Evaluating the monophyly and phylogenetic relationships of Brassolini genera (Lepidoptera, Nymphalidae)

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Abstract. This study uses 80 morphological characters and cladistic analysis to evaluate the monophyly and phylogenetic relationships of 18 genera that constitute the butterfly tribe Brassolini. Most characters derive from genitalia, confirming previous generic definitions based mainly on wing characters, and showing that 16 of 18 genera are monophyletic. Mimoblepia Casagrande, syn.n., was subsumed within Opoptera Aurivillius to address the paraphyly of the latter, but resolution of the status of Aponarope Casagrande requires further study. The results suggest that the Brassolini includes six suprageneric groups/clades. Although this study verifies some genus-level relationships put forward over 100 years ago, some new hypotheses of relationships are proposed. Tracing larval host plant use onto the Brassolini phylogeny indicates that species in this tribe retain the use of Arecaceae and Poaceae from their ‘satyroid’ ancestors.

Introduction

The conspicuous ventral eyespots, large size and somberly attractive colours of the brassolines make them some of the most familiar of all Neotropical butterflies (Figs 1–4), and they have long been of interest to naturalists. Brassolines feed on monocots as larvae, and genera may vary in both diet breadth and their association with plant families (Penz et al., 2000). Some are confined to a single plant family (e.g. Narope Doubleday and Opoptera Aurivillius on Poaceae), whereas others have a broad diet (e.g. Caligo Hübner has a host range that includes eleven genera in seven plant families). Except for the gregarious Brassolis Fabricius, larvae have characteristic head scoli and tails (but see below), and typically feed at night (DeVries, 1987 and references cited therein). Adult brassolines feed on rotten fruit juices, and their flight activity generally is crepuscular. Depending on the species, mating occurs either at dawn or dusk, and mating behaviours often are complex. Although several anecdotes exist concerning brassoline mating behaviour (e.g. Fruhstorfer, 1912; DeVries, 1987), only two field studies have provided detail on this. Freitas et al. (1997) and Srygley & Penz (1999) showed that male Caligo idomeneus (Linnaeus, 1758) and Caligo illioneus (Cramer, 1775) and Caligo oleus C. Felder & R. Felder, 1861 perch at forest edges to form mating leks. Virgin females visit these leks to select a male for copulation, and oviposition is crepuscular.

Recent phylogenetic analyses have indicated that the Brassolini constitutes a monophyletic group (Brower, 2000; Freitas & Brown, 2004; Peña et al., 2006), which is not surprising given the past 170 years of research (e.g. Boisduval, 1836; Stichel, 1904; Fruhstorfer, 1912; Ehrlich, 1958; Ackery, 1988; Casagrande, 1995). The DNA-based cladistic analysis by Peña et al. (2006) examined a broader sample of taxa within the Satyrinae, Brassolini, Morphini and Amathusiini than ever before, thus strengthening the support for the Brassolini as a natural group, and suggesting that these three tribes are basal lineages within the Satyrinae. Although many taxonomic rearrangements have been made throughout the history of the Brassolini (e.g. descriptions of new taxa, new combinations, etc.), the recent addition of the genus Bio Hübner (Vane-Wright & Bopper, 2004), formerly in the Satyrinae, was particularly significant, because it called into question characters used for tribal definition.

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Some adult characters present in \textit{Bia} conflict with the traditional diagnosis for the Brassolini, whereas others are homoplasious. The following character state combination has been used to define the Brassolini: (1) closed hindwing discal cell; (2) absence of swollen veins; and (3) presence of a hindwing precostal cell (Casagrande, 1995). Freitas & Brown (2004) showed that the ‘closed hindwing discal cell’ is highly homoplasious within Nymphalidae, being gained independently and lost in various nodes of their tree. Swollen veins are present in \textit{Bia}, hence its placement previously in the Satyrinae (e.g. Weymer, 1912), but this character state is not present universally in satyrines, and occurs in other nymphalid subfamilies (e.g. Biblidinae). As for the hindwing precostal cell, although Freitas & Brown (2004) coded this structure as absent for \textit{Bia}, Vane-Wright & Boppé (2004: 236) clearly showed a rudimentary precostal cell in \textit{Bia actorion} (Linnaeus, 1763). Nonetheless, in addition to brassolines, this character is present also in \textit{Hyantis} Hewitson and \textit{Morpheopsis} Oberthür (Amathusiini) (Frühstorfer, 1912; Miller, 1968; C. M. Penz, personal observation), and therefore seems to be homoplasious in the context of the ‘satyroid’ clade sensu Peña \textit{et al.} (2006).

Early-stage characters used traditionally to support the definition of the Brassolini are found in other nymphalid groups. Casagrande (1995) listed three early-stage characters that complement her adult-based definition of the Brassolini: (1) monocotyledonous food plants; (2) presence of head scoli; and (3) presence of a bifid anal plate (= caudae or tails). All of these characters occur in some ‘satyroid’ lineages, and also in other nymphalid subfamilies (e.g. Ackery, 1988; Freitas & Brown, 2004). In line with the study by DeVries \textit{et al.} (1985), Freitas & Brown (2004) used early-stage morphology to group \textit{Bia} with brassolines, where the following characters link \textit{Bia} with other genera: (1) absence of transverse ridges on egg; (2) in the last instar, colour of the anal plate (or caudae if present), dark (black, brown, red); (3) in the last instar, presence of three additional pairs of scoli on the head; and (4) crepuscular flight period. However, these four characters are also homoplasious within the Nymphalidae. In sum, although little doubt exists that the Brassolini constitutes a natural assemblage together with \textit{Bia}, at present there is a lack of unique, universal diagnostic characters for this group.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Sample of studied species; dorsal surface on the left side, except for \textit{Aponarope sutor}. From left to right and top to bottom: \textit{Bia actorion}, Bolivia; \textit{Narope cylastros}, no data; \textit{Aponarope sutor}, Brazil, Rondônia; \textit{Brassolis sophorae}, Brazil, Santa Catarina; \textit{Dynastor darius}, Paraguay.}
\end{figure}
An extensive body of systematic literature exists concerning brassolines (see Stichel, 1904 and Casagrande, 1995 for references to early works), but the contributions of four authors are of particular significance. The classifications by Stichel (1904, 1909, 1925, 1932), Fruhstorfer (1912), Miller (1968) and Casagrande (1982, 1995, 2002, 2004) provided the framework upon which all recent studies are based, including that presented here. Hand-drawn trees based on classifications by Fruhstorfer (1912), Stichel (1932), Miller (1968) and Casagrande (1995, 2004) are shown in Fig. 5, and these authors’ views on brassoline higher level classification are summarized below.

Deeply ingrained in the catalogue tradition, Stichel (1904, 1909) meticulously listed all of the then recognized brassoline taxa. Although Stichel characterized each genus and, in some cases, proposed infrageneric groups (his Sections and Cohors), his two seminal works did not include suprageneric classification for the eleven brassoline genera he considered valid. By contrast, Fruhstorfer (1912) divided ‘Brassolidae’ into two subfamilies (Fig. 5A), thus making an assertion of relationships between genera based on larval characters; the ‘Brassolininae’ lacked larval forked tails and head scoli, whereas these structures were present in the ‘Caligoninae’. However, these defining characters of the Brassolininae are valid only for the genus *Brassolis* – species of *Dynastor* Doubleday have well-developed tails and head scoli, which are reduced but present in *Penetes* Doubleday (Casagrande, 1995). Fruhstorfer (1912) largely maintained Stichel’s (1904; 1909) infrageneric groups, but downgraded four genera (Fig. 5A). Therefore, Fruhstorfer established a subgroup within his Caligoninae that represented his views of close affinities between these taxa.

Stichel (1925, 1932) based his tribal definitions on adult characters, such as the presence and nature of the androconial organs, venation and wing colour patterns. He divided the ‘Brassolininae’ into three tribes (Fig. 5B), an arrangement that differed only slightly from that proposed by Fruhstorfer (Fig. 5A). The two main modifications were

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**Fig. 2.** Sample of studied species, dorsal surface on the left side. From left to right and top to bottom: *Dasyophthalma rusina*, Brazil; *Mimoblepia staudingeri*, Costa Rica, Heredia; *Oopoptera fruhstorferi*, Brazil, Santa Catarina; *Oopoptera aorsa*, Brazil, Paraná (note body in ventral view to show character 14:1, see Appendix 1).
Fig. 3. Sample of studied species, dorsal surface on the left side. From left to right and top to bottom: *Eryphanis reevesii*, Brazil, São Paulo; *Caligopsis seleucida*, Bolivia, Cochabamba; *Eryphanis aesacus*, Mexico, Veracruz; *Caligo beltrao*, Brazil, Santa Catarina; *Eryphanis 'polyxena*', Colombia, Cali; *Caligo martia*, Brazil, Pinhal.
Fig. 4. Sample of studied species, dorsal surface on the left side. From left to right and top to bottom: *Selenophanes josephus*, Panama, Panama; *Mielkella singularis*, Mexico, Chiapas; *Penetes pamphanis*, Brazil, Paraná; *Orobrassolis ornamentalis*, Brazil, São Paulo; *Catoblepia orgetorix*, Ecuador; *Blepolenis bassus*, Brazil; *Opsiphanes tamarindi*, Mexico, Veracruz; *Opsiphanes boisduvalii*, Mexico.
placing Dynaster within ‘Caligonidi’, and creating ‘Naropidi’ for the genus Narope alone. In contrast to previous authors, Miller (1968) did not consider the morphological differences between brassoline genera sufficiently strong to warrant a tribal classification within his Satyridae. Based on eye pubescence, antennal club width, presence of tibial spurs on the middle and hindlegs, and the ratio of the forewing discal cell length over that of the costal vein, Miller established four ‘series’ within the Brassolinae (Fig. 5C). Of these, the Narope series corresponded to Stichel’s (1925) ‘Naropidi’, but the others represented novel arrangements. The Dasyophthalma series included its namesake genus that has pubescent eyes, the Caligo series included Caligo and Eryphanis Boisduval (Caligopsis Seydel was not mentioned), and the Brassolis series encompassed the seven remaining genera. Except for the Narope and Dasyophthalma series, however, the characters listed by Miller (1968) were insufficient to define the other two generic groups.

Modern brassoline classification owes much to Casagrande, who described the four newest genera of the group (Casagrande, 1982). Although Casagrande’s (1995) classification was based on previously used characters, her work was the first broad survey of brassoline male and female genitalia, and uncovered a wealth of morphological diversity. Casagrande (1995) retained Stichel’s (1925) ‘Naropidi’ (as Naropina), but included all remaining genera within the tribe Brassolini, following Boisduval (1836), not Frühstorfer (1912) (compare Fig. 5A, D). Her morphological characterization of the Brassolini is sufficiently broad to accommodate a wide range of genera, but no attempts were made to establish phylogenetic affinities between taxa. Casagrande’s (2004) most recent classification grants brassolines tribal status and includes the subtribes Biona (monotypic), Brassolina (15 genera) and Naropina (two genera), with all taxa listed alphabetically (Fig. 5D).

This study expands and refines our previous understanding of brassoline genitalic morphology by assessing character homology for male and female structures in the light of a phylogenetic analysis. Representatives of all Brassolini genera included in the most current classification (Casagrande, 2004) were examined to provide the first cladistic evaluation of the monophyly of each genus, and the phylogenetic relationships within the tribe. This analysis confirms some former notions of generic affinities and, like other recent work within Nymphalidae (e.g. Penz & Djunjianti, 2003; Brower et al., 2006), also provides some new hypotheses of relationships. To bridge phylogenetics and natural history, the tree resulting from the analysis was used to map and discuss patterns of larval host plant use within the Brassolini.

**Materials and methods**

**Taxon sampling**

Table 1 lists the species included in the analysis and the collection data for dissected specimens. Criteria for taxon sampling were as follows.

Outgroup: A recent study has indicated that the genus Elymnias is a closely related outgroup to Brassolini (Peña et al., 2006), and Elymnias hypermnestra (Linnaeus, 1763) (Satyrinae) was selected as outgroup for this analysis.
Table 1. Dissected specimens. Taxa are listed in alphabetic order and the number of valid species in each genus (in square brackets) follows Casagrande (2004).

<table>
<thead>
<tr>
<th>Taxon [number of species in genus]</th>
<th>Locality</th>
<th>Repository</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aponarope Casagrande, 1982 [1]</strong></td>
<td>1 m, Brazil Rondônia, Fazenda Rancho Grande</td>
<td>FLMNH</td>
</tr>
<tr>
<td><strong>Aponarope sutor (Stichel, 1916)</strong></td>
<td>1 m, Peru, Loreto, Iquitos; 1 f, Peru Satipo</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Bia Hu¨bner, 1819 [2]</strong></td>
<td>1 m, Brazil; 1 f, Brazil, [Santa Catarina] São Bento</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Blepolenis bassus (C. Felder &amp; R. Felder,1867)</strong></td>
<td>1 m, Brazil, Rio de Janeiro, Nova Friburgo; 1 f, Brazil</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Blepolenis batea (Hu¨bner, 1821)</strong></td>
<td>1 m, Brazil, Rio de Janeiro, Nova Friburgo; 1 f, Brazil</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Brassolis Fabricius, 1807 [4]</strong></td>
<td>1 m, Colombia, Cali; 1 f, Panama, Tocumen MPM, FLMNH</td>
<td></td>
</tr>
<tr>
<td><strong>Brassolis isthmia Bates, 1864</strong></td>
<td>1 m, Brazil, Santa Catarina, Nova Teutònía; 1 m Brazil, Ceará, Fortaleza; 1 f, Brazil, Rio de Janeiro, Nova Friburgo; 1 f, Brazil, Paraı´so; 1 f, Bolivia, Santa Cruz, Mineros</td>
<td></td>
</tr>
<tr>
<td><strong>Caligo Hu¨bner, 1819 [21]</strong></td>
<td>1 m, Colombia, Antioquia, Zaragosa; 1 f, no data MPM</td>
<td></td>
</tr>
<tr>
<td><strong>Caligo atreus (Kollar, 1850)</strong></td>
<td>1 m, Costa Rica, Heredia, Finca La Selva</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Caligo beltrao (Illiger, 1801)</strong></td>
<td>1 m &amp; 1 f, Brazil, Stuporanza</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Caligo illioneus (Cramer, 1775)</strong></td>
<td>1 m &amp; 1 f, Costa Rica, Heredia, Finca La Selva</td>
<td>MPM</td>
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<tr>
<td><strong>Caligo martia (Godart, 1824)</strong></td>
<td>1 m, Brazil, Pinhal; 1 f, Brazil</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Catoblepia Seydel, 1924 [1]</strong></td>
<td>1 m, Bolivia, Cochabamba, San Francisco; 1 f, Brazil, Amazonas, Madeira River MPM, USNM</td>
<td></td>
</tr>
<tr>
<td><strong>Catoblepia berecynthia (Cramer, 1777)</strong></td>
<td>1 m &amp; 1 f, Central Peru MPM</td>
<td></td>
</tr>
<tr>
<td><strong>Catoblepia orgetorix (Hewitson, 1870)</strong></td>
<td>1 m &amp; 1 f, Colombia, Antioquia, Zaragosa; 1 m, Ecuador, Oriente; 1 f, Ecuador, Pichincha, Santo Domingo de los Colouredos</td>
<td>MPM</td>
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<tr>
<td><strong>Catoblepia xanthus (Linnaeus, 1758)</strong></td>
<td>1 m, Brazil, Pará, Obidos; 1 f, British Guiana, Georgetown MPM</td>
<td>MPM</td>
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<tr>
<td><strong>Dasyophthalma Westwood, 1851 [4]</strong></td>
<td>1 m, South Brazil; 1 f, South Brazil MPM</td>
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<tr>
<td><strong>Dasyophthalma creusa (Hu¨bner, 1821)</strong></td>
<td>1 m, Brazil, Santa Catarina; 1 f, Brazil, Santa Catarina, São Bento do Sul</td>
<td>MPM</td>
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<tr>
<td><strong>Dasyophthalma rusina (Godart, 1824)</strong></td>
<td>1 m, Paraguay; 1 f, Brazil, Paraná, Ponta Grossa MPM</td>
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</tr>
<tr>
<td><strong>Dynastor Doubleday, 1849 [3]</strong></td>
<td>2 m, Brazil, Santa Catarina</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Dynastor darus (Fabricius, 1775)</strong></td>
<td>1 m, Paraguay; 1 f, Brazil, Paraná, Ponta Grossa MPM</td>
<td></td>
</tr>
<tr>
<td><strong>Dynastor napoleon Doubleday, 1849</strong></td>
<td>2 m, Brazil, Santa Catarina</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Eryphanis Boisduval, 1870 [5]</strong></td>
<td>1 m, Mexico, San Luis Potosi; 1 f, Mexico MPM</td>
<td></td>
</tr>
<tr>
<td><strong>Eryphanis aesacus (Herrich-Schäffer, 1850)</strong></td>
<td>2 m &amp; 1 f, Colombia, Cali; 1 m, Paraguay, Pedro Juan Caballero</td>
<td>MPM</td>
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<tr>
<td><strong>Eryphanis ‘polyxena’ (Meerburgh, 1780)</strong></td>
<td>1 m &amp; 1 f, Brazil, São Paulo, Pinhal</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Eryphanis reevesii (Doubleday, 1849)</strong></td>
<td>1 m, Mexico, Chiapas; 1 f, [Mexico] Santa Rosa Comitán</td>
<td>AMNH</td>
</tr>
<tr>
<td><strong>Mielkella Casagrande, 1982 [1]</strong></td>
<td>1 m &amp; 1 f, Costa Rica, Heredia, Puerto Viejo</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Mielkella singularis (Weymer, 1907)</strong></td>
<td>1 m, Mexico, Chiapas; 1 f, [Mexico] Santa Rosa Comitán</td>
<td>AMNH</td>
</tr>
<tr>
<td><strong>Minoblepis Casagrande, 1982 [1]</strong></td>
<td>1 m &amp; 1 f, Costa Rica, Heredia, Puerto Viejo</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Narope Doubleday, 1849 [18]</strong></td>
<td>1 m, Bolivia</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Narope cyllabirus Westwood, 1851</strong></td>
<td>1 m, Brazil, Paraná</td>
<td>MPM</td>
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<tr>
<td><strong>Narope cyllarius Westwood, 1851</strong></td>
<td>1 m, Brazil, Paraná, Ponta Grossa MPM</td>
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<tr>
<td><strong>Narope cyllastros Doubleday, 1849</strong></td>
<td>1 m, Paraguay; 1 f, Brazil, Paraná, Ponta Grossa MPM</td>
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<tr>
<td><strong>Narope nesope Hewitson, 1869</strong></td>
<td>1 m, Brazil, Paraná</td>
<td>MPM</td>
</tr>
<tr>
<td><strong>Narope panniculus Stiehle, 1904</strong></td>
<td>1 m, Ecuador, Oriente; 1 f, Brazil, Minas Gerais</td>
<td>AMNH</td>
</tr>
</tbody>
</table>

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Ingroup: The analysis included 38 species that constituted approximately 45% of all Brassolini species (see Casagrande, 2004). All 18 valid genera were represented (Table 1), six of which are monotypic (although Casagrande, 2004 indicated that *Caligopsis* may include two species). Species sampling was based on classifications by Stichel (1904, 1909) and Fruhstorfer (1912), who proposed supra- and infrageneric groups. Here, small genera are represented by two species (e.g. *Brassolis*), and three to five species were sampled from more diverse genera (e.g. *Narope*), representing different infrageneric groups proposed by previous workers (e.g. Stichel, 1904, 1909).

Casagrande (2004) suggested that *Eryphanis polyxena* (Meerburgh, 1780) should be considered a synonym of *Eryphanis automedon* (Cramer, 1775), and noted also that the former name is preoccupied. Here, *Eryphanis 'polyxena'* (in quotes) is used to emphasize the fact that the taxon distributed from Central America to Colombia, examined here (Fig. 3, see DeVries, 1987 for distribution and natural history), represents a species separate from *Eryphanis automedon*. A study in progress will address the taxonomic status of *Eryphanis automedon* and *Eryphanis 'polyxena'* (C. M. Penz, in preparation).

**Character sampling**

A list of 80 characters (illustrated in Figs 8–12) is presented in Appendix 1, and the associated character matrix is available as Table S1 (see 'supplementary material'). From these characters, eight refer to both sexes, 44 are from males and 28 are from females. Six uninformative characters (characters 8, 25, 48, 64, 75, 76) were included because they may be useful for future analyses of a larger number of taxa. Abdomens and legs (left female foreleg, left male midleg) were prepared using a 10% solution of KOH, and subsequently stored in glycerol–ethanol solution (3:1). Pinned adults were used to examine external morphology. All structures were examined using an optical stereomicroscope with light and dark field and up to 130× magnification. The terminology for the genitalia follows Klots (1970) with some modifications by Kristensen (2004).

**Analysis**

A cladistic analysis using parsimony as the optimality criterion was used to test the monophyly of, and to infer the phylogenetic relationships among Brassolini genera. All characters had equal weight, and multistate characters were set as unordered. Heuristic searches in PAUP 4.0b10 (Swoford, 2002) used stepwise addition with 500 tree bisection–reconnection random addition sequences. Estimates of branch support were calculated from 500 bootstrap replicates, excluding uninformative characters and with 100 trees retained at each bootstrap iteration. To reduce the number of equally parsimonious trees in the equal weights analysis, as a means of improving the resolution of the strict consensus tree, one round of successive approximation weighting (SAW) was run with characters re-weighted on the basis of the maximum value of their rescaled consistency index. Rescaled consistency indices (RC) for each character are given in Appendix 1. To assess the number of character changes per branch, the default algorithm for character

<table>
<thead>
<tr>
<th>Taxon [number of species in genus]</th>
<th>Locality</th>
<th>Repository</th>
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<tbody>
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<td>Opoptera Aurivillius, 1882 [6]</td>
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<td>Opoptera aorsa (Godart, 1824)</td>
<td>1 m, Bolivia; 1 f, Brazil, Rio de Janeiro, Nova Friburgo</td>
<td>MPM, FLMNH</td>
</tr>
<tr>
<td>Opoptera arsippe (Hopffer, 1874)</td>
<td>1 m, South Brazil; 1 f, [Brazil] Itaporanga</td>
<td>MPM, AMNH</td>
</tr>
<tr>
<td>Opoptera fruhstorferi (Röber, 1896)</td>
<td>1 m, Brazil, Rio de Janeiro, Nova Friburgo; 1 f, Brazil, [Rio de Janeiro] Petrópolis</td>
<td></td>
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<tr>
<td>Opoptera syme (Hübner, 1821)</td>
<td>1 m &amp; 1 f, Colombia, Cali</td>
<td>MPM</td>
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<tr>
<td>Orocassogloss Casagrande, 1982 [1]</td>
<td>1 m &amp; 1 f, Brazil, São Paulo, Umuarama</td>
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<td>Orocassogloss ornamentalis (Stichel, 1906)</td>
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<td>Penetes Doubleday, 1849 [1]</td>
<td>1 m, Brazil, Paraná, Curitiba; 1 f, Brazil</td>
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<td>Penetes pamphanis Doubleday, 1849</td>
<td>1 m, Brazil, Paraná, Rolanda; 1 f, Peru, Rio Hualiga</td>
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<td>Selenophanes cassiope (Cramer, 1775)</td>
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</tr>
<tr>
<td>Selenophanes josephus (Godman &amp; Salvin, 1881)</td>
<td>1 m, Panama, Canal Zone; 1 f, Panama, Darién, Caña</td>
<td></td>
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<tr>
<td>Selenophanes josephus (Godman &amp; Salvin, 1881)</td>
<td>1 m, Panama, Canal Zone; 1 f, Panama, Darién, Caña</td>
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AMNH, American Museum of Natural History; FLMNH, Florida Museum of Natural History; MPM, Milwaukee Public Museum; USNM, United States National Museum; f, female; m, male.
change reconstruction under parsimony in MacClade 4 was used (Maddison & Maddison, 2000; unordered characters, downpass/uppass/final passes).

Mapping larval host plant use

The resulting phylogeny was used to map larval host plant records, and to explore genus-level patterns within the Brassolini. To do so, the strict consensus of the SAW trees was used to build a topology in which brassoline genera appeared as terminal taxa. Larval host plant data are based on Penz et al. (2000), and include glasshouse records for Caligopsis seleucida (Hewitson, 1877) (Furtado & Campos-Neto, 2004). Each host plant family was considered a ‘character’, and the use of each plant family was scored for 12 brassoline genera. Using MacClade 4, host plant data were traced onto the genus-level tree (as explained above), and the results are discussed in the light of the topology for ‘satyroid’ lineages proposed by Peña et al. (2006).

Results

Phylogenetic analysis

The initial analysis of 80 equally weighted characters produced 288 equally parsimonious trees with a length of 224 steps. Figure 6 shows the strict consensus (inset) and tree statistics of these trees. SAW selected nine of these 288 trees, and their strict consensus is also illustrated in Fig. 6, together with bootstrap values from the analysis using equal weights. The analysis demonstrates that 16 of 18 brassoline genera are monophyletic, and can be divided into six groups that include one to seven genera (Fig. 6). Figure 7A–F includes all character state changes recovered by the analysis. Characters that support the monophyly of each genus, and the most important characters that can be used to establish taxon groups, are listed below. Appendix 1 should be consulted for the full explanation of the remaining character changes listed in Fig. 7, and for illustrations of character states. The terminology for character status follows MacClade 4, and abbreviations are as follows: uu, unique and universal (highlighted in bold type); c., changed above (i.e. within the group), not outside; ha, homoplasious above (i.e. within the group); ho, homoplasious outside (i.e. outside the group); hao, homoplasious above and outside.

Tribe Brassolini (Figs 6, 7A)

The present comparative study did not recover new characters that provide additional support to the monophyly of the tribe Brassolini. Although universal within the ingroup, the presence of a precostal cell (character 6:1) is uninformative in the context of this analysis because Elymnias hypermnestra (outgroup) is the only species which lacks the precostal cell.

Brassolini genera

Bia, Group 1 (Fig. 7A).

8:1 uu: Prominent HW tail at Cu2: present (Fig. 1).
26:0 ho: Heavier sclerotization of the dorsal edge of the valva: does not extend to the valva tip (Fig. 8H).
Fig. 7. Distribution of character state changes in the strict consensus of nine trees obtained with successive approximation weighting (SAW). The key for symbols and character status appears next to B. The most important characters supporting the groups are listed in the results, and others can be found in Appendix 1. A, Character changes supporting the six major groups of genera within the Brassolini. B, Group 2, Nanope clade. C, Group 3, Brassolis clade. D, Group 4, Opoptera clade. E, Group 5, Caligo clade. F, Group 6, Opsiphanes clade.
31:2 ho: Groove at the articulating point between the valva and appendices angulares: extended distally for c. one-third of the valva length (Fig. 8H).
35:0 ho: Distal opening of the phallus: dorsal.
43:0 ho: Distal portion of the posterior uncus process: blunt.
70:0 ho: Antrum: entirely membranous.

Narope + Aponarope, Group 2 (Fig. 7B). No characters were found to separate Narope from Aponarope Casa-grande. Narope neso Hewitson, 1869, Aponarope sutor (Stichel, 1916), Narope cyllastros Doubleday, 1849, Narope cyllabarus Westwood, 1851, and Narope cyllarus Westwood, 1851 were grouped by the unique character state 44:1 uu [Uncus ventrolateral spines: present (Fig. 9E)], suggesting that the genus Narope, as defined currently, is paraphyletic. The following character states group all species of Narope and Aponarope sutor.

18:1 hao: In lateral view, dorsal outline of tegumen: straight anteriorly and arched posteriorly (Fig. 10F).

23:1 ho: In lateral view, pedunculum and vinculum seamlessly amalgamated, synscleritous.
52:2 hao: Gnathos: adjoining tegumen exclusively (Fig. 10F, G).
54:1 uu: Female foreleg, number of tarsomeres: one (Fig. 9F). Note that this character state was found in Narope panniculus Stichel, 1904 and Narope cyllastros, and is predicted to occur in other species of this group.
67:1 uu: Lateral sections of sterigma: separated from remainder of sterigma (Fig. 11C).
68:0 ho: Posterior section of sterigma (‘lamella postvagina-lis’): absent (Fig. 11C).
77:1 ho: Posterior edge of sclerotized base of papilla anales: with a dorsal projection, appearing in-curved (Fig. 12F).
79:1 uu: In lateral view, papillae anales: deeper than tall (Fig. 12F).

Brassolis (Fig. 7C).

12:0 ho: Male midleg, tibial spurs: absent.
16:1 uu: In dorsal and dorsolateral views, anterolateral constriction of the tegumen: present (Fig. 10C).
34:1 uu: Basal, internal region of valva: conspicuously less sclerotized than distal region (Fig. 8J).

46:0 uu: Gnathos: absent.

68:0 ho: Posterior section of sterigma ('lamella postvagina-lis'): absent (Fig. 11C).

70:3 uu: Antrum: fully sclerotized.

78:1 ho: Setae on posterior edge of sclerotized base of papilla anales: present (Fig. 12E).

**Dynastor** (Fig. 7C).

39:1 ho: Lateral uncus wings, as they extend posteriorly into uncus process: merging to form a single, prominent dorsal keel (Fig. 10G).

**Opoptera + Mimoblepia** (Fig. 7D). No characters were found that separated Opoptera from Mimoblepia Casagrande. Two character states group Mimoblepia staudingeri (Godman & Salvin, 1894) with two species of Opoptera; 31:2 ho [Groove at the articulating point between gonopod and appendices angulares: extended distally for c. one-third of the gonopod length (Fig. 8H)]; and 52:1 ho [Gnathos: adjoining tegumen more extensively than lateral uncus wing (Fig. 10I)]. This indicates that, as defined currently, the genus Opoptera is paraphyletic. The following character states group all species of Opoptera and Mimoblepia staudingeri.

26:2 uu: Heavier sclerotization of the dorsal edge of the valva: encircles the valva tip (Fig. 8C, D).

28:0 uu: Heavily sclerotized portion of the dorsal edge of the valva: with minute ribbed serrations (Fig. 8C, D).

41:0 ho: In lateral view, posterior uncus process: straight at base and bending at tip.

57:1 ho: Female foreleg, basal tarsomere (tm1): clearly longer than the sum of the lengths of tarsomeres 2–5 (Fig. 9H).

**Dasyophthalma** (Fig. 7D).

1:1 uu: Pubescence of eyes: present.

60:1 ho: Degree of sclerotization of the intersegmental sac between Stn7 and sterigma: with an anteromedial scleritized region (Fig. 11C).

62:1 ho: Anterior ribs of the intersegmental sac between Stn7 and sterigma: broken (Fig. 11E).

65:2 ho: Anterior section of sterigma ('lamella antevagina-lis'): absent (Fig. 11D, E).

69:1 ho: Inward projections of sterigma: present (Fig. 11A, D).

**Caligopsis** (Fig. 7E).

38:0 ho: Coecum penis: absent (Fig. 9A).

51:1 ho: Gnathos, distal region: spiny (Fig. 10G).

53:1 ho: Female foreleg, dorsal spines on tibia: present.

**Eryphanis** (Fig. 7E).

32:2 uu: Thin, setae-bearing flange associated with the dorsal, basal region of the valva: present, sclerotized (Fig. 8E shows 32:1, the unsclerotized flange present in Caligo).
35:0 ho: Distal opening of phallus: dorsal.

_Caligo_ (Fig. 7E).

78:1 ho: Setae on posterior edge of sclerotized base of papilla anales: present (Fig. 12E).

_Selenophanes_ (Fig. 7F).

21:1 ho: Hairpencil on dorsolateral portion of tegumen: present (Fig. 10I).
35:2 ho: Distal opening of phallus: ventrolateral.
56:0 ho: Female foreleg, ventral tarsomere spines: short (Fig. 9I).
60:1 ho: Degree of sclerotization of the intersegmental sac between Stn7 and sterigma: with an anteromedial sclerotized region (Fig. 11C).
62:1 ho: Anterior ribs of the intersegmental sac between Stn7 and sterigma: broken (Fig. 11E).
77:1 ho: Posterior edge of sclerotized base of papilla anales: with a dorsal projection, appearing in-curved (Fig. 12F).

_Penetes_ (Fig. 7F).

19:0 ho: Hairpencil on dorsoposterior portion of tegumen: absent (see Fig. 10D for the location of this hairpencil).
35:0 ho: Distal opening of phallus: dorsal.
40:1 ho: In ventrolateral view, pocket of setae in the membrane ventral to the uncus wings: present (Fig. 9D).
52:1 ho: Gnathos: adjoining tegumen more extensively than lateral uncus wing (Fig. 10I).
74:0 ho: Signa: nearly as long as corpus bursa (Fig. 12D).
80:1 ho: In lateral view, posterior contour of papilla anales: straight or arched-in (Fig. 12G).

_Catoblepia_ (Fig. 7F).

31:2 ho: Groove at the articulating point between valva and appendices angulares: extended distally for c. one-third of the valva length (Fig. 8H).

70:2 uu: Antrum: with a sclerotized ring.

_Mielkella_ (Fig. 7F).

45:0 ho: Length of setae at posterior uncus process: all setae short.
53:0 ho: Female foreleg, dorsal spines on tibia: absent.
63:0 ho: Lateral section of sterigma: fused to anterolateral edge of eighth tergite (Tg8) (Fig. 11H).
77:1 ho: Posterior edge of sclerotized base of papilla anales: with a dorsal projection, appearing in-curved (Fig. 12F).

Fig. 10. Illustrations of characters. Scale bars, 1 mm. Male second pair of legs, left side. A, _Opsiphanes tamarindii_. B, _Caligo atreus_. Male tegumen and uncus in dorsal (C), dorsolateral (H) and lateral views (all others). C, _Brassolis sophorae_. D, _Orobrassolis ornamentalis_. E, _Bia actorion_. F, _Narope cyclastros_. G, _Narope cyclabarbus_. H, _Opoptera syme_. I, _Caligo atreus_. J, _Dasyophthalma rusina_.

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Orobrassolis (Fig. 7F). No character changes were attributed to *Orobrassolis ornamentalis* (Stichel, 1906) in this study.

Blepolenis (Fig. 7F).

77:1 ho: Posterior edge of sclerotized base of papilla anales: with a dorsal projection, appearing in-curved (Fig. 12F).

Opsiphanes (Fig. 7F).

10:1 ho: Male midleg, lateral rows of spines on tibia: on outer side only (i.e. one lateral row).
63:0 ho: Lateral section of sterigma: fused to anterolateral edge of eighth tergite (Tg8) (Fig. 11H).

Groups of genera

Characters for Group 1 (*Bia*) and Group 2 (*Narope + Aponarope*) are listed above. As shown in Fig. 7, nearly all branches of the strict consensus tree are supported by unique character states, including groups of genera and subgroups.

Group 3 (*Brassolis + Dynastor*) (Fig. 7C).

37:1 uu: Peg-like setae on phallus shaft: present (Fig. 9B).
53:1 ho: Female foreleg, dorsal spines on tibia: present.
55:1 ho: Female foreleg, pulvillar pad: present, round.
56:0 ho: Female foreleg, ventral tarsomere spines: short (Fig. 9I).

Group 4 (*Mimoblepia + Opoptera + Dasyophthalma*) (Fig. 7D).

14:1 ha: Mid- and hindlegs, colour of distal edge of each segment and tarsal subsegment: lighter, forming rings that contrast the colour of the segment/subsegment (Fig. 2). Although *Opoptera fruhstorferi* (Röber, 1896) and *Opoptera syme* (Hübner, 1821) lack this single character state that
supports Group 4, it does not occur in any of the other brassoline species examined here.

Group 5 ((Caligopsis + Eryphanis) + Caligo) (Fig. 7E).

32:1 ca: Thin, setae-bearing flange associated with the dorsal, basal region of the valva: present, membranous (changing to 32:2 in Eryphanis) (Fig. 8E).

47:1 hao: Gnathos, posterior expansion of proximal region: present (Fig. 10I).

60:3 ho: Degree of sclerotization of the intersegmental sac between Stn7 and sterigma: entire sac appears sclerotized (see Fig. 11D for a membranous intersegmental sac).

69:1 ho: Inward projections of sterigma surrounding ostium bursa: present (Fig. 11A, D).

73:1 uu: Signa: divergent (Fig. 12A).

Group 6 (((((Opsiphanes + Blepolenis) + Orobrassolis) + Mielkella) + Catoblepia) + Penetes) + Selenophanes) (Fig. 7F).

28:2 uu: Heavily sclerotized portion of the dorsal edge of the valva: with uneven-shaped spines or projections (Fig. 8F, G, H, K).

53:1 hao: Female foreleg, dorsal spines on tibia: present.

55:1 hao: Female foreleg, pulvillar pad: present.

71:1 hao: Corpus bursa: elongate (Fig. 12C).

Discussion

This study provides the first cladistic analysis of all brassoline genera. Although not designed to test the monophyly of the Brassolini, all species examined in this study share a character state that is universal but not unique to this tribe: the presence of a hindwing precostal cell (character 6:1, illustrated in Casagrande, 2002: 487 and Vane-Wright & Boppre´, 2004: 236). Studies in progress will address the issue of diagnostic characters for this butterfly group (see ‘Introduction’) and its phylogenetic relationships with Amathusiini and Morphini (C. M. Penz et al., in preparation). The analysis here supports the monophyly of all but two genera (Narope and Opoptera), and indicates that the Brassolini includes six natural suprageneric groups (Figs 6; 7). The results are compared below with four previous contributions to brassoline systematics and classification. Larval host plant use is then discussed, and future directions for studies within the Brassolini are indicated.

Brassolini systematics

Over time, the number of described brassoline genera has increased steadily, often separating species previously placed together. When most brassoline genera were described (1807–1906), emphasis was given to characters such as colour pattern, venation and male scent organs. Indeed, similarity in wing colour may be a product of common descent, in which case characters derived from wing colour patterns should be congruent with other phylogenetically informative characters (e.g. male and female genitalia, 75% of the characters analysed here). Therefore, in addition to elucidating brassoline phylogeny, this study can also be used to examine the usefulness of wing colour characters as the main foundation for generic definitions in this group.

Confirming previous studies (e.g. Freitas & Brown, 2004; Peña et al., 2006), in my analysis, Bia appears as the sister taxon to the remaining brassoline genera. Bia differs from
other brassolines in three external characters: the inflated forewing Sc vein (character 2:0), the absence of mid- and hindleg tibial spurs (character 12:0; absent also in Brassolis) and the absence of dorsal spines on the midleg tarsus (character 13:0). Species of Bia resemble several satyrines with respect to these character states [see Elymnias hypermnestra in Table S1 ('supplementary material')], and their small size and prominent hindwing tail at vein Cu2 further contrast the general brassoline gestalt. Therefore, it is unsurprising that Bia was transferred to Brassolini based mostly on early-stage characters (Freitas et al., 2002; Vane-Wright & Boppré, 2004).

The separation of Narope into its own tribe (currently subtribe) was proposed by Stichel (1925), and maintained subsequently (Miller, 1968; Casagrande, 1995, 2004). Although four unique character states support the monophyly of Narope + Aponarope (5:0, 54:1, 67:1, 79:1, see ‘Results’), the analysis here suggests that Narope is paraphyletic (Figs 6; 7B). Aponarope sutor probably was set apart from Narope because it has several autopatomorphic character states. Casagrande (1982, 2002) listed the following distinctive characters for this species: spiny meso- and metathoracic femora (character 9:1, also found in some species of Eryphanis, Orobrassolis Casagrande, Blepoloris Röber and Opsiphanes Doubleday), the broad tip of the male valva (unique within Naropina) and the sclerotized female lamella postvaginalis (females were unavailable for examination). Both Narope and Aponarope have similar wing size, shape and colour, including reduced ventral hindwing eyespots (Fig. 1). Although this study provides some evidence for subsuming Aponarope within Narope, taxonomic change should await a more comprehensive analysis.

Previous opinions on the relationships between Brassolis and Dynastor are difficult to unravel. Frustorfer (1912) grouped Brassolis with Dynastor and Penetes, but this assemblage was based on the absence of larval head scoli and tails – both incorrectly interpreted (see ‘Introduction’). By contrast, Stichel (1904) listed Brassolis and Dynastor sequentially in his catalogues, which could indicate either general similarity or close phylogenetic relationships. However, in his later works, Stichel’s Brassolidi (Fig. 5B) included only Brassolis and Penetes (Stichel, 1925, 1932). Although the dorsal colour pattern of Penetes pamphanis Doubleday, 1849 superficially resembles that of Brassolis, this species completely lacks the ventral hindwing eyespot present in most brassolines (Fig. 4). The analysis here shows that Brassolis and Dynastor are monophyletic, that together they form a natural group, and that neither genus is closely related to Penetes (Fig. 6). This relationship is interesting in terms of larval biology, because the absence of head scoli has been regarded as an indication that Brassolis should be considered as the ‘most basal’ genus within the brassolines (e.g. Frustorfer, 1912). More likely, the absence of head scoli may be associated simply with the gregarious habits of Brassolis larvae, with hundreds of larvae resting tightly together in a tent made of palm leaflets and silk (e.g. Dunn, 1917).

The analysis here suggests that, together, Opoptera and Mimoblepia constitute a natural group. Stichel (1904) divided Opoptera into two sections (see examples of sections and groups in Fig. 2): Desmicosometi, including the groups Aorsiformes (aorsa Godart, 1824, arișippe Hopfle, 1874) and Symiformes (fruhstorferi, syme, sulcius Staudinger, 1887); and Peragnosti, including a single species (staudingeri). Casagrande (1982) formalized Stichel’s separation of Staudingeri with the description of the genus Mimoblepia. In the analysis presented here, no character states unique to Opoptera were found, but two were unique and universal to Opoptera + Mimoblepia, both concerning the finely serrated ornamentation of the male valva (26:2, 28:0; Fig. 7D and Appendix 1). Although Stichel’s groups Aorsiformes and Symiformes were supported by the results presented here, Mimoblepia staudingeri appeared closely related to Fruhsorferi and syme, thus making Opoptera paraphyletic (Figs 6; 7D). These findings concur with the taxonomic arrangement by Stichel (1904), and the fact that syme is the type species of Opoptera argues against transferring syme and fruhstorferi to Mimoblepia (Fig. 7D).

Therefore, based on the analysis presented here, it is proposed that the monotypic genus Mimoblepia, syn.n., (type species: staudingeri) be subsumed within Opoptera (see ‘Revised generic classification of Brassossil’, below).

Although the grouping of Dasyophthalma Westwood and Opoptera is not strongly supported by the analysis presented here (Figs 6; 7D), these genera share a unique male leg character state (14:1, see ‘Results’ and Opoptera aorsa in Fig. 2). When this character is excluded from the analysis, the position of Dasyophthalma becomes unstable: in some trees it groups with Opoptera (as in Fig. 6), whereas in others it appears as the sister genus to Opoptera + other clades (not illustrated). As the name indicates, Dasyophthalma has pilose eyes, a unique character used to set it apart from other brassolines in previous classifications (e.g. Stichel, 1904; Frustorfer, 1912; Miller, 1968). The analysis here provides the first suggestion that Dasyophthalma and Opoptera may be closely related, thus suggesting that there has been strong divergence in wing shape and colour between these two genera (Fig. 2). Nonetheless, this hypothesis should be verified in future studies based on more comprehensive species and character sampling.

Caligo includes butterflies easily recognized by a large wingspan, shadowy blue dorsal wing colours and conspicuous ventral hindwing eyespots, and it is perhaps the most familiar of all brassoline genera (Fig. 3). In its early concept, Caligo included species classified currently in Eryphanis and Caligopsis. The description of Eryphanis set apart aescus Herrich-Schäffer, 1850 (type species) and other deep blue species with double ventral eyespots in hindwing cells m3 and cu1, plus a dorsal hindwing scent organ in cell cu2. Caligopsis seleucida lacks such a scent organ and has predominantly brown wing colours, which justified its separation from Eryphanis. These three monophyletic genera together form a natural group (Figs 6; 7A, E), which satisfyingly retrees their taxonomic history. Despite the diaspora of various taxa, Caligo remains the
largest Brassolini genus (21 species recognized by Casagrande, 2004), and its full range of morphological variation should be sampled more thoroughly in the future.

*Opsiphanes* and allies have a more convoluted history than *Caligo*. Stichel (1904, 1909) divided *Opsiphanes* into two species groups: Bateiformes and Cassiformes. The Bateiformes included species in which the basal to medial areas of the wings were yellow or orange, and contrasted with the dark brown postmedial and marginal areas [see *Blepolenis bassus* (C. Felder & R. Felder, 1867) in Fig. 4]. In the Cassiformes, the basal area of the wings usually is brown (see *Opsiphanes tamarindi* C. Felder & R. Felder, 1861 in Fig. 4), and the forewing has a postmedial orange or white band (except *boisduvalii* Doubleday, 1849; Fig. 4). These two groups correspond currently to the genera *Blepolenis* and *Opsiphanes* that appear as monophyletic sister taxa in the analysis presented here (Figs 6; 7A, F). On the basis of wing pattern, *singularis* Weymer, 1907 was first described as a member of *Opsiphanes*; it was moved to *Catoblepia* by Stichel (1909), and then re-included in *Opsiphanes* by Bristow (1991). Casagrande (1982) created the monotypic genus *Mielkella* Casagrande for *singularis* based on the morphology of the male genitalia and legs, and her assessment that *singularis* belonged neither in *Opsiphanes* nor in *Catoblepia* is confirmed (Figs 6; 7F). Furthermore, Casagrande (1982) erected the monotypic *Orobrassolis* for the unusually coloured *ornamentalis* that was previously in *Brassolis*, noting that *ornamentalis* differed from *Brassolis* by the presence of male abdominal scent organs and gnathos, and female signa. Indeed, *Orobrassolis ornementalis* is well separated from *Brassolis*, with support for grouping with *Mielkella*, *Blepolenis* and *Opsiphanes* (Figs 6; 7F). Although the study presented here did not recover unique synapomorphies for any of these four genera, one universal character state joins them as a monophyletic clade (character 30:1, see ‘Results’).

By contrast with that of Stichel (1904, 1909), Fruhstorfer’s (1912) definition of *Opsiphanes* included four groups; *Opoptera*, the nominal *Opsiphanes*, *Catoblepia* and *Selenophanes* Staudinger (Fig. 5A). Fruhstorfer’s quartet assemblage was based mostly on venation, although he noted that *Opoptera* was ‘nearly entitled to generic rank’. Keeping in mind that the ‘group *Opsiphanes*’ included Stichel’s (1904, 1909) Bateiformes and Cassiformes (currently *Blepolenis* and *Opsiphanes*), Fruhstorfer’s suggestion that *Opoptera*, *Opsiphanes*, *Catoblepia* and *Selenophanes* are closely related seems odd given the views of the time (i.e. Stichel, 1904, 1909). However, this view is supported by the results presented here, except for the placement of *Opoptera* (Figs 6; 7D, F).

The present study is the first to suggest explicitly that the enigmatic *Penetes pomphanis* is a member of the *Opsiphanes* clade (Fig. 7F). One homoplous character groups Penetes with *Catoblepia*, *Mielkella*, *Orobrassolis*, *Blepolenis* and *Opsiphanes* (26:9), the heavier sclerotization of the dorsal edge of the valva does not extend to the valva tip (Fig. 7F). When this character is excluded from the analysis, the positions of *Penetes* and *Selenophanes* are reversed (not illustrated). The relationships proposed here (Figs 6; 7F) therefore are tentative and should be verified in future studies including additional characters. Finally, potential genus-level synapomorphies included here should be confirmed with more comprehensive studies within each of the six clades revealed in the analysis. Studies in progress are directed towards this goal (C. M. Penz et al., in preparation).

### Revised generic classification of Brassolini

**Tribe Brassolini**

**Subtribe Biina**

- *Bia* Hübner, 1819

**Subtribe Naropina**

- *Narope* Doubleday, 1849
- *Aponarope* Casagrande, 1982

**Subtribe Brassolina**

- *Brassolis* Fabricius, 1807
- *Dynastor* Doubleday, 1849
- *Dasyophthalma* Westwood, 1851
- *Opoptera* Aurivillius, 1882
- *Mimobolepia* Casagrande, 1982
  - syn.n.
- *Caligo* Hübner, 1819
- *Caligopsis* Seydel, 1924
- *Eryphanis* Boisduval, 1870
- *Penetes* Doubleday, 1849
- *Selenophanes* Staudinger, 1887
- *Catoblepia* Stichel, 1901
- *Mielkella* Casagrande, 1982
- *Orobrassolis* Casagrande, 1982
- *Blepolenis* Röber, 1906
- *Opsiphanes* Doubleday, 1849

### Wing colour patterns

Available evidence supports the placement of Brassolini in the Satyrinae (Miller, 1968; Peña et al., 2006), and it is thus not surprising that brassolines share wing pattern elements with members of basal satyrine lineages. Let us consider two examples: the transverse dorsal forewing band and the ventral hindwing eyespots. The forewing band can be recognized easily in species of *Elymnias*, *Neorina*, *Ethope* and *Xanthotaenia* (see D’Abrera, 1985 for illustrations). When present, this band varies in colour, width, shape and position on the wing. Variation can be observed within and between genera, and across the entire Satyrinae, including brassolines (for illustrations, see Figs 1–4 and D’Abrera, 1985, 1987). Ventral hindwing eyespots are widespread and variable amongst the Satyrinae, and the full complement includes seven spots. Within Brassolini, *Narope* has a full complement of small spots, most genera have two or three large eyespots, and some (not all) species of *Selenophanes* and *Catoblepia* can have up to six (Fig. 4). A detailed study of wing colour pattern evolution within Brassolini would be of interest, and should incorporate all species in this tribe and also other tribes in the Satyrinae.

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Male scent organs

Brassolines have highly developed male pheromone organs located externally on the abdomen, plus patches of androconial scales on the wings (Wasserthal & Wasserthal, 1977; Vane-Wright & Boppré, 2004). Except for *Selenophanes cassiope* (Cramer, 1775), abdominal scent organs are ubiquitous in the *Caligo* and *Opsiphanes* clades (Groups 5 + 6; Fig. 7A). In these groups, the scent organs are located on the pleural membrane, whereas, in *Bia*, they are at the lower edges of the tergites (see character 15 in Appendix 1).

The diverse wing scent organs within the Brassolini belong to two general categories. First, scent scale patches consist of modified scales that do not differ noticeably in size from the scales of the surrounding wing areas (see hindwing scent patch of *Aponarope sutor* in Fig. 1). By contrast, ‘hairpencils’ or ‘hair tufts’ refer to thin, elongated, hair-like scales (see Vane-Wright & Boppré, 2004: 239). A preliminary inspection identified 12 types of scent organ in the species included in this study; three on the forewings (two scent scale patches and one hairpencil) and nine on the hindwings (five scent scale patches and four hairpencils). Some of these organs are unique to certain genera; for example, the scale patch in cell cu2-1a that occurs in all species of *Eryphanis* (Fig. 3). Others seem to be shared between genera, but vary in morphology and position along the wing membrane. For example, compare the scent patch inside the discal cell in *Bia actiorion* and above Rs in *Narope cyllastros* (Fig. 1), and over the basal portion of Rs in *Caligo beltrao* (Illiger, 1801) and *Caligo seleucida* (Fig. 3). A comprehensive study of scent patches and hairpencils is in progress that will assess their homology between genera (C. M. Penz et al., in preparation).

Host plant use

All brassolines feed on monocots as larvae, and genera vary in diet breadth and their association with plant families (Penz et al., 2000 and references cited therein). Although little is known about the association between herbivores and monocots in the Neotropics, the tracing of host plant use onto the brassoline genus-level tree revealed patterns of host use within this butterfly group (Fig. 13).

The use of plants in the family Arecaceae could be traced back to the ancestor of the Brassolini, and members of lineages that do not feed on palms generally use species in the Poaceae (Fig. 13). Two of the three largest Brassolini genera, *Caligo* (21 species) and *Opsiphanes* (11 species), expanded their host plant diet independently into the families Musaceae, Marantaceae and Cannaceae (Fig. 13), and there are records of *Caligo* larvae on Zingiberaceae. Possibly, a correspondence exists between species diversification and diet breadth within *Caligo* and *Opsiphanes*, as has been suggested for other taxa (e.g. *Morpho*; Penz & DeVries, 2002). However, countering this trend, *Narope* (18 species) is confined to the Poaceae. Interestingly, *Narope* females have an extremely reduced foreleg tarsus (character 54:1) and lack the patch of sensilla generally present in nymphalids and used for assessing host plant quality. The use of Arecaceae and Poaceae as hosts by species of the Brassolini corresponds to that reported for early ‘satyroid’ lineages (e.g. Ackery, 1988; Robinson et al., 2006).

The comprehensive phylogenetic analysis by Peña et al. (2006) suggested that the Amathusiini and Morphini + Brassolini were clades within the Satyrinae. Their results suggested that the Arecaceae-feeding Elymiina might be the sister group to all other ‘satyroid’ lineages. The next clade in their tree includes the Amathusiini, which mostly...
feed on Arecaceae, plus the Zetherina + Neorina series, reported to feed mainly on plants within the Poaceae. Host records for Pierrella and relatives include Arecaceae, Poaceae, Marantaceae and Heliconiaceae (DeVries, 1987; Ackery, 1988), a list of families that closely matches the host records of the Brassolini (Penz et al., 2000). Within the Morphini, the larvae of Antirrhea and Caerois feed on Arecaceae, the basal species of Morpho use Poaceae as hosts, but more derived species feed on dicots (DeVries, 1987; Penz & DeVries, 2002), which represents a unique and unusual colonization event in the context of monocot-feeding ‘satyroid’ taxa. In sum, feeding on Arecaceae and Poaceae seems to represent an ancestral pattern for ‘satyroid’ taxa, and the Marantaceae and Heliconiaceae were colonized repeatedly by various taxa, including Pierrella, Caligo and Opsiphanes. Taken together, Ackery (1988), Peña et al. (2006) and this study suggest that the prevalent use of Arecaceae and Poaceae within the Brassolini has probably been maintained from ‘satyroid’ ancestors.

Conclusions

The main conclusions of this study are as follows: 16 of the 18 Brassolini genera listed by Casagrande (2004) are monophyletic; the monotypic Mimoblepia is placed within Opoptera, and therefore is subsumed into Opoptera; the monotypic Aponarope is placed within Narope but requires a more comprehensive analysis to test the relationships between these two genera; and the monotypic Caligopsis, Penetes, Mielkella and Orobrassolis appear as discrete lineages. Genitalic characters confirm previous generic definitions based mostly on wing characters (colour, venation, androconial organs). Some generic relationships proposed in the early 1900s are confirmed here using cladistic analysis (e.g. Caligo plus Caligopsis and Eryphanis, and Selenophanes plus Catoblepia and Opsiphanes), whereas others represent novel hypotheses (e.g. Penetes included in the Opsiphanes group).

The relationships between Opoptera and Dasyophthalma, and Selenophanes and Penetes (within the Opsiphanes group), are weakly supported and should be assessed in future studies. Initial assessment of the monophyly of larger genera (e.g. Caligo) should also be tested in future analyses that include more species (C. M. Penz et al., in preparation).

Brassolini species retain ancestral use of Arecaceae and Poaceae as larval host plants, and patterns of host diversification within this tribe appear to be similar to those of other ‘satyroid’ lineages.

Supplementary material

The following material is available at http://www.blackwell-synergy.com under the DOI reference doi:10.1111/j.1365-3113.2007.00391.x

Table S1. Data matrix.

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Appendix 1. Character list

Head

1. Eye pubescence: absent (0), present (1). RC: 1.

Vantation

2. FW Sc: inflated (0), not inflated (1). RC: 1. Illustrated in Miller (1968: 34).


6. HW precostal cell: absent (0), present (1). Uninformative. Figs 1–4.

7. Prominent HW tail at M3: absent (0), present (0). RC: 1. Fig. 2.

8. Prominent HW tail at Cu2: absent (0), present (1). Uninformative. Fig. 1.

Male legs

9. Male midleg, dorsal spines on femur: absent (0), present (1). RC: 0.238. Fig. 10A.

10. Male midleg, lateral rows of spines on tibia: absent (0), on outer side only (i.e. one lateral row) (1), on both sides (i.e. two lateral rows) (2). RC: 0.16. Fig. 10B. Note: The male tibia possesses two ventrolateral rows of spines (see inset of Fig. 10B), and should not be confused with the lateral spines described here (marked with a double arrow).

11. Male midleg, outer, lateral row of spines on tibia: limited to the lateral portion of the tibia (0), slightly extended dorsally (1), fully extended dorsally (2). RC: 0.201. Fig. 10B.

12. Male midleg, tibial spurs: absent (0), present (1). RC: 0.333. Fig. 10B.


14. Male mid- and hindlegs, colour of distal edge of each segment and tarsal subsegment: similar to the colour of
the segment/subsegment (0), lighter, forming rings that contrast
the colour of the segment/subsegment (1). RC: 0.375. Fig. 2. Opoptera aorsa. Note: pinned specimens
were used to score this character.

Male abdomen and genitalia

15. Abdominal scent organs located on the pleural mem-
brane of the fourth abdominal segment: absent (0), present (1). RC: 0.472. Note: Vane-Wright & Bopp (2004)
described and illustrated the abdominal scent organs of Bia actio, which consist of clusters of short
scales surrounded by a rim of longer scales, and are permanent (not deciduous). Although in most brasso-
line species these organs seem to be located on the pleural membrane, in Bia they are positioned at the
lower edges of the tergites. The difference in position of the abdominal pheromone organs between Bia and
members of the Caligo and Opsiphanes clades explains the narrow definition of character 15, and here Bia was
scored ‘0’ for this character [see Table S1 ('supplementary material')].

16. In dorsal and dorsolateral views, anterolateral constrict-
ion of the tegumen: absent (0), present (1). RC: 1. Fig. 10C.

17. In dorsal and dorsolateral views, lateral protrusion of
the tegumen: absent (0), present (1). RC: 0.333. Fig. 10H.

18. In lateral view, dorsal outline of tegumen: arched both
anteriorly and posteriorly (0), straight anteriorly and
arched posteriorly (1), straight both anteriorly and
posteriorly (2). RC: 0.166. Fig. 10E–G.

19. Hairpencil on dorsoposterior portion of tegumen:
absent (0), present (1). RC: 0.138. Fig. 10D. Note: The 'hairpencils' described in characters 19–22 consist of
tufts of extremely thin scales inserted in sockets.

20. Hairpencil on dorsomedial portion of tegumen: absent
(0), present (1). RC: 0.187. Fig. 10D, J.

21. Hairpencil on dorsolateral portion of tegumen: absent
(0), present (1). RC: 0.166. Fig. 10I.

22. Hairpencil on dorsomedial portion of tegumen: short
(0), long (1). RC: 0.181. Fig. 10D, J.

23. In lateral view, separation between pedunculum and
vinculum visible (0), pedunculum and vinculum seam-
lessly amalgamated, synscleritous (1). RC: 0.238.

24. In ventral view, saccus: shorter than the genital
icapule is wide (0), equal to, or longer than the genital
icapule is wide (1). RC: 0.458. Fig. 8A, B.

25. Heavier sclerotization of the dorsal edge of valva:
absent (0), present (1). Note: In Elymnias hypermnestra
(outgroup), the ventral edge of the valva is heavily
sclerotized. Uninformative.

26. Heavier sclerotization of the dorsal edge of valva: does
not extend to the valva tip (0), extends just to the valva
tip (1), encircles the valva tip (2). RC: 0.263. Fig. 8C, D,
F, H, I.

27. Heavily sclerotized valva tip: broader than shaft (0),
narrower than shaft (1). RC: 1. Fig. 8C, D.

28. Heavily sclerotized portion of the dorsal edge of valva:
with minute ribbed serrations (0), with even-shaped
spines (1), with uneven-shaped spines or projections (2).
RC: 1. Fig. 8C–I, K.

29. Even-shaped spines of the dorsal edge of valva: expand-
ing for up to half the valva length (0), expanding for
almost the entire valva length (1). RC: 1. Fig. 8E, I.

30. Uneven-shaped spines or projections of the dorsal edge
of valva: spread apart (0), clustered together (1). RC: 1.
Fig. 8H, K.

31. Groove at the articulating point between valva and
appendices angulares: absent (0), limited to the base of
valva (1), extended distally for c. one-third of the valva
length (2). RC: 0.254. Fig. 8G, H.

32. Thin, seta-bearing flange associated with the dorsal,
basal region of valva: absent (0), present membranous
(1), present sclerotized (2). RC: 1. Fig. 8E.

33. Finger-shaped protuberance at the dorsal, basal third of
valva: absent (0), present (1). RC: 1. Fig. 8E.

34. Basal, internal region of valva: similar in sclerotization
to distal region (0), conspicuously less sclerotized than
distal region (1). RC: 1. Fig. 8J.

35. Distal opening of phallus: dorsal (0), dorsolateral (1),
ventrolateral (2). RC: 0.183.

36. Thin, dorsal prong of phallus: absent (0), present (1).
RC: 1. Fig. 9A.

37. Peg-like setae on phallus shaft: absent (0), present (1).
RC: 1. Fig. 9B. Note: Although it is unclear whether
these minute integument structures are true setae, under
light microscopy their insertion points resemble setal
sockets.

38. Coecum penis: absent (0), present (1). RC: 0.4. Fig. 9A.

39. Lateral uncus wings, as they extend posteriorly into
uncus process: vanishing gradually (0), merging to form
a single, prominent dorsal keel (1), forming two dorso-
lateral keels (2), forming two dorsolateral humps (3).
RC: 0.25. Fig. 10G.

40. In ventral view, pocket of setae in the membrane
ventral to the uncus wings: absent (0), present (1). RC:
0.375. Fig. 9D.

41. In lateral view, posterior uncus process: straight at base
and bending at tip (0), evenly arched (1). RC: 0.133. Fig. 10J.

42. In dorsal view, posterior uncus process: mid region
similar to or narrower than base (0), mid region clearly
broader than base (1). RC: 1. Fig. 9C.

43. Distal portion of posterior uncus process: blunt (0),
sharp, sclerotized point (1), bifid (2). RC: 0.357.

44. Uncus ventrolateral microtrichia: absent (0), present
(1). RC: 1. Fig. 9E. Note: these are minute spines that
do not arise from sockets.

45. Length of setae at posterior uncus process: all setae
short (0), all setae medium length (1), some or all setae
long (2). RC: 0.206.


47. Gnathos, posterior expansion of proximal region:
absent (0), present (1). RC: 0.238. Fig. 10I, J.

48. Gnathos, dorsal expansion of proximal region: smooth
(0), spiny (1). Uninformative.

49. Gnathos, anterior expansion of proximal region: absent
(0), present (1). RC: 1. Fig. 10H.
50. Gnathos, distal region: clearly narrower than proximal region (0), similar to or wider than proximal region (1). RC: 0.222. Fig. 10E, G.
51. Gnathos, distal region: smooth (0), spiny (1). RC: 0.125. Fig. 10D, G.
52. Gnathos: adjoining lateral uncus wing and tegumen approximately equally (0), adjoining tegumen more extensively than lateral uncus wing (1), adjoining tegumen exclusively (2). RC: 0.222. Fig. 10E–G, I.

Female foreleg
53. Female foreleg, dorsal spines on tibia: absent (0), present (1). RC: 0.2.
54. Female foreleg, number of tarsomeres: five (0), one (1). RC: 1. Fig. 9F.
55. Female foreleg, ventral tarsomere spines: short (0), long (1). RC: 0.222. Fig. 9G, I.
56. Female foreleg, basal tarsomere (tm1): approximately the same length as the sum of the lengths of tarsomeres 2–5 (0), clearly longer than the sum of the lengths of tarsomeres 2–5 (1). RC: 0.416. Fig. 9H, J.
57. Female foreleg, third tarsomere (tm3): approximately 1.5× longer than tm4 (0), nearly 4× longer than tm4 (1). RC: 1. Fig. 9I. Note: In species that fit the pattern described by character state (1), tm2 is also long.

Female genitalia
58. Sclerotized plates between seventh abdominal sternite (Stn7) and sterigma: rounded (0), with two lateral pockets (1). RC: 0. Fig. 11B, C, E.
59. Degree of sclerotization of the intersegmental sac between Stn7 and sterigma: entirely membranous (0), with an anteromedial sclerotized region (1), with a postero-medial sclerotized region (2), entire sac appears sclerotized (3). RC: 0.363. Fig. 11C.
60. Orientation of the anterior ribs of the intersegmental sac between Stn7 and sterigma: transverse (0), longitudinal (1). RC: 0. Fig. 11B–D.
61. Lateral section of sterigma: fused to anterolateral edge of eighth tergite (Tg8) (0), not fused to anterolateral edge of Tg8 (1). RC: 0.333. Fig. 11B, D, E.
62. Anterior ribs of the intersegmental sac between Stn7 and sterigma: continuous (0), broken (1). RC: 0.333. Fig. 11B, D, E.
63. Lateral section of sterigma: fused to anterolateral edge of eighth tergite (Tg8) (0), not fused to anterolateral edge of Tg8 (1). RC: 0.333. Fig. 11H.
64. Lateral section of sterigma: broad (0), thin (1). Uninformative. Fig. 11B, H.
65. Anterior section of sterigma (‘lamella antevaginalis’): continuous (0), interrupted by a gap at midpoint (1), absent (2). RC: 0.254. Fig. 11A, B, D, E, G, H. Note: the terms ‘anterior section’ and ‘posterior section’ of the sterigma are used instead of the traditional ‘lamella antevaginalis’ and ‘postvaginalis’.
66. Anterior section of sterigma (‘lamella antevaginalis’), broad flap extended to cover ostium bursa: absent (0), present (1). RC: 0.166. Fig. 11F. Note: The ‘flap’ described here represents a broadening of the anterior section of the sterigma that protrudes ventrally and covers the ostium bursa (e.g. Brassolis sophorae, Fig. 11F). In some species (e.g. Otpoptera aorsa, Fig. 11B), the anterior section of the sterigma does not form a protruding ‘flap’ and does not cover the ostium bursa.
67. Lateral sections of sterigma: continuous with remainder of sterigma (0), separated from remainder of sterigma (1). RC: 1. Fig. 11C, F.
68. Posterior section of sterigma (‘lamella postvaginalis’): absent (0), present (1). RC: 0.4. Fig. 11C, F.
69. Inward projections of sterigma: absent (0), present (1). RC: 0.259. Fig. 11A, D, G.
70. Sclerotized plates that differ from the anterior section in the strict sense, as clearly shown in Caligo beltrao in Fig. 11A.
71. Antrum: entirely membranous (0), with a sclerotized spot (1), with a sclerotized ring (2), fully sclerotized (3). RC: 0.375.
72. Corpus bursa: oval (0), elongate (1). RC: 0.416. Fig. 12A, C.
73. Signa: absent (0), present (1). RC: 0.133.
74. Signa: parallel (0), divergent (1). RC: 1. Fig. 12A, D.
75. Corpus bursa: oval (0), elongate (1). RC: 0.416. Fig. 12B, D.
76. Antrum: entirely membranous (0), with a sclerotized layer (1), fully sclerotized (3). RC: 0.375.
77. Anterior edge of the papilla anales: broad (0), narrower (1). RC: 0.375.
78. Posterior edge of the papilla anales: broad (0), narrower (1). RC: 0.375.
79. Inward projections of papilla anales: included in the papilla anales (0), protruding (1). RC: 0.166.
80. In lateral view, anterior contour of papilla anales: broad (0), narrow (1). RC: 0.166.