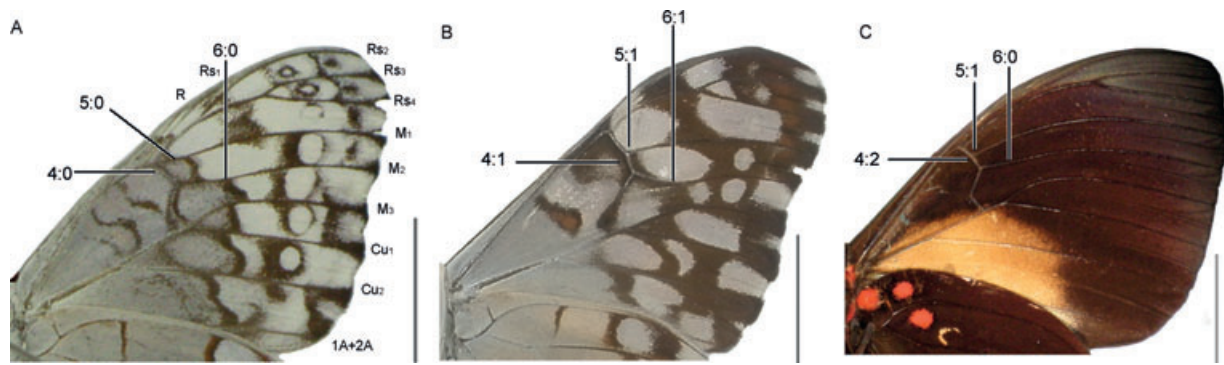
*chloe* Stoll (Hübner, 1819). Lacodaire (1833) erected *Peridromia* for *Papilio arethusa* Cramer, and Felder (1861) created *Amphichlora* from *Papilio feronia* Linnaeus. Based on wing venation, Godman & Salvin (1883) moved three of the four species originally placed in *Ageronia* by Hübner (1816) to



**Fig. 2.** Wing colour characters used in this study plotted on selected species of *Hamadryas*; dorsal side on the left, ventral side on the right. Featured species: A, *Hamadryas chloe*; B, *Hamadryas iphthime*; C, *Hamadryas amphinome*; D, *Hamadryas fornax*; E, female *Hamadryas laodamia*; F, male *H. laodamia*. Scale bars: 1 cm.

*Peridromia*. In their arrangement *Peridromia* included the type species of *Hamadryas*, *Papilio amphinome* plus 12 other species. Godman & Salvin (1883) also listed four other species in *Ageronia* (one of which was new), which now included seven species. The use of the name *Hamadryas* in a taxonomic

context was negligible for the first part of the twentieth century (Bouton, 1962; Lamas *et al.*, 1995), and all subsequent authors used the name *Ageronia*. Accordingly, the name *Hamadryas* was not used by Fruhstorfer (1916), and although his species groups followed the same arrangement proposed by Godman



**Fig. 3.** The three patterns of male forewing venation found in *Hamadryas*, indicating the location of the sound organ and some of the characters used in this study: A, forewing of *Hamadryas glauconome*; B, forewing of *Hamadryas iphthime*; C, forewing of *Hamadryas laodamia*. Scale bars: 1 cm.

& Salvin (1883), he classified all species in two species groups within *Ageronia*: the *Ageronia* and *Peridromia* groups. Jenkins (1983) grouped all species within *Hamadryas*, and synonymized two-thirds of the previously described species and subspecies names. Although Hemming (1967) recognized all four generic names (*Hamadryas*, *Ageronia*, *Peridromia* and *Amphichlora*) as valid genera, currently *Hamadryas* is the only valid name used in reference to the 20 species Jenkins maintained inside the genus (Jenkins, 1983; Lamas, 2004), whereas *Ageronia*, *Peridromia* and *Amphichlora* are treated as junior synonyms (Lamas, 2004).

Given its taxonomic history, some species groups have been maintained inside *Hamadryas*. Based on wing venation and male genitalia, Jenkins (1983) divided the genus into three species groups (vaguely suggested as subgenera) that agree mostly with earlier arrangements by Godman & Salvin (1883) and Fruhstorfer (1916) (left-hand and middle columns in Table 1, respectively). These species groups were (right-hand column in Table 1): the *februa* group, which corresponds to the subgenus *Ageronia* (seven species); the *feronia* group, equivalent to the subgenus *Hamadryas* (ten species); and the *laodamia* group, corresponding to the subgenus *Peridromia* (three species). Although Jenkins (1983) presented the only comprehensive taxonomic study for *Hamadryas* to date, no phylogenetic hypothesis of species relationships was provided and the monophyly of putative species groups was untested.

Although inferring species relationships is the immediate outcome of phylogenetics, the phylogeny also allows the study of ecological characters and their variation under a historical context. Many examples of such studies have come from arthropods (Kuntner & Coddington, 2009; see Miller & Wenzel, 1995 for a review). For example, butterflies exhibit interesting life histories, and placing them in a phylogenetic framework has improved our understanding of mimicry (Brower, 1995, 1997; Jiggins *et al.*, 2006; Elias *et al.*, 2008; Oliver & Prudic, 2010) and sexual dimorphism (Kunte, 2008).

Here I use morphology and wing colour data to provide the first species-level phylogenetic hypothesis for *Hamadryas* to: (i) test the monophyly of *Hamadryas* and identify its apomorphies; (ii) infer the sister group of genus; (iii) test the

**Table 1.** Composition of species groups suggested for *Hamadryas*.

<i>Hamadryas</i> species groups		
Godman and Salvin, 1883	Fruhstorfer, 1916	Jenkins, 1983
<b><i>Ageronia</i></b>	<b><i>Ageronia</i></b>	<b><i>Hamadryas</i></b>
a:	Species of the <i>Ageronia</i> group:	<i>februa</i> species group:
<i>februa</i>	<i>februa</i>	<i>atlantis</i>
<i>glauconome</i>	<i>glauconome</i>	<i>chloe</i>
b:	<i>ferox</i>	<i>albicornis</i>
<i>atlantis</i>	<i>atlantis</i>	<i>februa</i>
<b><i>Peridromia</i></b>	<i>chloe</i>	<i>amphichloe</i>
a:	<i>albicornis</i>	<i>glauconome</i>
<i>laodamia</i>	Species of the <i>Peridromia</i> group:	<i>honorina</i>
b:	<i>feronia</i>	<i>feronia</i> species group:
<i>amphinome</i>	<i>guatemalena</i>	<i>feronia</i>
<i>arinome</i>	<i>iphthime</i>	<i>guatemalena</i>
a':	<i>epinome</i>	<i>iphthime</i>
<i>fornax</i>	<i>fornax</i>	<i>epinome</i>
c:	<i>alicia</i>	<i>fornax</i>
<i>feronia</i>	<i>rosandra</i>	<i>alicia</i>
<i>guatemalena</i>	<i>amphinome</i>	<i>rosandra</i>
<i>iphthime</i>	<i>arinome</i>	<i>amphinome</i>
	<i>belladonna</i>	<i>arinome</i>
	<i>laodamia</i>	<i>belladonna</i>
	<i>velutina</i>	<i>laodamia</i> species group:
	<i>arete</i>	<i>laodamia</i>
		<i>arete</i>
		<i>velutina</i>

validity of suggested subgenera as monophyletic units; and (iv) determine if selected natural history traits resulted from common ancestry or convergent evolution.

## Material and methods

### Taxon sampling

This study includes 19 of the 20 *Hamadryas* species. Except for the female of *Hamadryas belladonna* Bates, and female

*Hamadryas albicornis* Staudinger, male and female specimens were obtained from the following collections: American Museum of Natural History (AMNH); Florida Museum of Natural History, McGuire Center for Lepidoptera and Biodiversity (FLMNH); Milwaukee Public Museum (MPM); Smithsonian Institution, National Museum of Natural History (NMNH); DeVries Collection (PJD); and Natural History Museum of Los Angeles County (LACM). Taxonomic determinations followed Jenkins' (1983) revision. The specimens examined are listed in Table S1.

*Batesia hypochlora* Felder & Felder, *Panacea prola* Doubleday, *Panacea divalis* Bates (sensu Hill *et al.*, 2002), and all the four species of *Ectima* Doubleday were used as outgroups. These three genera together with *Hamadryas* comprise the tribe Ageronini, which is considered monophyletic based on the most recent phylogeny of Nymphalidae (Wahlberg *et al.*, 2009). *Batesia hypochlora* was used to root the tree: this results in *Panacea* Godman & Salvin appearing as a sister group of *Hamadryas* plus *Ectima*; however, a sister relationship between *Batesia* and *Panacea* has been shown previously (Hill *et al.*, 2002; Wahlberg *et al.*, 2009).

### Characters

Leg and genitalia dissections were made following standard procedures using a 10% KOH solution, and were kept in a 3 : 1 solution of 70% ethanol and glycerol. Examination of characters and drawings were conducted using a stereomicroscope equipped with a camera lucida. The terminology for external morphology and genitalia follows Kristensen (2003), and homologies of wing pattern elements (Fig. 1E) follow Nijhout (1991).

The matrix includes 93 characters (88 of which are informative): characters 1–15 refer to venation (Figs 1, 3), wing shape and androconia; characters 16–49 describe wing colour (Figs 1, 2), characters 50–92 concern male and female genitalia (Figs 4–6); and one character (character 93) describes oviposition patterns. Information about the patterns of oviposition was taken from the literature. The character list, matrix and literature records of the patterns of oviposition are provided in the Appendix and Tables S2 and S3, respectively. Abbreviations used throughout the text are: DFW, dorsal forewing; DHW, dorsal hindwing; FW, forewing; HW, hindwing; VFW, ventral forewing; VHW, ventral hindwing.

### Cladistic analysis

The matrix was analysed under equal weights (hereafter EW) and implied weights (hereafter IW) (Goloboff, 1993) in TNT 1.1 (Goloboff *et al.*, 2008a). EW remains as the traditional approach used in systematics, and as such in this study EW was used for exploratory purposes. Under IW, I explored a wide range of IW concavity values ( $k = 1–100$ ). Regardless of the weighting scheme, tree searches included 500 replicates of random addition sequence, holding ten trees per replication,

tree bisection reconnection (TBR) for branch swapping and 90 ratchet iterations (Nixon, 1999). After the search, branches of length zero were collapsed and duplicate trees were discarded (coll rule 4; condense; unique). All characters were considered unordered, although making multistate characters additive was explored. When dealing with polymorphisms TNT treats the states as either/or, and thus only adds a step when the ancestral state is not included within the polymorphism.

Three measures of group support were calculated: the absolute Bremer support (ABS; Bremer, 1994), which measures the total volume of favourable evidence; the relative Bremer support (RFD; Goloboff & Farris, 2001), which provides an estimate based on the volume of evidence in favour and against each node; and symmetric resampling (SR) or symmetrical jackknife, which uses the same probability for character deletion and character inclusion, thus eliminating the influence of weighting against homoplasy (Goloboff *et al.*, 2003).

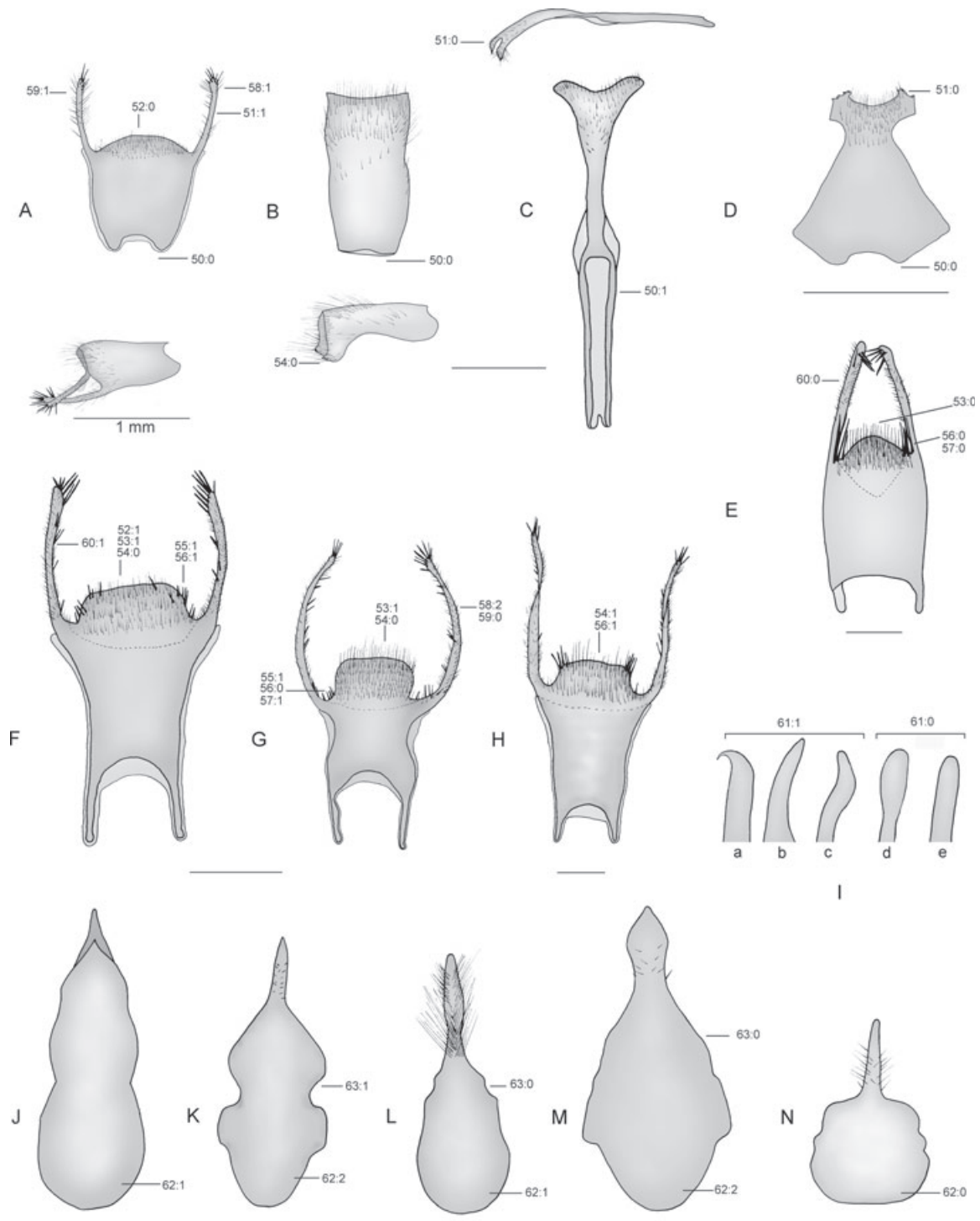
The Bremer support values (ABS and RFD) were calculated by retaining up to 3000 trees with different suboptimal lengths, from 0.2 to 4 steps longer than the optimal tree, and running 500 replications of Wagner while retaining all the trees from each replication. The relative Bremer support is calculated as the relative fit difference (RFD) between two trees. If RFD is 0, the volume of evidence supporting the group equals the volume of evidence contradicting the group, and if RFD is 1, the group has never been contradicted. SR was conducted by generating 1000 pseudoreplicates of the matrix, and the results are expressed in differences of group frequency (GC, for group present/contradictory) values, instead of straight group frequencies. Using GC provides the advantage of knowing the support of groups with low resample values (with less than 50%), which are otherwise collapsed under the standard calculation of frequencies (Goloboff *et al.*, 2003). GC represents the difference between the frequency of the group in question and the frequency of its most frequent contradictory group (Goloboff *et al.*, 2003). A GC value of  $-1$  indicates maximum contradiction, GC = 0 indicates indifference and GC = 1 represents complete support.

The trees were explored and edited with MACCLADE 4.08 (Maddison & Maddison, 2005). MacClade was used also to reconstruct the ancestral state of selected natural history traits and to optimize the minimum number of changes for these traits under traditional parsimony.

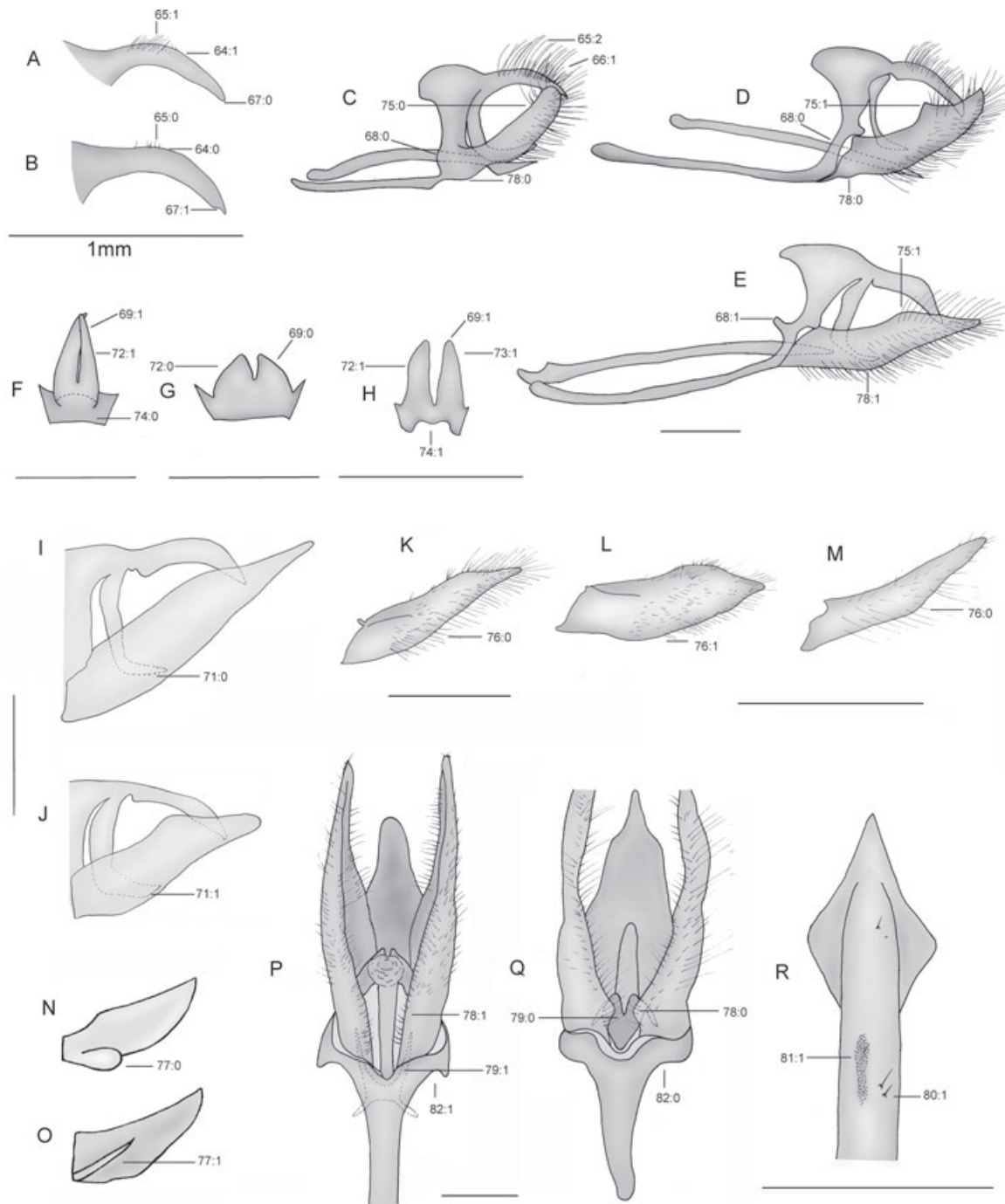
### Results

Using EW generated ten equally parsimonious trees of 260 steps, the strict consensus of which is shown in Fig. 7A. In contrast, IW concavity values from  $k = 2$  to 100 or higher found one single most parsimonious tree (MPT) (Fig. 7B). This tree, together with the optimization of all the unambiguous transformations and branch support, is shown in Fig. 8. This tree corresponds to one of the ten MPTs under equal weights. Making multistate characters additive had no effect on the topology under either analysis.

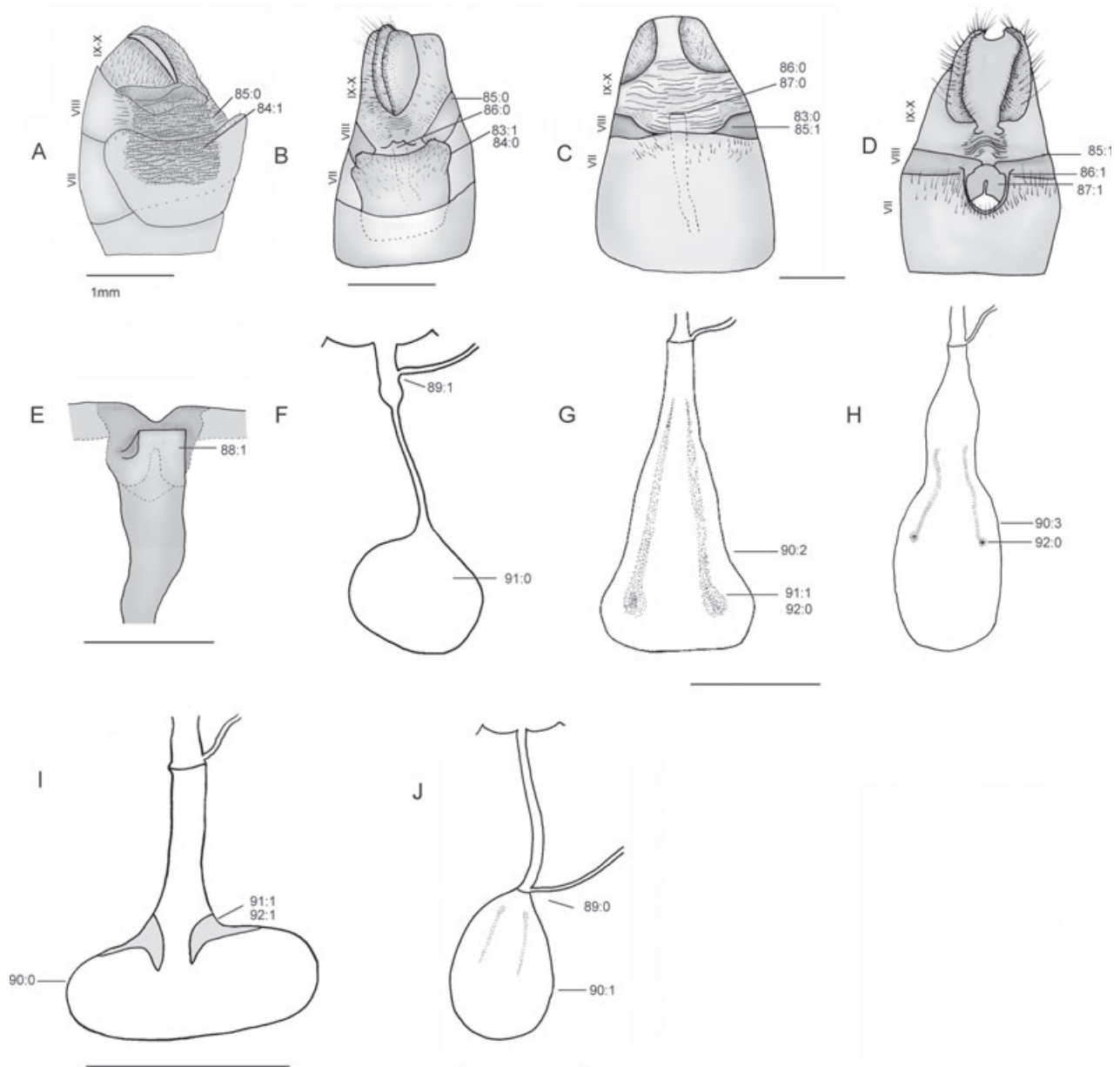
Here the optimal solution found by IW is preferred because IW assigns weights to characters according to their reliability



**Fig. 4.** Characters from male genitalia used in this study: A, hypandrium of *Hamadryas chloe* in ventral view, lateral view of the hypandrium below; B, hypandrium of *Panacea prola* in ventral view, lateral view below; C, hypandrium of *Ectima thecla* in ventral view, lateral view above; D, hypandrium of *Batesia hypochlora*; E, hypandrium of *Hamadryas velutina* in ventral view; F, hypandrium of *Hamadryas februa* in ventral view; G, hypandrium of *Hamadryas epinome* in ventral view; H, hypandrium of *Hamadryas arinome* in ventral view; I, tip of rami (a, *Hamadryas atlantis*; b, *Hamadryas arete*; c, *Hamadryas alicia*; d, *Hamadryas februa*; e, *Hamadryas albicornis*); J, tegumen of *Batesia hypochlora* in dorsal view; K, tegumen of *H. arete* in dorsal view; L, tegumen of *Hamadryas chloe* in dorsal view; M, tegumen of *Hamadryas fornax* in dorsal view; N, tegumen of *E. thecla* in dorsal view. Scale bars: 1 mm.



**Fig. 5.** Continuation of male characters used in this study: A, uncus of *Hamadryas alicia* in lateral view; B, uncus of *Hamadryas glauconome* in lateral view; C, lateral view of the genitalia of *Hamadryas albicornis*; D, lateral view of the genitalia of *Hamadryas atlantis*; E, lateral view of the genitalia of *Hamadryas fornax*; F, gnathos of *Hamadryas velutina* in ventral view; G, gnathos of *Hamadryas arinome* in ventral view; H, gnathos of *H. alicia* in ventral view; I, diagram of the genitalia of *Hamadryas amphinome* in lateral view; J, diagram of the genitalia of *Hamadryas feronia* in lateral view; K, valva of *H. amphinome* in lateral view; L, valva of *Hamadryas guatemalena* in lateral view; M, valva of *Hamadryas chloe* in lateral view; N, diagram of internal side of the valva in species of *Panacea* and *Batesia*; O, diagram of internal side of the valva in species of *Hamadryas*; P, genitalia of *Hamadryas amphinome* in ventral view; Q, genitalia of *Batesia hypochlora* in ventral view; R, phallus of *Hamadryas arete* in ventral view. Scale bars: 1 mm.



**Fig. 6.** Characters from female genitalia used in this study: A, sterigma of *Ectima erycinoides*; B, sterigma of *Hamadryas chloe* in ventral view; C, sterigma of *Hamadryas amphinome* in ventral view; D, sterigma of *Hamadryas arete* in ventral view; E, sclerotized plate in antrum of *Hamadryas arete* in ventral view; F, bursa copulatrix of *Panacea prola*; G, bursa copulatrix of *Hamadryas februa*; H, bursa copulatrix of *Hamadryas arinome*; I, bursa copulatrix of *Hamadryas chloe*; J, bursa copulatrix of *Hamadryas atlantis*. Scale bars: 1 mm.

along the search of the topology (weighting against their homoplasy), instead of assuming a priori that all characters bear the same importance as evidence of phylogenetic relationships. Furthermore, IW has been shown empirically to produce more stable hypotheses than EW and to improve jackknife frequencies, especially in morphological data (Goloboff *et al.*, 2008b).

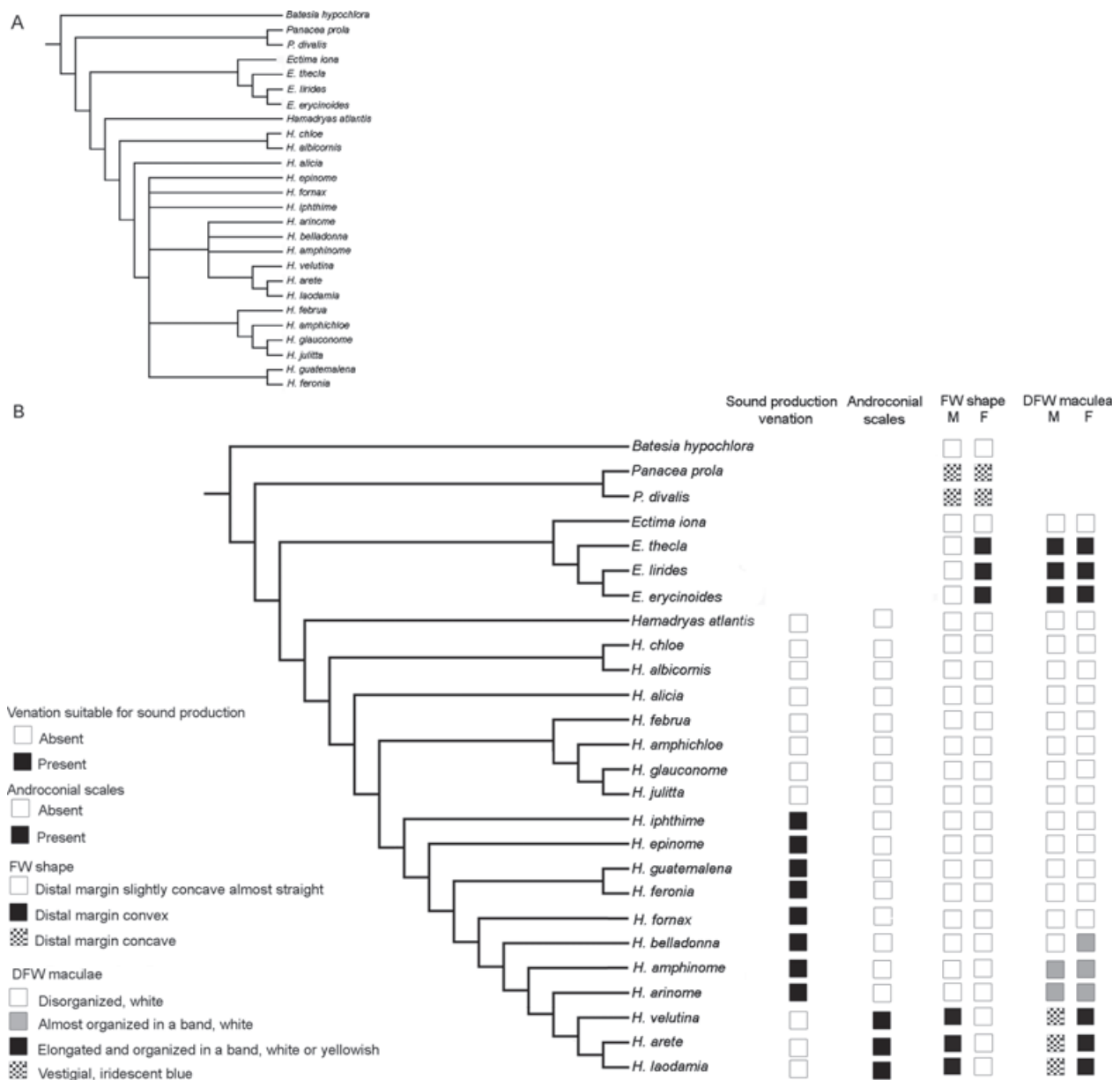
Below I describe the optimal tree found under IW and present the apomorphies for selected groups of taxa (nodes A–E in Fig. 8). The names used throughout the text

for some groups of species are not intended to have any taxonomic value: they are used only to facilitate the description of the results.

#### *The position of Hamadryas within the Ageroniini*

*Ectima* is the sister group of *Hamadryas* (see also Wahlberg *et al.*, 2009), a relationship supported by three synapomorphies





**Fig. 7.** A, strict consensus of the ten most parsimonious trees (MPTs) obtained under equal weights. B, unique MPT found under implied weights.

and two homoplasious characters [1(1) and 81(1); Fig. 5R; RFD = 0.74, ABS = 0.37, GC = 0.97]. The synapomorphies of *Ectima* plus *Hamadryas* are: internal side of the base of valva with a projection that smoothes into an internal fold [77(1); Fig. 5O]; juxta slightly sclerotized and not projected posteriorly [79(1); Fig. 5P]; and the presence of signa [91(1); Fig. 6G–I].

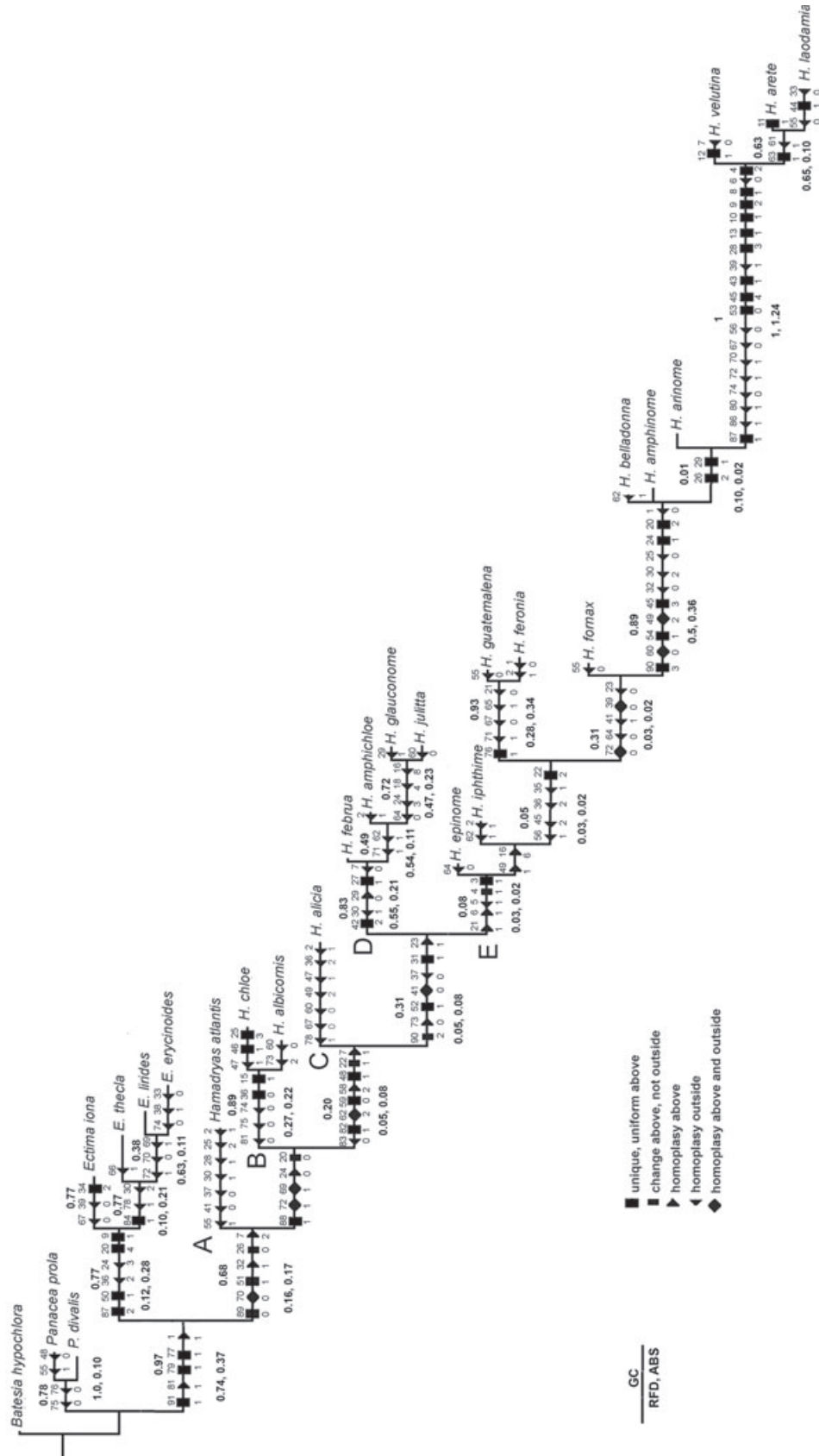
*Monophyly and apomorphies of Hamadryas*

*Hamadryas* is monophyletic, as indicated by three apomorphies and three homoplastic character changes [7(2), 32(1);

Fig. 1F, Fig. 2C–E; 70(0)]. The apomorphies of *Hamadryas* are: DFW spot at R<sub>3</sub>, R<sub>4</sub> white [26(0); Fig. 2B–F]; hypandrium with lateral edges projected into elongated rami [51(1); Fig. 4A, C, D]; and ductus seminalis connecting very near to the corpus bursa [89(0); Fig. 6J]. The monophyly of *Hamadryas* has an RFD of 0.16, an ABS of 0.17 and a GC value of 0.68.

**Species relationships**

In *Hamadryas* the first split in the tree corresponds to a single species branch (Fig. 8 branch labeled A): *Hamadryas atlantis*



**Fig. 8.** Most parsimonious tree under implied weights ( $k = 2-100$ ) with unambiguous optimization of all the characters. Support values are shown above (GC from symmetrical resampling) and below (relative Bremer support, RFD and absolute Bremer support, ABS) branches.

Bates. This is one of the most distinctive species in the genus, as demonstrated by the considerable number of character changes on its branch. Two colour pattern and three genitalia characters separate *H. atlantis* from other *Hamadryas*. In *H. atlantis* the DFW band inside the discal cell between elements c and d is green, as in species of *Panacea* [20(3)], and the the antrum is entirely membranous [88(0)], unlike all other species of *Hamadryas*, in which the dorsal side of the antrum has a small sclerotized plate [88(1); Fig. 6E].

After the split of *H. atlantis* there is a clade composed of *Hamadryas chloe* and *H. albicornis* (Fig. 8, branch labelled B). This clade is supported by two apomorphies, three homoplastic character changes and has an RFD of 0.27, an ABS of 0.22 and a GC of 0.89. The apomorphies are: HW CuA<sub>2</sub> vein noticeably longer than CuA<sub>1</sub> and 1A+2A [15(1); Fig. 2A]; and DHW pattern element e formed by red scales [36(0); Fig. 2A–D].

*Hamadryas alicia* (Fig. 8, branch labelled C) is the second single species branch in the topology, and appears as the sister taxon to the remaining species. This relationship is supported by four apomorphies: a vestigial spot composed of blue scales in the proximal portion of the DFW band between pattern elements c and d [22(1); Figs 1F, 2B–F]; the presence of DHW pattern element k [48(1); Figs 1F, 2C, E]; the presence of short and fine setae along the rami [59(0); Fig. 4G], which are long and thick in *H. atlantis*, *H. chloe*, and *H. albicornis*; and a triangular base of the saccus [82(1); Fig. 5P; 82(0); Fig. 5Q], in contrast to a squared base present in *H. atlantis*, *H. chloe* and *H. albicornis*. The most important characters that differentiate *H. alicia* from the rest of the *Hamadryas* are: colour of the distal band to pattern element e, which is blue in *H. alicia* [31(0); Fig. 2A; 31(1); Fig. 2C] and white in the rest of the species; the posterior edge of the hypandrium is short in *H. alicia* [52(0); Fig. 4A; 52(1); Fig. 4F], which is extended considerably in the rest of the species; and finally the shape of the ductus bursa, which is shortened in *H. alicia* [90(0); Fig. 6I] and pear-shaped or elongated in the rest of *Hamadryas* [Fig. 6G, H].

Clade D (Fig. 8, branch labelled D) is comprised by *Hamadryas februa* Hübner, *Hamadryas amphichloe* Boisduval, *Hamadryas glauconome* Bates and *Hamadryas julitta* Fruhstorfer. This clade is supported by two apomorphies and three homoplastic character changes [7(0), 29(0), 30(1)]. The apomorphies are presence of an ocellus on the dorsal side of forewing in cell R<sub>3</sub> [27(1); Fig. 1F, G] and internal ring of the pattern element h white [42(2); Fig. 1F]. This is a well-supported clade (RFD = 0.55, ABS = 0.21, GC = 0.83).

Clade E is supported by two apomorphies and three homoplastic character changes [5(1), 6(1), 21(1)]: four are structural and one pertains to wing colour. The synapomorphies are: forewing veins R and Rs<sub>1</sub> stalked in males [3(1); Fig. 1A, C]; and FW veins Rs<sub>1</sub>–Rs<sub>2</sub>+Rs<sub>3</sub>+Rs<sub>4</sub>, Rs<sub>3</sub>+Rs<sub>4</sub>–M<sub>1</sub> and M<sub>1</sub>–M<sub>2</sub> swollen in males [4(1); Fig. 3B], this last character changes in the *laodamia* clade (below). Within *Hamadryas*, the transformation of M<sub>1</sub> arising from the same point as Rs<sub>2</sub>+3+4 [5(0); Fig. 3A] to M<sub>1</sub> arising at the midpoint between Rs<sub>2</sub>+3+4 and M<sub>2</sub> [5(1); Fig. 3B] is unique and constant to this

clade. However, this character does not appear as a unique apomorphy because state '1' is present also in *B. hypochlora*. Clade E has low support (RFD = 0.03, ABS = 0.02, GC = 0.08). This group includes all the species in *Hamadryas* that possess all or some of the venation components for sound production.

Nested within clade E is the only species group proposed by Jenkins (1983) that was found to be monophyletic, the *laodamia* clade. Together with *H. laodamia* this clade also includes *Hamadryas arete* Doubleday and *Hamadryas velutina* Bates. It is supported by ten apomorphies [4(2), 8(1), 9(2), 10(1), 13(1), 28(3), 43(1), 45(4), 53(0), 87(1)] and nine homoplastic character changes; it has an RBS of 99 and an SR of 100. Three of the most interesting apomorphies of this clade are: FW veins Rs<sub>1</sub>–Rs<sub>2</sub>+Rs<sub>3</sub>+Rs<sub>4</sub>, R<sub>3</sub>+R<sub>4</sub>–M<sub>1</sub> and M<sub>1</sub>–M<sub>2</sub> thick but not swollen in males [4(2); Fig. 3C]; ostium bursa extending into the seventh sternite [87(1); Fig. 6D; 87(0); Fig. 6C]; and presence of androconial scales [10(1); Fig. 1C].

#### Pattern of character change: sound production

The production of sound involves four venation characters (3–6) present in the FW of males (Figs 1, 3). When the minimum number of unambiguous transformations of these characters is traced onto the topology (Table 2), only character 6 requires an extra step. Based on the distribution of these four characters, sound production evolved once and was lost once at the node subtending *H. laodamia* and its relatives. In Fig. 7B dark boxes indicate species with venation suitable for sound production.

#### Pattern of character change: sexual dimorphism

Here, species were considered sexually dimorphic when the differences between male and female led to an unambiguous visual sex determination. Sexual dimorphism (SD) in *Hamadryas* is obvious and affects two features: wing shape and colour pattern. In some species males and females differ in wing shape only, in others the organization of the white

**Table 2.** Evidence of clade E. The theoretical minimum number of steps is shown together with the number of extra steps in implied weights (IW), as opposed to equal weights (EW), topology. Synapomorphies are set in bold. The numbers of extra steps were calculated inside *Hamadryas* only, and were obtained from the unambiguous optimization of each character using MACCLADE 4.08 (Maddison & Maddison, 2005).

Evidence of clade E			
Character number	Minimum number of steps	Extra steps in EW	Extra steps in IW
<b>3</b>	1	1	0
<b>4</b>	2	1	0
5	1	1	0
6	1	2	1
21	1	2	1

bands in the DFW (maculae) varies between the sexes, and there are also some species in which SD involves changes in both features. Because wing shape and presence/absence of DFW maculae are encoded in the data matrix, including the presence or absence of sexual dimorphism as an additional character would be redundant.

Figure 7B represents the variation of SD across the topology in wing shape (third column) and organization of the DFW maculae (right column). Forewing shape is sexually dimorphic in two instances: in *Ectima* (except for *Ectima iona* Doubleday) and in the *laodamia* clade. In both cases the FW distal margin is modified into a convex shape departing from the more generalized form of *Hamadryas*, in which males and females both show a slightly concave (almost straight) wing margin. The SD of the wing margin in *Ectima* and the *laodamia* clade differs, however, as in *Ectima* the females exhibit a concave margin whereas in the *laodamia* clade this feature is found in the male.

The pattern of organization in the DFW maculae is sexually dimorphic in *H. belladonna* and in the *laodamia* clade. In the males of *H. belladonna* the DFW maculae are disjointed, which is the generalized condition in the other (monomorphic) *Hamadryas*, whereas the DFW maculae in the males of the *laodamia* clade are reduced to small iridescent blue spots. The females of *H. amphinome*, *Hamadryas arinome* Lucas, and *H. belladonna* have DFW elongated maculae that are almost organized into a diagonal postmedial band, in the females of the *laodamia* clade the DFW maculae are fully aligned to form a similar band.

## Discussion

### *Species groups within Hamadryas*

Although the phylogenetic hypothesis proposed here supports some of the species affinities suggested by Jenkins (1983), the only species group supported is the *laodamia* clade (*Peridromia*). This clade is the most morphologically distinctive group of *Hamadryas*, as these three species are the only ones in the genus that exhibit conspicuous sexual dimorphism, male scent organs and also unpalatability, at least in *H. laodamia* (Chai, 1990). Members of this group have the gnathos conspicuously elongated, and these are the only species that possess a complete female sterigma (with lamellae ante and postvaginalis), deviating noticeably from the rest of the genus.

Attempts to separate the species now grouped in *Hamadryas* into different genera, subgenera or species groups were based initially in the variation of one venation character (character 3; Fig. 1A, B; Godman & Salvin, 1883; Fruhstorfer, 1916), and later on the combination of a few genitalia and venation characters (Jenkins, 1983). All the authors cited above noticed that males of some species exhibited a different state of character 3 than their conspecific females. Accordingly, Godman & Salvin (1883) included in *Ageronia* all the species in which both males and females have the FW veins R and Rs<sub>1</sub>

separate, and placed the species in which the males exhibit veins R and Rs<sub>1</sub> stalked in *Peridromia*. Fruhstorfer (1916) followed the same organization but, unlike Godman & Salvin (1883), he classified all species under *Ageronia*, which was then partitioned into species groups.

Although the diagnosis of *Peridromia* sensu Godman & Salvin (1883) was correct, *H. alicia* was included erroneously in this group. *Hamadryas alicia* does not exhibit the venation character used to define *Peridromia*, and it does not share the most recent common ancestor of the other members of *Peridromia* (Fig. 8). Jenkins (1983) split *Peridromia* into the *feronia* and *laodamia* species groups because of the morphological departure of *H. laodamia* and relatives, but the diagnosis of the *feronia* group lacked unique characters. Furthermore, he did not consider that the two groups could be nested (the *laodamia* inside the *feronia* species group, as has been shown here). Granting them the same rank (e.g. subgenera) would render the *feronia* species group non-monophyletic (Fig. 8). Finally, this study found no apomorphies to support a common origin of the species placed in *Ageronia* sensu Godman & Salvin (1883). Moreover, the main character state used to define *Ageronia* is symplesiomorphic.

The dataset in this study included a set of different character systems. Venation and other features of the wing provided 15 characters, wing colour included 33 characters and genitalia accounted for 42 characters: three data partitions. Not one of these partitions alone included enough informative characters to resolve the relationships among species or species groups (results not shown).

### *Sound production*

Published records suggest that ten species produce cracking sounds; however, only eight species have all of the venation components required for sound production (left-most column in Fig. 7B). Otero (1990) noted that the sound production venation was present in *H. feronia*, but was absent in *H. februa*. However, Monge-Nájera & Hernández (1991) and Jenkins (1983) reported individuals of *H. februa* producing sounds in the field. Recently Marini-Filho & Benson (2010) showed that *H. februa* does not produce sound, based on hand tests (Otero, 1990) and observations of caged individuals. Jenkins (1983) also reported sound production in *H. amphichloe*, but this species lacks the suitable venation. Given the fast and erratic flight of *Hamadryas*, and that species that do not produce sound fly together with those that do, field observations could be based on erroneous identifications. However, a so-far undiscovered mechanism of sound production in these species cannot be eliminated.

The context in which sound production occurs varies within butterflies. For example, Kane (1982) described acoustic signals in *Pharneuptychia* nr. *pharnabazos* in the presence of mates and/or food. More recently, female *Heliconius cydno* were observed producing audible wing clicks during interactions with conspecifics during the day and at roosting time (Medina Hay-Roe & Mankin, 2004). After some debate

about the purpose of sound in *Hamadryas* (Darwin, 1871; Monge-Nájera *et al.*, 1998), three studies seem to have led to a consensus supporting the use of sound as an aid in sexual recognition (Otero, 1988, 1990; Marini-Filho & Benson, 2010). All behavioural studies agree that sound occurs during aerial encounters. These encounters can occur between two (or more) males or between a male and a female, and in both cases sound is produced. However, in male–male interactions sound is produced continually, whereas in male–female interactions sound is produced only during the initial phase of the pursuit (Marini-Filho & Benson, 2010). This observation led Marini-Filho & Benson (2010) to suggest that in male–female encounters, once sexual recognition occurs, there is a shift of behaviour from sexual recognition to courtship, which is accompanied by the cessation of sound production.

The pattern of species relationships presented here has interesting implications for the evolution of sound production in *Hamadryas*. First, it suggests that sound production is a derived condition that evolved only once, but it also suggests that sound production was lost in the *laodamia* clade. The loss of sound production is accompanied by two other transformations: the presence of male scent organs (not present in any other species in the genus), and the presence of sexual dimorphism in wing shape and colour pattern (Fig. 7B). The congruence of these characters suggests that sound production cues have been replaced by visual and olfactory signals in *H. laodamia*, *H. arete* and *H. velutina*. If sound production is used as an aid for sexual recognition, as has been suggested, sexual dimorphism could have replaced sound production in the species of the *laodamia* clade (Marini-Filho & Benson, 2010). The presence of androconial scales could further facilitate species recognition. Given that the three transformations occur at the node of *H. laodamia* and its relatives, it is impossible to know the sequence of the transformations, but the potential association of these three characters deserves further investigation. This result reinforces the importance of knowledge of phylogenetic (cladistic) relationships in the study of character evolution, without which unexpected losses cannot be assessed reliably.

#### Sexual dimorphism

The main hypotheses about the origin of sexual dimorphism (SD) date back to Darwin (1871) and Wallace (1889), with a third and more recent hypothesis proposed by Silberglied (1984). Silberglied suggested that because brilliant colour patterns of males could work as signals of communication (e.g. recognition of other males and advertisement of his own sex), SD could originate from interactions between males. Although it would have been interesting to discuss the SD of *Hamadryas* in terms of Silberglied's hypothesis, we know very little about the aerial interactions between males, and almost nothing about these interactions in the sexually dimorphic species. Therefore I restrict the discussion to Wallace's and Darwin's hypotheses, which seem more appropriate given our data.

According to Darwin's view, SD was the result of sexual selection based on female preference for specific male

attributes. Over time female choice causes the deviation of the male phenotype from the ancestral pattern. According to Wallace, however, SD could also result from natural selection acting on female traits. He suggested that females could evolve a protective coloration (camouflage or mimicry), and would therefore deviate from the ancestral condition to obtain a fitness benefit. Both hypotheses have received some support from butterflies, and are not mutually exclusive. The origin of SD through sexual selection has been demonstrated in *Bicyclus anynana* (Robertson & Monteiro, 2005) and in *Hypolimnas bolina* (Kemp, 2007), and SD arising from female-limited mimicry was shown in a number of *Papilio* species (Kunte, 2008). The phylogeny together with the distribution of SD in *Hamadryas* suggests that the colour pattern of females in the *laodamia* clade is ancestral (although modified towards the alignment of the DFW maculae into an almost straight diagonal band), and that the male colour pattern is the novel condition. Although this study does not provide direct evidence of sexual selection, these results support Darwin's model for the origin of SD.

#### Closing remarks

Although the optimal tree presented here was resilient to different strengths of the concavity function, overall the topology has very low support. The GC values give moderate support for some internal nodes and for some species groups, but in general show indifference, particularly for clade E. Although *Hamadryas* exhibits variation in ecological traits, it is otherwise rather uniform in terms of structures such as genitalia. This translates into a lack of informative characters, reflected here by low values of ABS. Phylogenetic studies of *Hamadryas* could be improved in the following ways: (i) with a comparative morphological study of early stages, in the hope that they would provide additional informative characters as has been the case for other groups (e.g. Penz & Pegg, 2003); and (ii) by the addition of molecular markers that will offer a larger source of characters.

Based on the phylogeny, my study identifies a shift in the signals used for sexual recognition inside *Hamadryas* (from sound to sexual dimorphism and androconial scales), but the aids used for sexual recognition prior to the appearance of sound remain undetermined. Although aerial interactions (in the form of spiral flights) are present in all species, there is some evidence suggesting that these interactions might not be so significant in the sexual recognition for the species that produce sound (D. Otero, personal communication). More field observations and cage experiments will be crucial to determine if this is the case.

#### Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-3113.2011.00599.x

**Table S1.** Examined material, with repository collection number in parentheses.

**Table S2.** Character matrix.

**Table S3.** Records of type of oviposition and literature references.

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## Appendix

1. White scales on antennae: (0) absent; (1) present.
2. Female, foreleg tibial spines: (0) absent; (1) present.

### Venation and wing characters

3. In males, FW veins R and  $Rs_1$  (Jenkins, 1983): (0) separated; (1) share a common stem. Figure 1A–C. Note that regardless of the species, females have R and  $Rs_1$  arising separately; however, a few females of *Hamadryas iphthime* and *Hamadryas fornax* have R and  $Rs_1$  arising from a single point.
4. In males, FW  $Rs_1-Rs_{2+3+4}$ ,  $Rs_{2+3+4}-M_1$ , and  $M_1-M_2$ : (0) same width as other veins; (1) fully swollen; (2) thick but not swollen. Figure 3A–C. Note: this corresponds to the sound production ‘organ’ described by Otero (1990). The females of *H. epinome*, *H. iphthime* and *H. fornax* have thick veins, but not swollen. State 2 implies that the veins are thicker than in the species scored with state 0, but they are not swollen as in the species scored with state 1.
5. In males, FW vein  $M_1$ : (0) arising from the same point as  $Rs_{2+3+4}$ ; (1) arising at midpoint length between  $Rs_{2+3+4}$  and  $M_2$ . Figure 3A–C. Note: all species were sexually monomorphic for this character, except for *H. fornax*, in which there are females where  $M_1$  and  $Rs_{2+3+4}$  arise from the same point.
6. In males, FW vein  $M_2$ : (0) mildly curved towards  $M_3$ ; (1) conspicuously curved towards  $M_3$ . Figure 3A–C. Note: there are a few modifications associated with the presence of the sound production organ, such as  $M_1$  arising from a different point than  $Rs$  and a bowed  $M_2$ ; however, this last modification is not present in *H. arete*, *H. laodamia* and *H. velutina*. Generally, females have a straight  $M_2$  regardless of the species (females of *H. epinome* have a bowed  $M_2$  and females of *H. iphthime* and *H. fornax* are polymorphic for this character).
7. In males, FW crossvein  $M_2-M_3$  (Jenkins, 1983): (0) joins the  $M_3-CuA_1$  fork; (1) joins the  $Cu_1-Cu_2$

- crossvein; (2) joins  $M_3$ . Figure 1A, B. Note: *H. ipthime* and *H. epinome* are sexually dimorphic for this character, with the females having state 0.
8. In males, FW anal margin (modified from Jenkins, 1983): (0) straight; (1) convex. Figure 1A, C.
  9. Males and females distal margin of FW: (0) mildly concave; (1) sexually dimorphic, with margin convex in females and almost straight in males; (2) sexually dimorphic, with margin almost straight in females and convex in males. Figure 1C.
  10. Androconial scales on VFW surface from  $Cu_2$  to the anal margin: (0) absent; (1) present. Figure 1C.
  11. VFW extension of the androconial scale patch: (0) patch on and above  $Cu_2$ ; (1) patch does not reach  $Cu_2$ . Figure 1C.
  12. Androconial scales in the costal margin of DHW surface: (0) absent; (1) present. Figure 1D.
  13. Androconial scales on DHW surface from  $Sc$  until  $M_2$ : (0) absent; (1) present. Figure 1D.
  14. Colour of androconial scales on DHW surface from  $Sc$  until  $M_2$ : (0) dark brown/black; (1) light brown.
  15. HW  $Cu_2$  vein: (0) as long as  $Cu_1$ ; (1) noticeable longer than  $Cu_1$  and  $1A+2A$ . Figure 2A, B.
  16. VHW, predominant coloration: (0) black; (1) red/orange; (2) brick; (3) yellow; (4) mustard; (5) gold-brown; (6) flax; (7) beige; (8) chalk. Figures 1G, 2A–D, F. Note: the ventral colour of *Panacea divalis* is not as bright as the one of *Panacea prola*; however, they are coded with the same state because there is no doubt about the monophyly of *Panacea* and this avoids adding homoplasy to the character.
  17. If predominant VHW coloration is gold-brown, flax, beige or chalk, then pattern elements are: (0) opaque brown; (1) copper/iridescent brown. Figure 1G, 2A, B.
  18. Ventral coloration of thorax: (0) red; (1) mustard; (2) brown; (3) flax; (4) chalk.
  19. When ventral colour of thorax is red: (0) completely red; (1) red patches on a brown background.  
*The next characters are based on Nijhout's (1991: fig. 2.17) nomenclature, and refer to the dorsal wing pattern.*
  20. Colour of scales in DFW band inside discal cell between elements c and d: (0) red to brick; (1) orange; (2) black (indistinguishable from background); (3) green; (4) brown. Figures 1G, 2A–F. Note: although there is variation in the tone of this band because of age and some intraspecific variation (*H. ipthime* is coded polymorphic), very few individuals of *H. feronia*, *H. guatemalena* or *H. fornax* had an orange band, and no individuals of *H. februa*, *H. epinome*, *H. glaucome* and *H. amphichloe* had a red band. This character was coded in the females for *H. glaucome*, *H. laodamia*, *H. arete* and *H. velutina*.
  21. In species where DFW band inside discal cell between elements c and d is distinguishable from background, this band: (0) reaches the coastal margin; (1) is vestigial, and does not reach the coastal margin of the discal cell. Figures 1G, 2A, B, D.
  22. Spot of blue scales in the proximal portion of the DFW band between elements c and d: (0) absent; (1) vestigial; (2) well developed. Figures 1F, 2B–F.
  23. DFW band between elements c and d bordered by: (0) black/dark scales; (1) blue scales. Figure 2A, D.
  24. In females, colour of DFW discal cell band between pattern elements d and e: (0) white; (1) blue; (2) iridescent green; (3) beige/light brown. Figures 1F, 2C–E. Note: individuals of *H. chloe* vary from a whiter band to a darker band, but there are still white scales inside the band. This band also varies in *H. amphinome* from a light blue to an iridescent green band; however, blue is the most common colour. In *H. glaucome* this band is brown, although it is covered by scattered grey scales.
  25. In females, DFW pattern element e: (0) composed of dark scales; (1) composed of (brown) light scales; (2) composed of blue scales; (3) composed of red scales. Figures 1F, 2A–E.
  26. DFW band/spot at  $R_3$ ,  $R_4$ : (0) white; (1) blue; (2) absent. Figure 2B–F.
  27. In females, DFW ocellus at  $R_3$ ,  $R_4$ : (0) absent; (1) present. Figures 1F, G, 2B, D, E.
  28. In females, DFW  $R_4$ ,  $R_5$  ocellus: (0) absent; (1) vestigial ocellus; (2) ocellus fully present; (3) only pupil visible. Figures 1F, G, 2A, B.
  29. In females DFW band/spot between  $R_5$  and  $M_1$ : (0) cream; (1) white; (2) blue; (3) absent. Figure 2B, D, F.
  30. DFW in females, width of space between margin of the discal cell and  $M_1$ – $M_2$  band: (0) narrow; (1) wide; (2) no space, reaching distal margin of discal cell. Figures 1F, 2B–E.
  31. DFW  $M_2$  cell in females, colour of next distal band to pattern element e: (0) blue; (1) white; (2) brown; (3) green. Figure 2A, C.
  32. DFW in  $M_2$  cell in females, shape of band distal to pattern element e: (0) entire, elongated towards distal margin of wing and pattern element f not visible; (1) split by pattern element f, proximal band oval. Figures 1F, 2C–E.
  33. In species where DFW band in the  $M_2$  cell is entire, inner margin of postmedian diagonal band: (0) straight; (1) irregular. Figure 2E.
  34. In species where DFW band at  $M_2$  is entire, postmedian diagonal band extends: (0) from  $R_3+R_4+R_5$  to  $CuA_2$ ; (1) from coastal margin to  $CuA_2$ ; (2) from  $R_3+R_4+R_5$  to  $CuA_1$ ; (3) from coastal margin to  $1A+2A$ . Figure 2C, E.
  35. DHW in females, pattern element d: (0) centre of element d composed of light scales; (1) element d composed of dark scales only; (2) centre of element d composed of red scales. Figures 1F, 2A–D.
  36. DHW in females, colour of the distal edge of discal cell (pattern element e): (0) red; (1) light; (2) dark brown. Figures 1F, 2A–D.



37. DHW in females, proximal portion of element f: (0) scattered; (1) continuous. Figure 2A, B.
38. DHW shape of pattern element f: (0) not continuous across the wing (broken); (1) continuous across the wing and narrowed into an almost straight line; (2) slightly continuous across the wing but dislocated (irregular line). Figures 1F, 2A, C, D.
39. When adjacent edges do not match, width of DHW pattern element f: (0) wide; (1) intermediate; (2) thin. Figures 1F, 2B–D. Note: next characters about the border ocelli were coded based on ocellus number 6 in Figure 1E.
40. DHW pattern element h (border ocelli): (0) absent; (1) present. Figure 2A, C–E. Note: although *Panacea* does have border ocelli, the ocelli are reduced and do not exhibit as many elements as do the ocelli in *Ectima* or *Hamadryas*. This is even more obvious in *Panacea prola* and *Panacea divalis*, in which the ocelli are vestigial, and therefore the next characters were coded as inapplicable.
41. DHW pattern element h (border ocelli): (0) with internal ring; (1) without internal ring. Figures 1F, 2A–D. Note: *H. feronia* has a very small almost vestigial internal ring only visible through the stereoscope.
42. DHW pattern element h (border ocelli) in species with internal ring, colour of internal ring: (0) greenish; (1) blue; (2) white. Figures 1F, 2A, B.
43. Most external ring of DHW pattern element h: (0) as a complete circle; (1) as an incomplete circle. Figure 2C–F.
44. DHW of males in species where external ring of pattern element h is incomplete, proximal portion of the external ring present only in ocellus: (0) 4–5–6; (1) 5–6. Figure 2F.
45. Composition of DHW ocellus 2 (Rs cell): (0) complete ocellus; (1) blurred ocellus; (2) only the most external ring present (empty ocellus); (3) external ring and a pupil present; (4) pupil only. Figures 1G, 2A–D, F.
46. Composition of DHW ocellus 4 (M<sub>3</sub> cell): (0) ocellus present; (1) empty ocellus. Figures 1F, 2A.
47. DHW ocellus 7 (Cu<sub>2</sub> cell): (0) pupil present; (1) empty ocellus. Figures 1G, 2A–D.
48. DHW pattern element k: (0) absent; (1) present. Figures 1F, 2C, E.
49. DHW pattern element j: (0) both lines continuous; (1) both lines broken; (2) only one line visible and broken; (3) two lines, joined in the middle of the cell. Figures 1F, 2A, C, D, F. Note: element j is composed of two lines of scales in some species or only one broad line in others. There is some intraspecific variation in this character, but the differences between species hold. *Hamadryas laodamia*, *H. velutina* and *H. arete* have a wide broken band at the edges of each cell, which I believe could be the result of filling up the empty space between both lines present in *H. fornax*: hence the coding.

### *Hypandrium*

The term hypandrium has traditionally been used in reference to the modified eighth sternite present in the males of Biblidinae. It is in this same sense that I am using it in this paper.

50. Anterior edge of hypandrium: (0) slightly extended anteriorly; (1) conspicuously extended anteriorly reaching internally almost the mid length of the abdomen. Figure 4B, C, D.
51. Lateral edges of posterior margin of hypandrium: (0) composed of tooth-like serrations; (1) projected into elongated rami. Figure 4A, C, D.
52. In species with rami, in ventral view, extension of posterior edge of hypandrium: (0) to a small degree from the base of rami; (1) considerably beyond the base of rami. Figure 4A, F.
53. In species with hypandrium extended beyond the base of rami, posterior margin of hypandrium: (0) rounded; (1) squared. Figure 4E–G.
54. When squared, posterior margin of hypandrium: (0) moderately straight; (1) conspicuously irregular (wavy). Figure 4F–H.
55. Macrochaete setae on hypandrium: (0) absent; (1) present. Figure 4F, G. Note: Jenkins (1983) refers to these setae as spines; these setae arise from a socket, and are pointy, heavily sclerotized and restricted to the hypandrium. I am naming them macrochaetes to distinguish them from other types of setae, although whether they are mechanoreceptors or not is unknown.
56. Location of macrochaete setae on hypandrium: (0) present only at the base of rami; (1) present on lateroposterior margin and some setae reaching the posterior margin of the sternite. Figure 4E–H.
57. Macrochaete setae at the posterior margin of hypandrium (base of rami): (0) as long as the macrochaete at the tip of rami; (1) smaller. Figure 4E, G.
58. Setae on lateral surface of rami: (0) absent; (1) present but few; (2) present in large numbers. Figure 4A, G.
59. When present, setae on external side of rami: (0) short and fine; (1) long and thick. Figure 4A, G.
60. Macrochaete setae along the rami: (0) absent; (1) present. Figure 4E, F. Note: these setae are very long and curved in *H. chloe*.
61. Tip of rami: (0) rounded; (1) pointed. Figure 4I.

### *Male genitalia*

62. In dorsal view, anterior edge of tegumen: (0) approximately squared; (1) rounded; (2) elongated. Figure 4J–N.
63. In dorsal view, constriction of tegumen at the point of attachment with gnathos: (0) small; (1) large. Figure 4K–M.
64. In lateral view, dorsal outline of uncus: (0) curved; (1) angled. Figure 5A, B.
65. Length of setae on the basal section of uncus: (0) short; (1) medium; (2) long. Figure 5A–C.

66. Long setae in the distal portion of uncus: (0) absent; (1) present. Figure 5C.
67. Posterior portion of uncus (tip): (0) width smoothly decreases towards the tip; (1) width decreases conspicuously at the tip (giving the tip the appearance of a claw). Figure 5A, B.
68. Latero-anterior margin of vinculum: (0) straight; (1) extended anteriorly. Figure 5D, E.
69. In ventral view, length of gnathos: (0) not longer than broad; (1) longer than broad. Figure 5F–H. Note: this character refers to the general appearance of the complete gnathos, i.e. the proximal and distal portions.
70. In lateral view gnathos arms (modified from Jenkins, 1983): (0) thin; (1) wide.
71. In lateral view, ventral projection of distal portions of gnathos: (0) absent; (1) present. Figure 5I, J. Note: in lateral view, the distal portion of gnathos is extended ventrally.
72. In ventral view, length of the distal processes of gnathos, from the point where they are fused to the tip: (0) short; (1) elongated. Figure 5F–H.
73. In dorsal view, species with distal portion of gnathos elongated, width of posterior portions: (0) broad; (1) medium; (2) thin. Figure 5H.
74. In ventral view, sclerotization of the distal portions of gnathos: (0) complete (all the distal portion of gnathos is fully sclerotized); (1) interrupted (only the sides of the distal portions are sclerotized). Figure 5F, H.
75. In lateral view, coastal edge of valva: (0) straight; (1) with a coastal projection at midpoint. Figure 5C–E.
76. In lateral view, distal portion of valva: (0) excavated or straight; (1) projected. Figure 5K–M.
77. In ventrolateral view, internal side of base of valva: (0) with a projection extended ventrally; (1) with a projection that smoothes into an internal folding of the valva. Figure 5N, O.
78. In ventral view, internal outline of valva: (0) with a projection close to the base of the valvae; (1) with a projection at midlength. Figure 5D, E, P, Q.
79. In dorsolateral view, juxta: (0) heavily sclerotized and projected posteriorly; (1) slightly sclerotized and not projected. Figure 5P, Q.
80. Setae on phallus shaft: (0) absent; (1) present. Figure 5R.

81. Cornuti: (0) absent; (1) present. Figure 5R.
82. In ventral view, base of saccus: (0) squared; (1) triangular. Figure 5P, Q.

#### *Female genitalia*

83. Posterior edges of abdominal seventh sternite: (0) straight; (1) slightly projected posteriorly. Figure 6B, C. Note: In *H. chloe* the seventh sternite seem to have been extended posteriorly, it looks extended a little bit in *H. atlantis* too and has small sclerotizations at the lateral edges. State 1 is developed to a greater degree in species of *Myscelia*, *Temenis* and *Nica*.
84. Membranous pocket between the sclerotized portions of the abdominal segments 7 and 8: (0) absent; (1) present. Figure 6A, B.
85. Eighth sternite: (0) free; (1) fused to the seventh sternite. Figure 6A–D.
86. Lamella antevaginalis: (0) absent; (1) present. Figure 6B–D.
87. Ostium bursa: (0) free; (1) contained to sternite 7; (2) contained to sternite 8. Figure 6C, D.
88. In dorsal view, heavily sclerotized plate on dorsal portion of antrum: (0) absent; (1) present. Figure 6E.
89. Ductus seminalis connecting to ductus bursa (character 52 in Hill *et al.* (2002): (0) very near of corpus bursa; (1) far from corpus bursa, and near ostium bursa. Figure 6F, J.
90. Shape of corpus bursa: (0) short and wide; (1) rounded; (2) cone shaped (narrow near ductus bursa); (3) pear shaped. Figure 6G–J.
91. Signa: (0) absent; (1) present. Figure 6F, G, I.
92. Shape of signa: (0) elongated; (1) two spine-shaped invaginations. Figure 6G–I.

#### *Natural history characters*

93. Oviposition pattern: (0) eggs laid singly; (1) eggs laid in clusters. Note: this character is based on literature descriptions of life cycles (Young, 1974; Muyschondt & Muyschondt, 1975a, b, c; Jenkins, 1983; DeVries, 1987; DeVries *et al.*, 2000).