JOURNAL OF
THE LEPIDOPTERISTS' SOCIETY

Volume 56 2002 Number 4

Journal of the Lepidopterists' Society
56(4), 2002, 193–198

EXPERIENCE-RELATED CHANGES IN THE BRAIN OF *AGRAULIS VANILLAE* (L.) (NYMPHALIDAE)

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ABSTRACT. In the brain of *Agraulis vanillae*, the size of the brain regions involved in the processing of olfactory information was found to depend on the butterfly's experience. Butterflies collected in nature have olfactory glomeruli and mushroom body calyxes of larger relative size than do butterflies reared and kept in the laboratory in isolation from normal environmental stimuli. No size difference was found in the optic lobes or the central body in either males or females.

Additional key words: mushroom body, neuropil, olfactory lobes.

The brain of an insect is the principal associative center of the body. It receives sensory information from a variety of sense organs, processes it and controls all functions of the organism, including complex forms of behavior. Several regions of the brain differing in morphology and function are recognized and referred to as neuropils (Fig. 1). Neuropils are the centers of the regions and are formed by a complex of densely packed nerve fibers. The neurons, which compose a region, lie at its periphery. On histological sections of the brain neuropils appear as much denser, darker than the rest of the brain areas.

The neuropils of particular significance in the processing of information in insect brains are the mushroom bodies and antennal lobes. Mushroom bodies receive signals from different sense organs and experiments on *Drosophila* (Heisenberg et al. 1985, Han et al. 1992) and *Apis* (Erber et al. 1980, Menzel et al. 1974, Hammer & Menzel 1998) suggest that they are implicated in olfactory memory formation. They are composed of three types of cells: cells that direct signals to the mushroom bodies, cells that deliver signals from the mushroom bodies to other parts of the nervous system, and the intrinsic cells (Kenyon cells) that connect the first two types between themselves. The Kenyon cells occupy the area around the mushroom body neuropil.

All information from the organs of smell (olfactory organs) is received in another brain region: antennal lobes, which are critically important in the delivery of olfactory information to the mushroom bodies. Antennal lobes are composed of a series of neuropils-olfactory glomeruli, which receive and process olfactory signals from the antennae.

Insect species with complex and flexible behavior possess well-developed mushroom bodies and antennal lobes, and larger insects have larger brains and more complex histological brain structure and generally exhibit greater complexity of behavior (Goossen 1949, Bernstein & Bernstein 1969). The largest mushroom bodies (relative to the rest of the brain) are found in social Hymenoptera. The morphological plas-

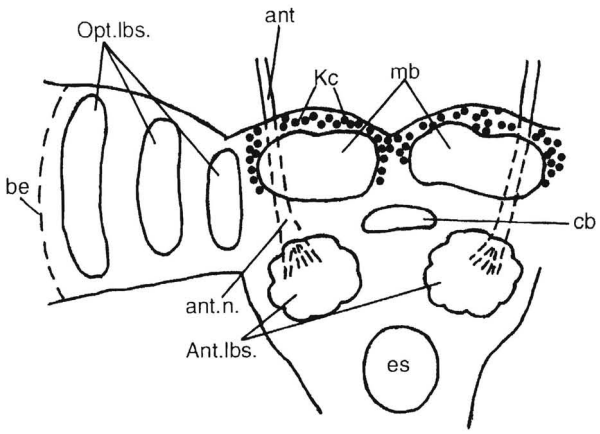

FIG. 1. Diagram of the butterfly brain showing the most important neuropils. **Opt.lbs.**—optic lobes, **Ant.lbs.**—antennal lobes, **mb**—mushroom bodies, **Kc**—Kenyon cells, **cb**—central body, **be**—back of the eye, **ant.n.**—antennal nerve, **ant.**—antenna, **es**—esophagus.

ticity of these brain structures has been demonstrated in bees (Withers et al. 1993, Winnington et al. 1996, Robinson 1998) and ants (Gronenberg et al. 1996). Mushroom bodies increase in size when these insects begin to perform complex and behaviorally more demanding tasks. Neuropil growth related to behavioral changes has also been observed in non-social insects, such as fruit flies and rove beetles (Bieber & Fuldner 1979, Technau 1984, Heisenberg et al. 1995). This growth was found to represent the further arborization and proliferation of existing brain cells, and not the production of new neurons.

Flexibility of behavior and learning have been demonstrated in different species of Lepidoptera (Swihart & Swihart 1970, Papaj 1986, Weiss 1995, 1997, Hartlieb 1996, Fan et al. 1997). Butterflies and moths have well-developed mushroom bodies (Ali 1974, Sivinsky 1989), and large antennal lobes (Matsumoto & Hildebrand 1981). Both olfactory and visual learning have been described in *Agraulis vanillae* (Weiss 1995, Kroutov et al. 1999).

Here we studied brain morphology in two groups of *Agraulis*. One group comprised butterflies collected in nature ("experienced" group) and the other group was reared and maintained in the laboratory in isolation from normal environmental stimuli ("naïve" group). We investigated the hypotheses that the sizes of brain structures involved in information processing and learning vary according to the individual experience of butterflies, and that such structures should be larger in butterflies exposed to various environmental stimuli than in butterflies deprived of those.

MATERIALS AND METHODS

Adults and larvae of *Agraulis vanillae* were collected in Gainesville, Florida. All butterflies used in experiments were collected during the 3–4 day period of the abundance peak of the species. Larvae were reared in the laboratory on their natural host-plant *Passiflora incarnata* (L.), picked in the same area where the larvae were found. Laboratory reared adults spent 48 hours after eclosion in 25 × 25 × 25 cm screen cages. The laboratory conditions were 25°C, 65% relative humidity, L:D 16:8 h. Butterflies were fed a 25% sugar solution.

For the preparation of the histological specimens butterfly heads were removed and fixed in Bouin's fixative, prepared 24 hours prior to usage, for 2 days. They were then rinsed in 70% ethanol and embedded in paraffin. Heads of 16 reared males, 10 reared females, 17 wild males and 22 wild females were sectioned. The frontal microtome sections were 10 µm thick and were stained with hematoxylin-eosin.

Volumetric analysis was performed with an AIS/C image analysis system (Imaging Research, Inc.) interfaced to a Zeiss Axiophot microscope via a Dage 72 CCD camera. The following areas were measured in selected spaced sections on both sides of the brain: whole brain, antennal lobes, olfactory glomeruli, optic lobes, central body, mushroom body calyces, and the regions occupied by Kenyon cells. When areas were measured, this was done without awareness of the group to which that individual belonged. The volume of a brain structure was calculated using the formula

$$\mathrm{Vol}_{(\mathrm{object})} = \sum_{i=1}^{n} A_i \times t \times N$$

where **n** is the number of sections on which measurements were made, **A** is the area of a measured section, **t** is the distance between adjacent sections (e.g., section thickness), and **N** is the number of sections represented by the section $\mathbf{A_i}$. Between 10 and 20 evenly spaced sections were used to determine the volume of each region. This corresponded to 50–100% of all the sections containing each measured structure. The relative volume of each brain structure was calculated as a percentage of the volume of the whole brain.

For statistical analysis of the data a fixed effects linear model (ANOVA) was fit with PROC GLM (SAS v.8). That is, size was modeled as a function of the fixed effects 'brain region', 'butterfly gender' and 'butterfly group' ("experienced", "naïve" and "control"). All relevant assumptions such as constant variance and normality were formally assessed. Due to the large number of multiple Bonferroni comparisons we tested at the 0.01 level of significance throughout.

To exclude the possible effect of age on the changes in *Agraulis* brain, a control group of 10 males and 10 females, reared in the laboratory was kept in cages for 20–25 days after eclosion under the same conditions as described for the experimental group. The heads of

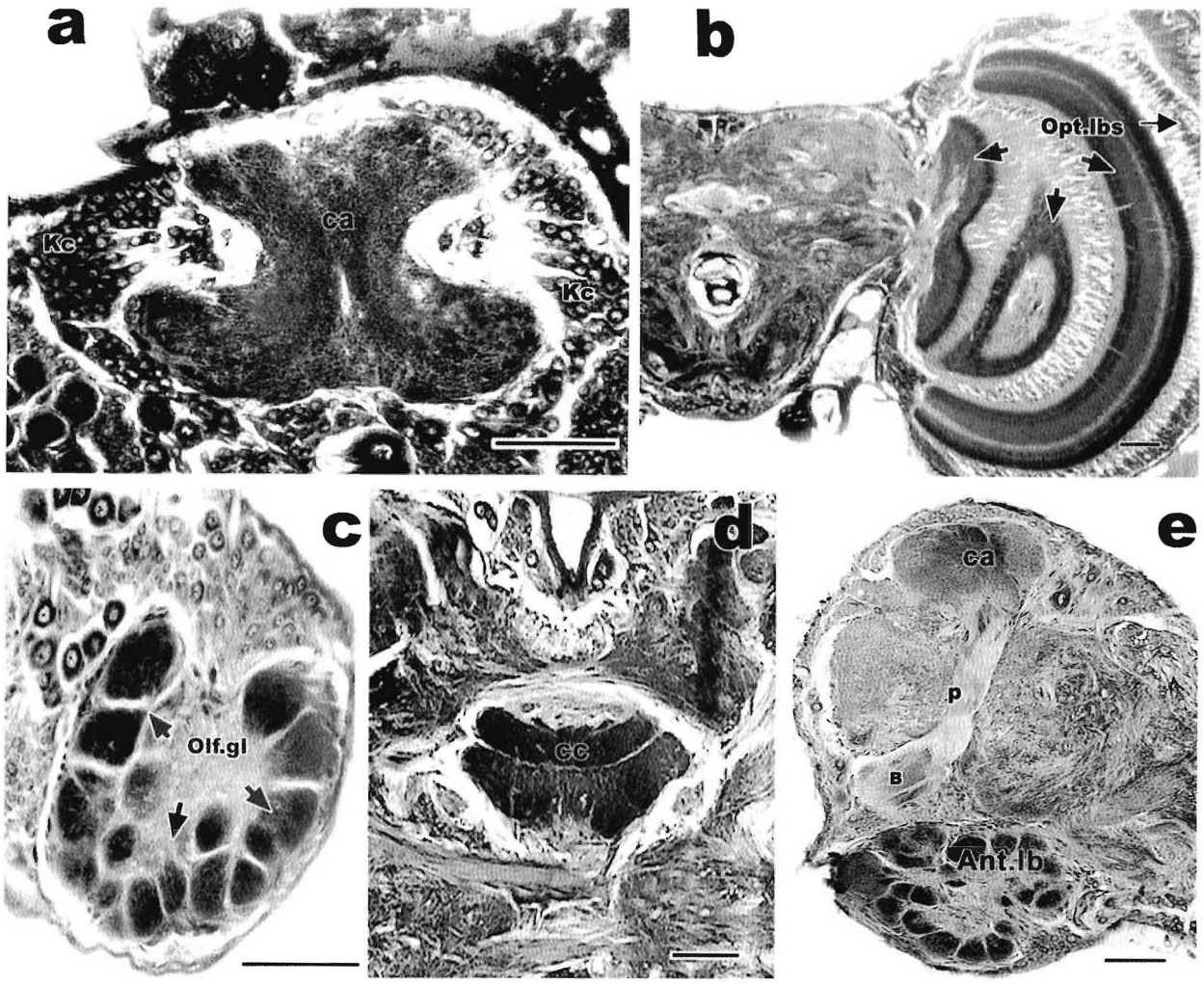

FIG. 2. Sections of the brain of *Agraulis vanillae*. **a:** mushroom body calyx (ca) and Kenyon cells (Kc); **b:** optic lobes (Opt.lbs); **c:** antennal lobe with glomeruli (Olf.gl.); **d:** central body (cc); **e:** mushroom body—calyx (ca), pedunculus (p), β-lobe (B) and antennal lobe (Ant.lb.). **a,b,c,d**—frontal sections, **e**—sagittal section. Scale bars—100 μm.

control butterflies were sectioned, sections stained and brains measured as described above.

RESULTS

Figure 2 shows the sections of the measured brain structures in *Agraulis vanillae*. Most of the regions exhibit clearly defined boundaries. Because of the absence of a clear boundary between the mushroom body's pedunculus and lobes, and the surrounding neuropil, attributable to the staining method chosen for the study, only mushroom body calyces were measured.

Whole brain volume of *Agraulis* showed no significant variation according to group (Fig. 3). There was found to be a significant interaction of gender *group* brain region ($p < 0.0001$). Multiple pairwise comparisons revealed the following patterns: "experienced" individuals of both sexes exhibited significantly larger mushroom bodies and olfactory glomeruli than did "naïve" or "control" individuals (Fig. 4; Table 1). The relative volume of mushroom body calyces in "experienced" butterflies was greater than in "naïve" ones by 36% in males, and by 38% in females. Olfactory glomeruli were larger in "experienced" *Agraulis* by 48% in males, and 24% in females.

The Kenyon cells region and antennal lobes showed mixed outcomes. Within the Kenyon cells region, there were no significant differences in volume among the male groups, but "experienced" females exhibited smaller volumes than did "controls". For the antennal lobes, "experienced" males have larger volumes than do "naïve" males. There were no differences among the female groups. The central body and optic lobe regions exhibited no significant difference for any pairwise comparison.

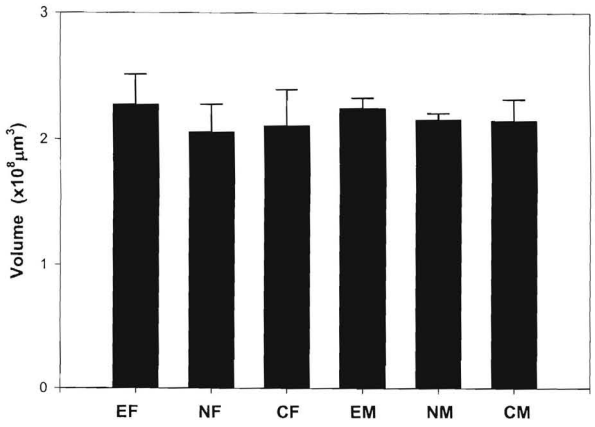

FIG. 3. Whole brain volume in *Agraulis vanillae*. **EF**—"experienced" females, **NF**—"naïve" females, **CF**—"control" females, **EM**—"experienced" males, **NM**—"naïve" males, **CM**—"control" males.

For most brain regions assessed, there was no significant difference between male and female volumes. However, the antennal lobes exhibited the following pattern: "naïve" and "control" females have larger antennal lobes than their male counterparts ($p < 0.0001$ in each case), but "experienced" females do not differ significantly from "experienced" males.

For all brain regions, the "naïve" and "control" groups exhibited no significant differences in volume, within males or females.

DISCUSSION

The results of our research demonstrate that in *Agraulis* differences in experience are correlated with changes in the volume of several of its brain regions involved in sensory information processing and memory formation. During their adult stage (2–4 weeks), *Agraulis vanillae* butterflies must perform various activities, the success of which can be enhanced by learning. Location of feeding sites with flowers that offer sufficient nectar reward, and recognition of potential danger are of importance to both sexes. Female *Agraulis* need to find suitable host-plants on which to lay eggs. This involves not only recognition of the proper plant amongst a variety of other plants, but also memory of the location of the host *Passiflora* patch, because butterflies of this species utilize vast habitats and linger at one spot for no longer than is necessary to complete either feeding or egg-laying. Males, in turn, need to locate the host-plant area to encounter females and mate.

Detailed analysis of the captivity conditions and their specific influence on *Agraulis*'s experience, learning and associated morphological changes in its brain was not attempted. However, it seems evident that captive laboratory-reared butterflies would have a greatly reduced range of external stimuli, being deprived of space, visual stimuli, contacts with host-plant, flowers and sex partners. It was also impossible to determine precisely the age of "experienced" butterflies, collected in nature. But because we collected them in a period of 10–14 days after the beginning of their abundance peak, we can estimate all collected butterflies to be of approximately the same age.

Generally, measured brain structures were larger (relative to the volume of the whole brain) in "experienced" butterflies. But no difference in the relative volume of optic lobes and central body was recorded between two groups of *Agraulis*. The most dramatic increases in relative volume occurred in the mushroom bodies and olfactory glomeruli, whereas no size difference in optic lobes between "experienced" and "naïve" butterflies was observed. Therefore, olfactory stimuli may be of primary importance in driving the structural changes in the *Agraulis* brain. Although butterflies reputedly rely heavily on visual stimuli (Swihart 1970, Silberglied 1979, 1984), and *Agraulis* is capable of visual as well as olfactory learning (Weiss 1995), their optic lobes only pass visual information to the central brain, where processing and integration of this information takes place. Therefore such a result is predictable.

The relative decrease in volume of the Kenyon cells region is rather hard to explain. However, because there was no change in the whole brain volume, this region's relative decrease could represent an actual compression of the Kenyon cell clusters by the ex-

TABLE 1. Relative volumes of brain regions as percentage of the whole brain volume in *Agraulis vanillae*. Within each box, different small case letters indicate significant differences, whereas the same letters indicate no difference.

	Mushroom body calyx	Olfactory glomeruli	Kenyon cells region	Antennal lobes	Central body	Optic Lobes
"Experienced" males	2.01 ± 0.08 a	1.45 ± 0.09 a	0.69 ± 0.07 a	3.74 ± 0.35 b	0.61 ± 0.09 a	64.4 ± 3.0 a
"Naïve" males	1.48 ± 0.05 b	0.98 ± 0.05 b	0.75 ± 0.05 a	3.41 ± 0.10 a	0.68 ± 0.04 a	69.7 ± 8.3 a
"Control" males	1.38 ± 0.07 b	0.98 ± 0.09 b	0.72 ± 0.04 a	3.50 ± 0.10 ab	0.66 ± 0.05 a	65.1 ± 2.3 a
"Experienced" females	2.18 ± 0.10 a	1.44 ± 0.08 a	0.56 ± 0.03 a	3.90 ± 0.10 a	0.63 ± 0.07 a	61.8 ± 2.6 a
"Naïve" females	1.58 ± 0.05 b	1.16 ± 0.05 b	0.73 ± 0.05 ab	4.00 ± 0.20 a	0.64 ± 0.03 a	62.4 ± 2.2 a
"Control" females	1.58 ± 0.12 b	1.11 ± 0.04 b	0.84 ± 0.08 b	4.00 ± 0.12 a	0.66 ± 0.02 a	61.1 ± 1.9 a

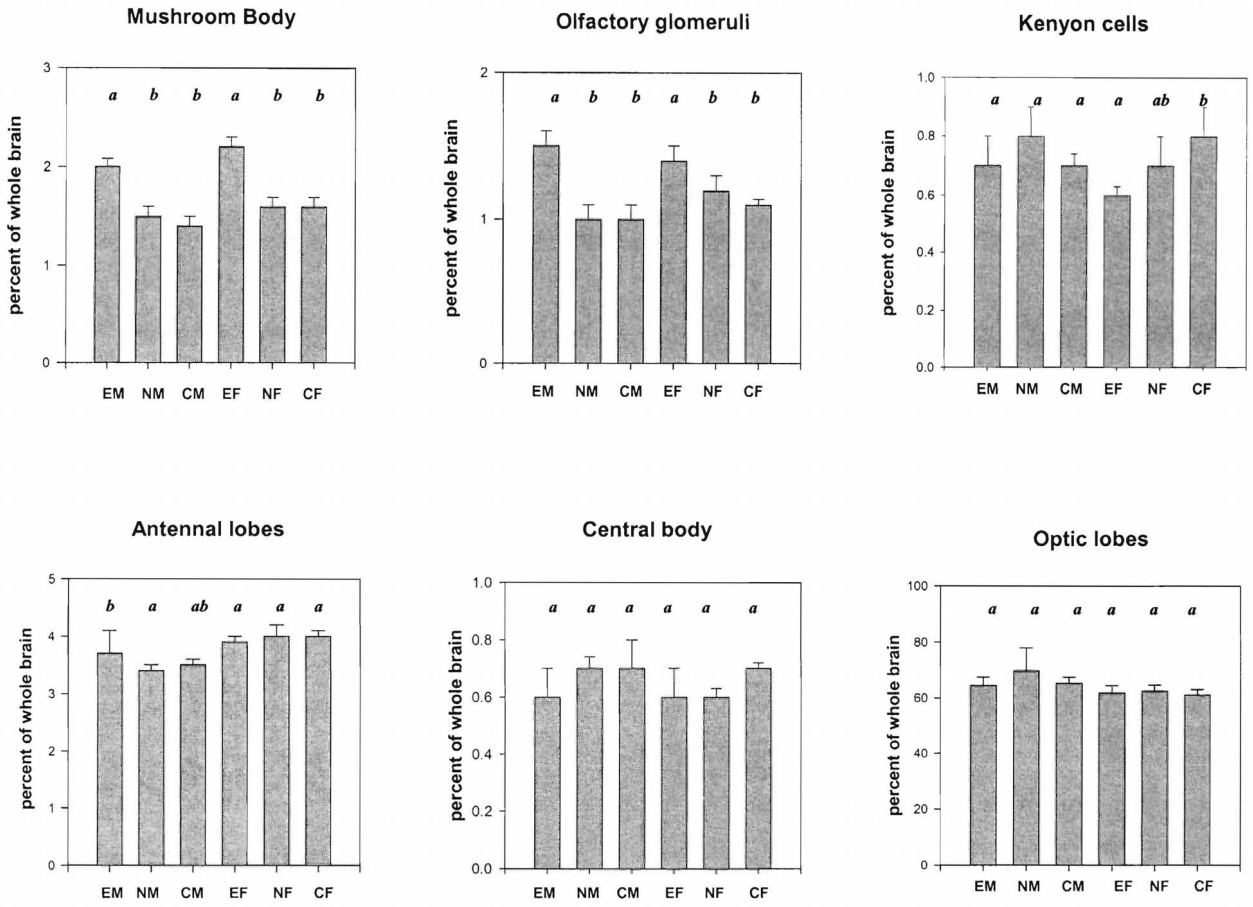

FIG. 4. Relative volumes of brain regions as percentage of the whole brain volume in *Agraulis vanillae*. **EM**—"experienced" males, **NM**—"naïve" males, **CM**—"control" males. **EF**—"experienced" females, **NF**—"naïve" females, **CF**—"control" females. Different small case letters indicate significant differences, whereas the same letters indicate no difference for each sex.

panding mushroom body calyces. This problem could be addressed by more detailed experimental analysis, for example assessment of cell packing density.

The data presented here are similar in many ways to those of studies in other species of insects, which measured the size differences in brain regions caused by different experience and behavioral repertoire. As in the present study, an increase in relative volume of mushroom bodies, and decrease in relative volume of the Kenyon cells region were reported for ants (Gronenberg et al. 1996) and bees (Withers et al. 1993). Also, there was no increase in the relative volume of the optic lobes in either of these insects. Olfactory glomerular volume was found to differ between 1-day-old and nurse bees (larger in nurses), but the increase was not maintained in foragers. For rove beetles mushroom body volume increase and no changes in optic lobe volume were recorded (Bieber & Fuldner 1979).

The sexual dimorphism found in the reorganization of some brain structures in *Agraulis*, namely the difference in olfactory glomeruli volume between "naïve" and "experienced" males being twice as great as that in females, can perhaps be explained by the differences in behavior of males and females. Females need to locate host-plants and determine their suitability for oviposition, and males need to search for females and recognize proper chemical cues from suitable partners. Thus, each sex may rely on different environmental stimuli. The change in the intensity of these stimuli may effect butterflies of different sexes differently, and cause the observed dissimilarity in the brain reconstruction. This dimorphism corresponds with our earlier findings in *Agraulis* learning (Kroutov et al. 1999), where different learning capability was recorded for the two sexes.

Measurements in the control group show that morphological changes in the brain of *Agraulis vanillae* are not age-related, but experience-related, since the relative volumes of the studied brain structures in 2-day ("naïve") and 20–25-day ("control") butterflies were not significantly different. These changes occur in only

a few brain compartments that are noted for their role in information processing and learning in insects. This further supports the hypothesis that growth of these brain regions is related to learning experience and behavioral complexity of a butterfly.

Further experiments involving the manipulation of various elements of the environment may lead to a better understanding of the exact relation between particular types of information, how they are processed, and changes they cause in the brain of *Agraulis vanillae*. It would be especially interesting to analyze the specific effects of various environmental "deprivations" and, inverted, the effect of additional stimuli on the changes in *Agraulis'* brain structures.

ACKNOWLEDGMENTS

We thank Dr. M. S. Mayer for his helpful comments on our paper, Scott Whittaker for his help with the preparation of the illustrations and Galin Jones for helping us with the statistical analysis of our data.

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Received for publication 6 March 2001, revised and accepted 21 February 2002.

Journal of the Lepidopterists' Society
56(4), 2002, 199–215

PHYLOGENETIC ANALYSIS AND REVIEW OF *PANACEA* AND *BATESIA* BUTTERFLIES (NYMPHALIDAE)

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ABSTRACT. Phylogenetic analysis of 53 morphological characters for five species of *Panacea* and *Batesia hypochlora* supports the separation of the two genera and showed that the monotypic genus *Batesia* is basal to *Panacea*. Male genitalia were uniform within *Panacea* and characters informative for phylogeny reconstruction were restricted to wing coloration. Illustrations of adults and genitalia, a brief diagnosis, and distributions are provided for each species.

Additional key words: *prola*, *procilla*, *regina*, *divalis*, *bleuzeni*, *chalcothea*, *lysimache*, *bella*, *hypochlora*, *Caryodendron*, Euphorbiaceae.

By possessing distasteful wings or body fluids, brightly colored butterflies are generally avoided by many vertebrate predators in nature. This phenomenon is particularly well known in various genera of Nymphalidae (e.g., *Acraea*, *Heliconius*, many Danainae and Ithomiinae), Papilionidae (e.g., *Battus*, *Parides*) and Pieridae (e.g., *Mylothris*, *Delias*, *Appias*, *Perrhybris*, *Itaballia*) among others (see Poulton 1908, Sywnnerton 1919, Carpenter 1942, Fisher 1958, Chai 1986). Nevertheless, a great many of these same butterflies are eagerly sought after and prized by a different group of predators, human collectors. Although collector value may provide a metric of how garishly colored a particular butterfly might be, it is often a poor measure of how well we understand that species. Therefore, when considering biological or evolutionary understanding of particular butterflies, it is likely that drab ones are equally as well known as those that are brightly colored. Although well represented in museum collections, and available as virtual specimens on the internet, nymphalid butterflies in the genera *Batesia* Felder and Felder, 1862 and *Panacea* Godman and Salvin, 1883 are good examples of this phenomenon.

The Neotropical genus *Batesia* occurs from central Colombia to eastern Ecuador, southeast Peru, western Brazil, and likely into northeast Bolivia; effectively an upper Amazonian distribution. On the other hand, members of *Panacea* are found from Costa Rica south across Venezuela and the Guianas, throughout the Amazon basin, and into Bolivia.

Both *Batesia* and *Panacea* were originally described as monotypic genera, but only *Batesia* with its single species, *hypochlora* Felder and Felder, 1862 has remained so. The history of *Panacea* is somewhat convoluted. *Panacea prola* (Doubleday, 1848) was initially designated the type species of *Pandora* Doubleday, 1848—a name used previously for different insect genera by at least seven different authors, and thus, an invalid homonym (see Hemming 1967). In an attempt to settle this quandary, Kirby (1871) transferred all species of *Pandora* to *Batesia*. Godman and Salvin (1883), however, felt that all species formerly in *Pandora* warranted separation from *Batesia*, and erected the genus *Panacea* to accommodate them—thus providing a panacea to the *Pandora* problem. Eight species have been described in *Panacea*—*P. prola*; *P. procilla* (Hewitson, 1852); *P. regina* (Bates, 1864); *P. divalis* (Bates, 1868); *P. chalcothea* (Bates, 1868); *P. lysimache* Godman and Salvin, 1883; *P. bleuzeni* Plantrou and Attal, 1986; and *P. bella* D'Abrera, 1987, not all that are currently regarded as valid species (see synonymies below).

The vicissitudes of nomenclature aside, nearly all natural history studies suggest that *Batesia* and *Panacea* are distinct, but closely related genera. At present they are classified in the Biblidini along with *Hamadryas*, *Ectima*, *Eunica*, *Myscelia*, *Dynamine*, *Colobura* and other genera (Godman & Salvin 1883, Seitz 1916, Ackery 1984, Harvey 1991).

Recent observations indicate that *Batesia* and *Panacea* share *Caryodendron* spp. (Euphorbiaceae) as host plants, and that their immature stages are very similar (DeVries et al. 1999). The correspondence of immature biology, classification, and the fact that these genera have never been assessed using cladistic methods led us to ask whether *B. hypochlora* was separate from *Panacea*, or if it represented a derived species within *Panacea*. Accordingly, this study tests both hypotheses through phylogenetic analysis of five species of *Panacea* plus *Batesia hypochlora*. Based on adult morphology we show that *Batesia hypochlora* is basal to *Panacea*, and that together they form a monophyletic group. We then present characters to aid in species identification, and provide notes relevant to future work on their taxonomy and natural history.

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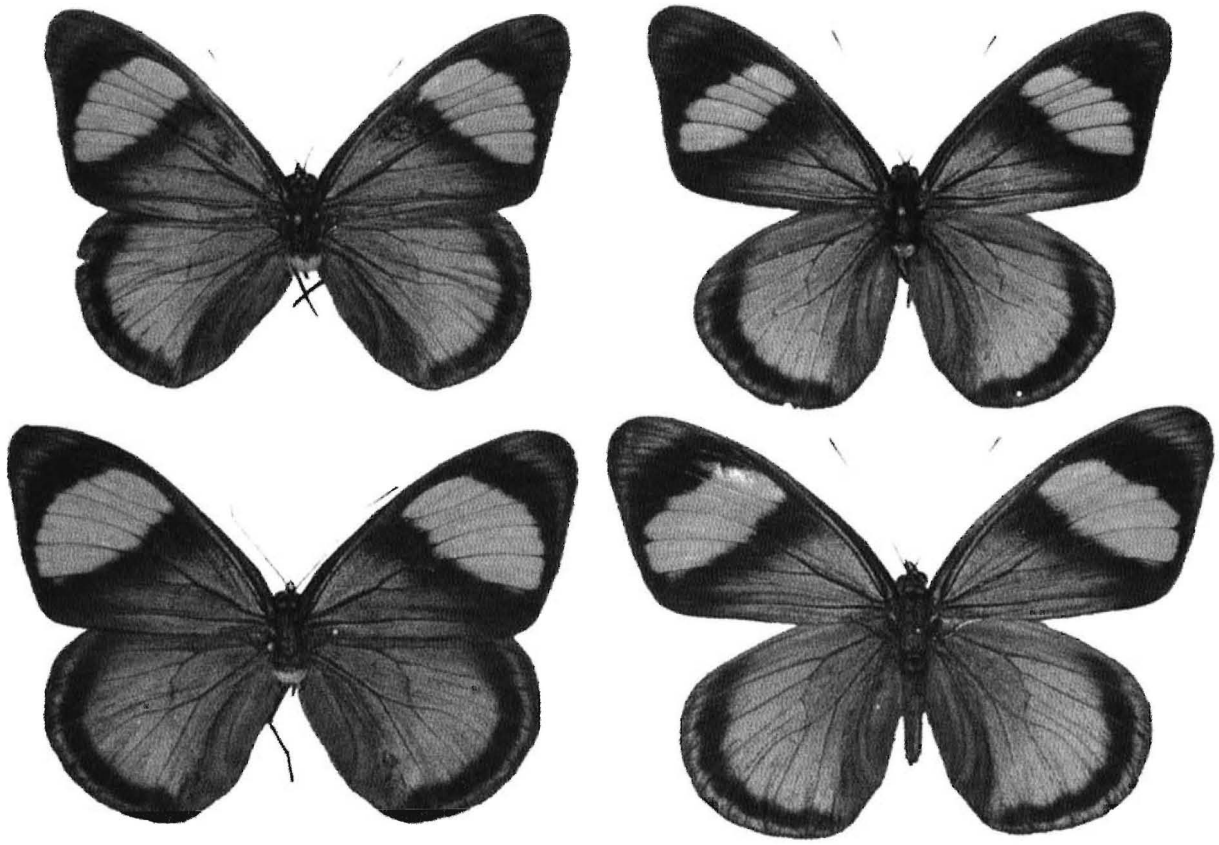

FIG. 1. *Batesia hypochlora*, dorsal. Top row, males; bottom row, females. Left column, Garza Cocha, Ecuador; right column, Rondonia, Brazil.

MATERIALS AND METHODS

Species studied. Excepting *P. chalcothea* (see identification section below), our phylogenetic analysis included all valid species of *Panacea* (*P. prola*, *P. procilla*, *P. regina*, *P. divalis*, and *P. bleuzeni*) and *Batesia hypochlora* (Figs. 1–10).

To assess intra-specific variation in wing pattern and genitalia, we examined specimens from five distinct localities. Abundant material from a single site in eastern Ecuador (*P. prola*, n = 57; *P. divalis*, n = 55; *P. regina*, n = 43; and *B. hypochlora*, n = 24) allowed us to evaluate morphological and phenotypic variation within a single population (see DeVries & Walla 2001 for site description). Whenever possible individuals from different localities were dissected to evaluate morphological variation in the genitalia. Although a small number of specimens were available of *P. procilla* (n = 4) and *P. bleuzeni* (n = 2), these species are phenotypically distinctive from other *Panacea* and characters could be scored with confidence. For *P. bleuzeni*, one specimen of each sex was used to score genitalia characters directly, but wing and body characters were scored using the description of Plantrou and Attal (1986), the illustrations in D'Abrera (1987:487, as *P. bella*) and photographs from the private collection of G. Attal. Characters 22 and 23 were scored as "missing" for *P. bleuzeni* due to lack of material. Table 1 lists the examined taxa, number of dissected individuals, and locality data.

We used *Biblis hyperia* (Cramer, 1780) and *Hamadryas arinome* (Lucas, 1853), *H. amphinome* (Linnaeus, 1767), *H. laodamia* (Cramer, 1777), and *H. feronia* (Linnaeus, 1758) as outgroup taxa for phylogenetic analysis. Based on larval and adult morphology, and host plant use (Euphorbiaceae) these taxa are considered closely related to *Batesia* and *Panacea* (Seitz 1916, Ackery 1984, Harvey 1991).

Preparation of material. Genitalia were prepared with a standard treatment of 10% potassium hydroxide, examined with a stereomicroscope, and subsequently stored in glycerol. Illustrations are given in Figs. 11–13.

Characters and terminology. Our character matrix

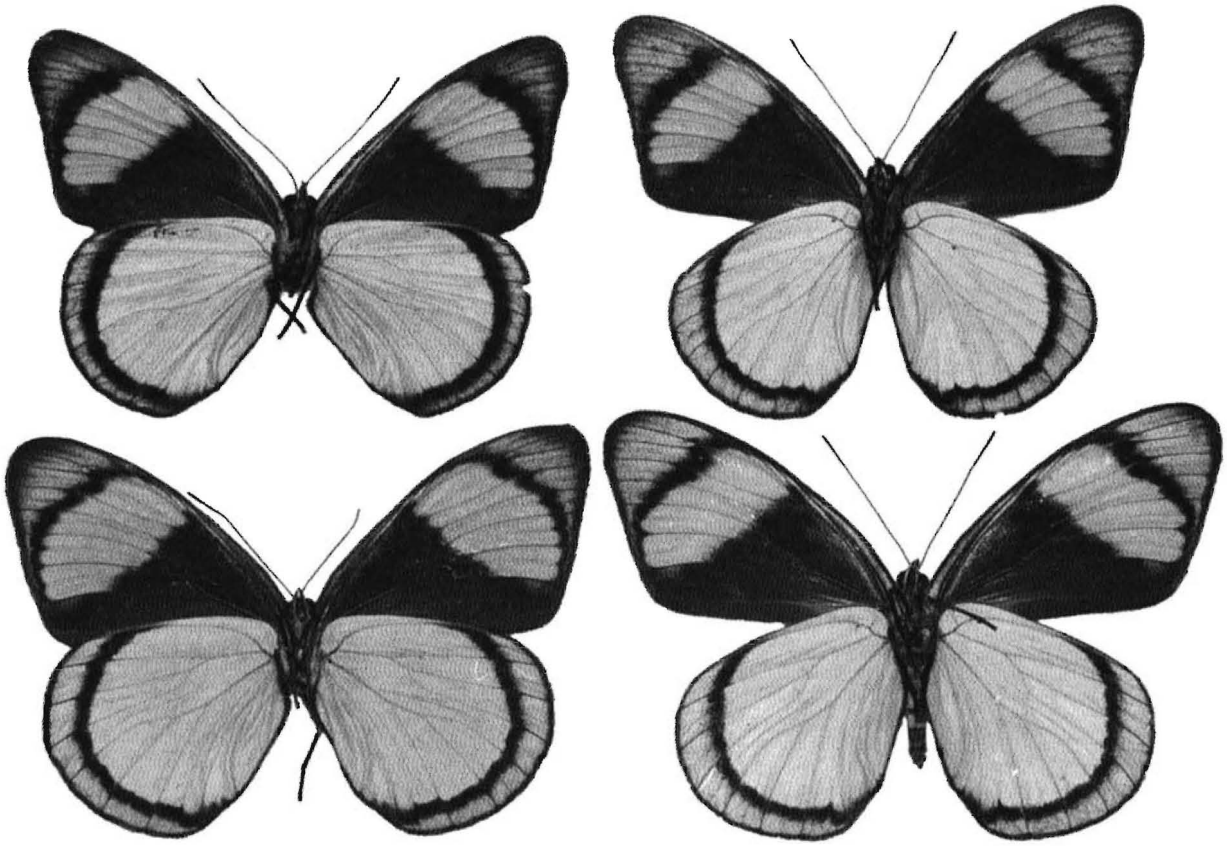

FIG. 2. *Batesia hypochlora*, ventral. Left column, Garza Cocha, Ecuador; right column, Rondonia, Brazil.

includes 53 characters (43 binary and 10 multistate), of which 24 were derived from males (23 from genitalia, one from wing coloration), 7 derived from females (6 from genitalia and one from wing coloration), and 22 from both sexes (16 from wing patterns, four from venation, one from forelegs and one from body scales).

Terminology for adult external morphology follows Scoble (1992). Terminology for male and female genitalia follows Klots (1970) except for the use of hypandrium and ramus, which follow the definitions in the glossary of Tuxen (1970) and Jenkins (1986, 1987, 1990). We use hypandrium to mean "a male subgenital plate," and ramus as "lateral or ventro-lateral process of male eighth sternite, directed posteriorly" (see glossary in Tuxen 1970; Jenkins 1983, 1986). In character 10 we follow D'Abrera (1987) where a "complete ocellus" consists of a spot surrounded by a round ring (e.g., *P. procilla*, Fig. 6), and an "incomplete ocellus" is a spot without a round outer ring (e.g., *P. bleuzeni*, Fig. 7).

Phylogenetic analysis. We used a heuristic search in PAUP 3.1 (Swofford 1993) with all characters given equal weight, multi-state characters unordered, polymorphic characters treated as exhibiting both states, and the search used a TBR branch swapping routine. Following analysis, *Biblis hyperia* was used to root the tree. Branch support was estimated by 500 bootstrap replicates, and we used MacClade 3.01 (Maddison & Maddison 1992) to identify character changes along the branches of the tree. The character list and data matrix are in Appendix 1 and 2.

RESULTS

Phylogeny

Our analysis indicates that *Panacea* and *Batesia* are monophyletic, sister taxa. The single most parsimonious tree (tree length = 79, CI = 0.82, RI = 0.88) suggests that *Batesia hypochlora* is a sister species to *Panacea*, a relationship supported by four characters (Fig. 14; Table 2, clade 1). We found 11 autapomorphies for *B. hypochlora* (Table 2, clade 2), and nine characters that justify the monophyly of *Panacea* (Table 2, clade 3). Our analysis also showed that all members of *Panacea* are morphologically similar, but they differ strongly from *Batesia hypochlora*.

Among *Panacea* the genital morphology was notably conservative, and characters providing the basis for inferring species relationships were derived mostly from wing morphology. Only one male genital character (hy-

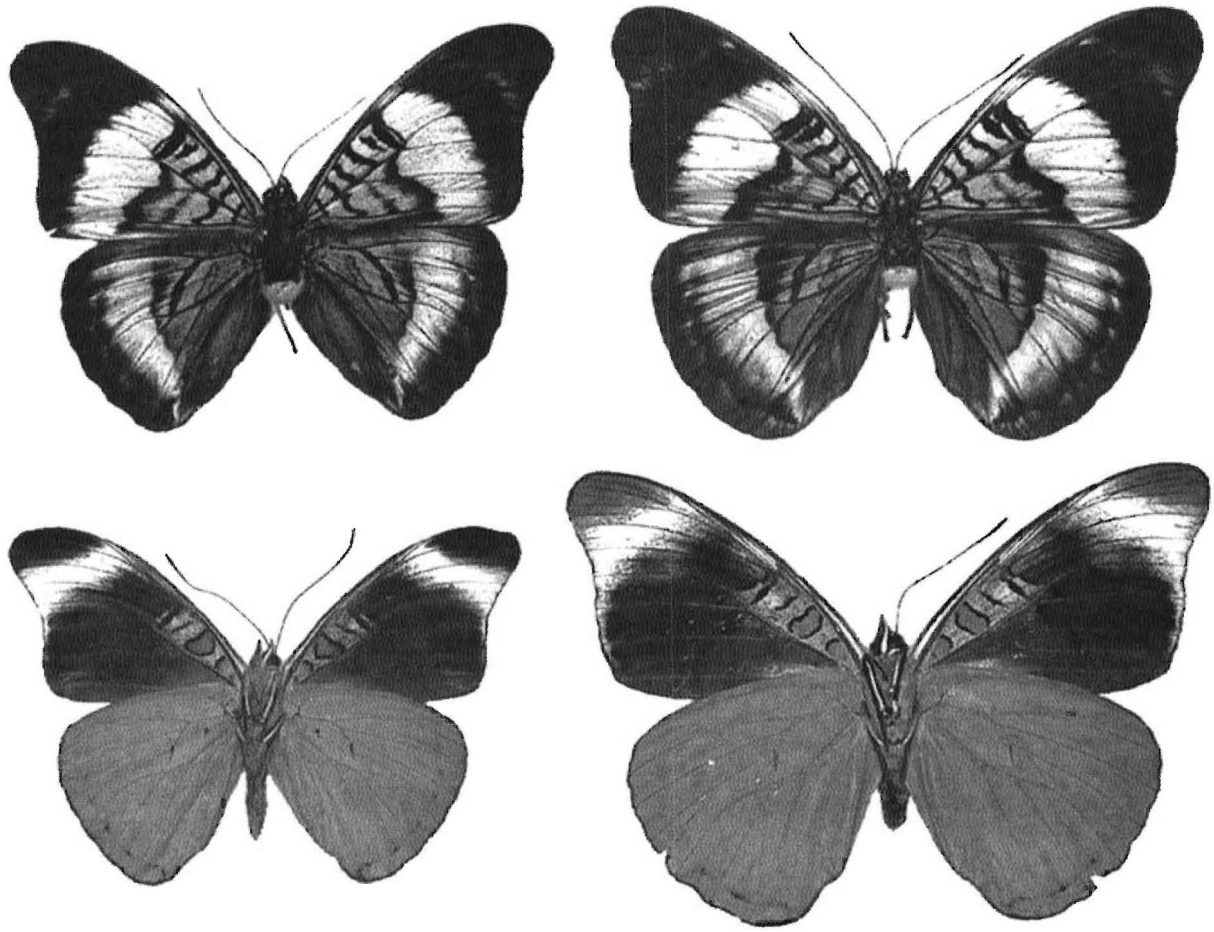

FIG. 3. *Panacea prola*, dorsal and ventral. Top row, left, male; right, female. Bottom row, left male; right, female. All from Garza Cocha, Ecuador.

pandrium, character 28) could be used to distinguish among *Panacea* species. However, as it represents an autapomorphy for *P. divalis*, character 28 was uninformative for establishing phylogenetic relationships within *Panacea*. The grouping of *P. regina*, *P. divalis*, *P. bleuzeni* and *P. procilla* was supported by seven characters, all derived from wing pattern morphology (Table 2, clade 4). One character justified grouping *P. divalis*, *P. bleuzeni* and *P. procilla* (Table 2, clade 5) and a single character grouped *P. bleuzeni* and *P. procilla* (Table 2, clade 6).

Identification and Taxonomy

Here we provide synonymies, characters for identification of the study taxa, approximate geographical distributions, and comments on phenotypic variation of the species included in our analysis. For completeness, we also provide taxonomic notes on *P. chalcothea*, although we did not examine this taxon directly.

Batesia Felder and Felder, 1862

Batesia Felder and Felder, 1862. Wien. ent. Monats. 6:112.

Batesia hypochlora Felder and Felder, 1862 (Figs. 1, 2, 11, 13)

Batesia hypochlora Felder and Felder, 1862. Wien. ent. Monats. 6:113

Batesia hypochlora hypoxantha Salvin and Godman, 1868. Ann. Mag. Nat. Hist. (4)2:147

Batesia hypochlora hemichrysa Salvin and Godman, 1868. Ann. Mag. Nat. Hist. (4)2:147

Batesia hypochlora chrysocantha Fruhstorfer, 1915. Soc. ent. 30(12):66

Batesia hypochlora f. *intermedia* Michael, 1931. Ent. Zeit. 44(20):309–312

Species characters. Forewing dorsal surface dark iridescent blue from basal to submedial areas, a prominent postmedial red band surrounded by black, apex iridescent blue. Hindwing dorsal surface mostly iridescent blue, with a postmedial black band and an iridescent blue marginal band from apex to tornus. Forewing ventral surface dark brown from basal to submedial areas and tornus, postmedial red band surrounded by brown, subapex yellow. Hind-

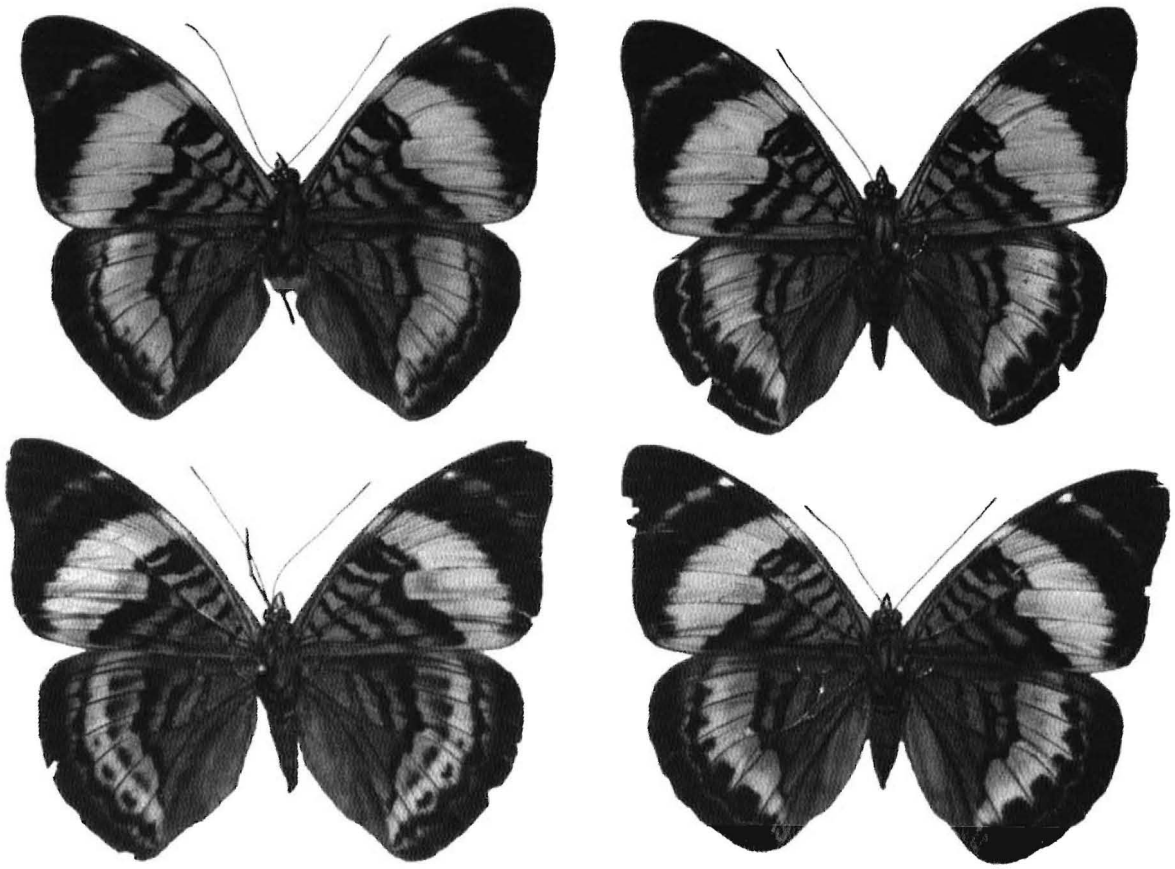

FIG. 4. *Panacea regina*, dorsal. Top row, male; bottom row, female. All from Garza Cocha, Ecuador.

wing ventral surface chalky yellow with a distinct black postmedial band and yellow marginal band from apex to tornus.

Distribution. Western Amazonas, Brazil; Ecuador, Peru (Seitz 1916, D'Abrera 1987, Austin & Emmel 1990, Robbins et al. 1996).

Variation. Judging by the named subspecies (see synonomic list) the intensity of yellow on the ventral surface of the HW may vary. However, whether these names are biologically meaningful remains uncertain. We found little variation in our samples from Garza Cocha, Ecuador, although we note that Ecuadorian and Brazilian material differ in the respective width of the forewing subapical band (Fig. 1).

Panacea Godman and Salvin, 1883

Pandora Doubleday, 1848. Gen. Diurnal Lep. p. 300 Pl. 3 fig 5

Panacea Godman and Salvin, 1883. Biol. Centr. Am. pp. 274–275

Panacea prola (Doubleday, 1848) (Figs. 3, 11, 13)

Pandora prola Doubleday, 1848. Gen. Diurnal Lep. p. 300 Pl. 3 fig. 5

Panacea prola female f. *dubia* Kretzschmar 1894. Deutsche ent. Zeit. "Iris" 6(2):158–160

P. prola zaraja Fruhstorfer, 1912. Ent. Rundschau 29(6):46

P. prola amazonica Fruhstorfer, 1915. Soc. ent. 30(12):66

P. prola prolifica Fruhstorfer, 1915. Soc. ent. 30(12):66

P. prola amazonica f. *bronzina* Bryk, 1953. Arkiv. Fur Zool. 5(1):1–268

Species characters. Dorsal surface with broken blue-green iridescent bands. Forewing dorsal surface without a subapical line in both sexes, but some females with a faint greenish-white subapical band. Hindwing dorsal surface without ocelli or blue submarginal line. Hindwing ventral surface bright red, generally without black markings, but sometimes with a faint black submarginal line.

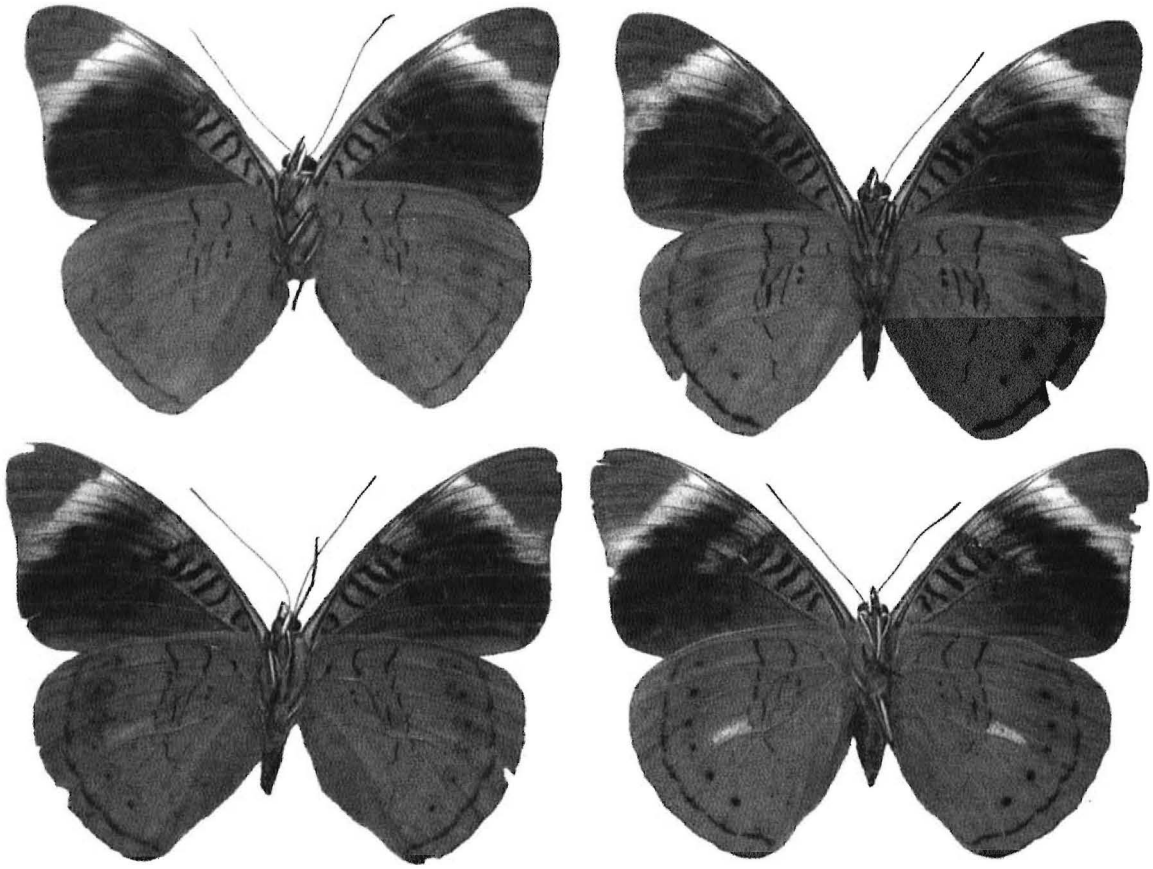

FIG. 5. *Panacea regina*, ventral. Top row, male; bottom row female. All from Garza Cocha, Ecuador.

Distribution. Panama, Colombia, Venezuela, Guianas and upper Amazon basin (Seitz 1916, D'Abrera 1987, Emmel & Austin 1990, Otero & Romero 1992, Lamas 1994, Robbins et al. 1996, Neild 1996).

Variation. We found wide variation in wing length, but little variation in color pattern in large samples from Garza Cocha, Ecuador. Small individuals appear to be the result of caterpillars feeding on poor quality *Caryodendron* leaves, or those that were semi-starved (pers. obs.).

Subspecies. *Panacea prola zaraja*, from Venezuela, Merida; *P. p. amazonica*, from the upper Amazon; *P. p. prolifica*, from Ecuador.

Panacea regina (Bates, 1864)
(Figs. 4, 5, 11, 13)

Pandora regina Bates, 1864. J. Entom. 2(10):213.
Panacea regina victrix Fruhstorfer, 1915. Soc. ent. 30(12):66.

Species characters. Dorsal surface with broken blue-green iridescent bands. Forewing ventral surface with reddish apex and white subapical band but without the distinct red spots outlined by black in discal cell (see *P. divalis*). Hindwing dorsal surface with a blue medial band adorned with incomplete black ocelli that vary in size, and may reach the distal margin of the band; submarginal wavy line sometimes faint. Hindwing ventral surface red with broken submedial to medial transverse black lines, the most distal starting at Sc + Rs and ending at Cu_2; faint post-medial ocelli in almost all cells; conspicuous black submarginal line. Females often with a short, white longitudinal stripe in ventral hindwing cell M_2–M_3, nearly at the center of wing.

Distribution. Western and upper Amazon (Ecuador, Peru, Brazil) (Seitz 1916, D'Abrera 1987, Lamas 1994, Robbins et al., 1996).

Variation. In Ecuadorian and Brazilian samples we found that the medial ocelli on the dorsal hindwing vary considerably within populations. In females we found the ventral hindwing ocelli were sometimes incomplete.

Subspecies. *Panacea regina victrix*, from Ecuador; see also *P. chalcothea* (below).

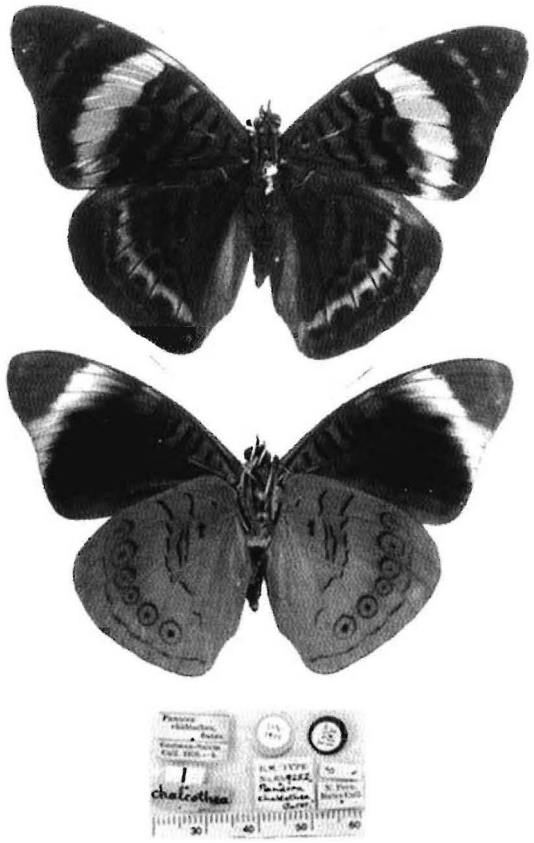

FIG. 6. *Panacea chalcothea*, male, dorsal and ventral, plus label. This specimen is an apparent syntype (see Identification and Taxonomy). Note: whether *chalcothea* is a subspecies of *P. regina* or a valid species remains to be resolved.

Panacea chalcothea (Bates, 1868) (Fig. 6)

Pandora divalis Bates, 1868. Ent. mon. Mag. 4(44):170.

This somewhat obscure taxon figures importantly in the history of *Panacea*, and its taxonomic status is unresolved. Although we were unable to examine material of *chalcothea* directly, the photo provided by G. Lamas (Fig. 6) may serve as a starting point for identifying this taxon. Here we excerpt correspondence received from G. Lamas that bears directly on the taxonomic interpretation of *Panacea chalcothea*:

"Bates (1868:170) described *chalcothea* based on at least 2 specimens, one female (?) illustrated by Hewitson ([1854], Ill. exot. Butts 1. pl. [42], fig. 4), and thought by the latter to be the female of *procilla*; and one male from "southern Equador". Hewitson's "female" belonged to the collection of the Entomological Society of London, and that specimen is almost certainly lost, while Bates' male would have been in his collection, and should have gone to the BMNH through Godman and Salvin. There seems to be no Bates specimen of *chalcothea* from southern Ecuador at the BMNH. However, there is a male specimen from Bates' collection, labeled *chalcothea* by Bates himself, but from "N Peru", and I interpret this as a possible syntype of *chalcothea*, agreeing very well with the written description of the male given by Bates in his original paper. Bates may well have confused "S Ecuador" with "N Peru". Anyway, that specimen from "N Peru" most probably came from Amazonas department in Peru. . . . Now, [it] seems to me that *chalcothea* (based on Bates' o.d. and the syntype referred to above) is . . . very probably a subspecies of *regina*, or could even be a full species. For the time being, I'm calling those 2 specimens as *Panacea regina chalcothea*, though I wouldn't be too surprised if they were to represent a high altitude species distributed from Colombia to N Peru (if Hewitson's "New Granada" locality for his specimen is correct, which is quite doubtful)."

TABLE 1. Number of dissected individuals and locality data. Abbreviations for source collections are: P. J. DeVries (PJD); G. Austin (GTA); G. Attal (GA); Los Angeles County Museum (LACM); Milwaukee Public Museum (MPM).

Taxa	Source of dissected material
Ingroup	
Batesia hypochlora	2 males: Brazil (GTA)
	8 males: Ecuador, Sucumbios, Garza Cocha (PJD)
	1 female: Brazil (GTA)
	1 female: Ecuador, Sucumbios, Garza Cocha (PJD)
Panacea bleuzeni	1 male: French Guyana (GA)
	1 female: French Guyana (GA)
Panacea divalis	5 males: Ecuador, Sucumbios, Garza Cocha (PJD)
	2 males: Brazil, Rondonia (GTA)
	3 females: Ecuador, Sucumbios, Garza Cocha (PJD)
Panacea procilla	2 males: Brazil (n = 1) and Colombia (n = 1) (LACM)
	1 male: Colombia (MPM)
	1 female: Colombia (MPM)
Panacea prola	5 males: Ecuador, Sucumbios, Garza Cocha (PJD)
	3 females: Ecuador, Sucumbios, Garza Cocha (PJD)
Panacea regina	5 males: Ecuador, Sucumbios, Garza Cocha (PJD)
	3 females: Ecuador, Sucumbios, Garza Cocha (PJD)
Outgroups	
Biblis hyperia	1 male: Ecuador, Sucumbios, Garza Cocha (PJD)
	1 female: Ecuador, Sucumbios, Garza Cocha (PJD)
Hamadryas amphinome	1 male: Ecuador, Sucumbios, Garza Cocha (PJD)
	1 female: Ecuador, Sucumbios, Garza Cocha (PJD)
Hamadryas arinome	1 male: Ecuador, Sucumbios, Garza Cocha (PJD)
	1 female: Ecuador, Sucumbios, Garza Cocha (PJD)
Hamadryas feronia	1 male: Ecuador, Sucumbios, Garza Cocha (PJD)
	1 female: Ecuador, Sucumbios, Garza Cocha (PJD)
Hamadryas laodamia	1 male: Ecuador, Sucumbios, Garza Cocha (PJD)
	1 female: Ecuador, Sucumbios, Garza Cocha (PJD)

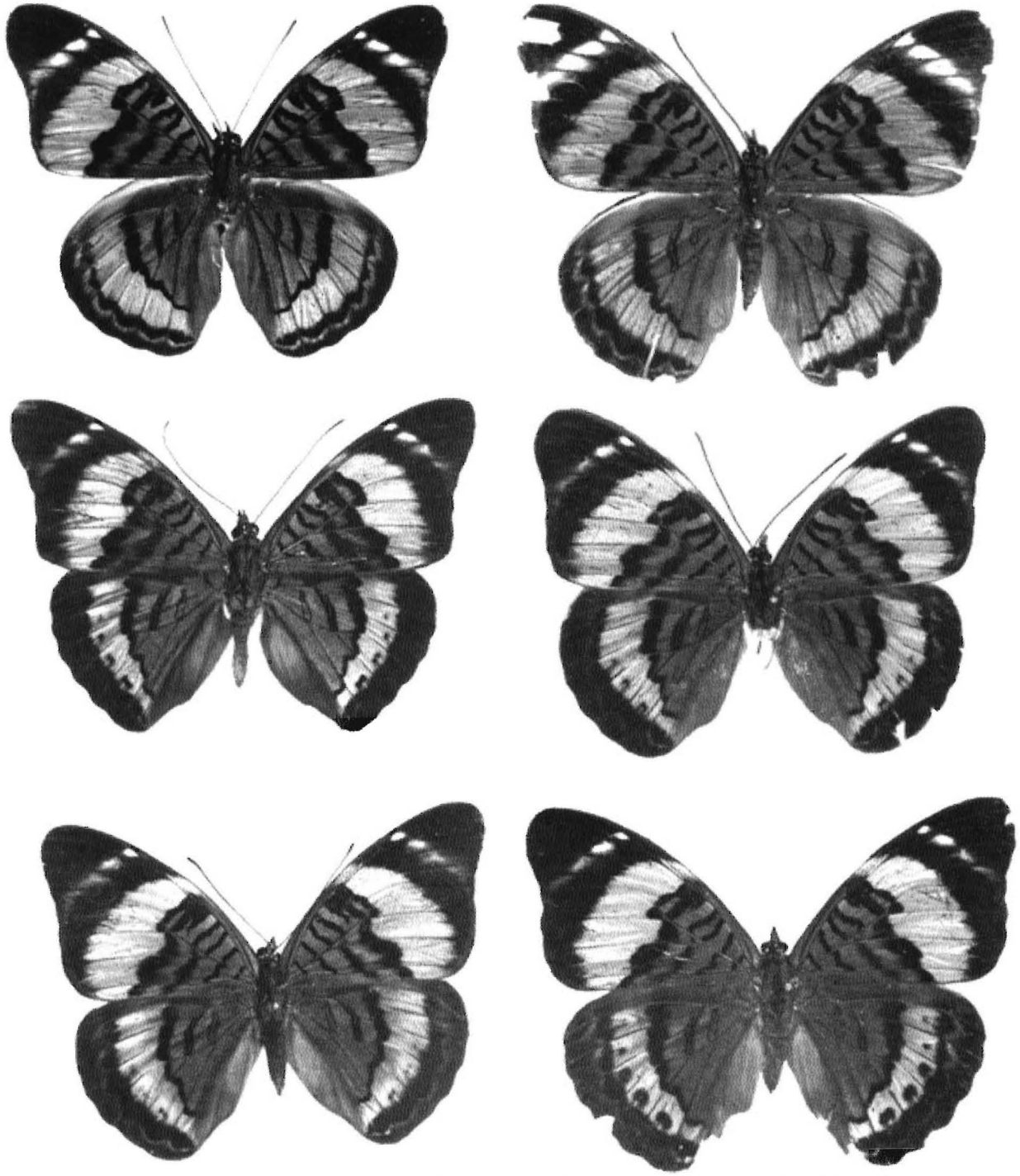

FIG. 7. *Panacea divalis*, dorsal. Left column, males; right column, females. Top row, Rondonia, Brazil; middle and bottom rows, Garza Cocha, Ecuador. Note variation in medial bands and submarginal ocelli.

Distribution. Apparently Western Amazonas (Ecuador, Peru) and Colombia (?).

Panacea divalis (Bates, 1868)
(Figs. 7, 8, 12, 13)

Pandora divalis Bates, 1868. Ent. mon. Mag. 4(44):171.
Panacea procilla divalis Seitz, 1916. Die Gross Schmetterlinge der Erde p. 537.

Species characters. Dorsal surface with broken iridescent blue-green bands. Forewing ventral sur-

TABLE 2. Characters justifying the groupings of species and genera. MacClade 3.01 was used to map character changes on the most parsimonious tree. Characters indicated in bold type were unique to the group they support (independent of reversals).

Clade 1. *Panacea* and *Batesia*
(2:0) Fringe of scales in forewing and hindwing outer margin solid dark color
(16:0) Ventral surface of hindwing with black submarginal line that is discrete in anal area and more diffuse toward costal area
(24:0) Thorax: ventral portion completely covered with red-orange scales
(27:1) In lateral view: Hypandrium without anterior rod-like projections
Clade 2. *Batesia hypochlora*
(8:2) Males: Ventral surface of forewing apex dark, with a yellow band
(19:0) Forewing venation: M_1 arched toward anal margin
(25:0) Hypandrium: narrow, plate like, with obvious constriction near the middle of its long axis
(29:0) In lateral view, anterior portion of tegumen extremely projected
(30:1) Uncus tip in lateral view sharply hooked
(32:1) Uncus short
(33:0) In lateral/ dorso-lateral view, base of uncus with obvious large dorsal ridges
(34:1) In lateral view, tip of uncus not reaching or extending beyond tip of valva
(37:0) Distal portion of gnathos small and projected ventrally
(38:0) In ventral view, distal portion of gnathos with a rounded invagination
(43:1) Distal portion of valva with small bare chitinous tip
(53:0) Antrum mostly membranous
Clade 3. *Panacea*
(4:1) Forewing postmedial band expressed dorsally only
(5:1) In dorsal view, forewing subapical white band reduced
(7:0) Ventral surface of forewing with white subapical band
(10:0) Ventral surface of hindwing largely colored red-orange, with or without purplish sheen
(17:0) Ventral surface of hindwing with dark line imposed upon cross-vein m_2–m_3 (at distal edge of discal cell)
(23:0) Foreleg with white scales laterally
(42:0) Distal portion of valva curving ventrally
(44:0) In lateral view, basal portion of valva with large conspicuous ventrally produced rounded projection
(46:1) In lateral view, distal portion of saccus straight to slightly projected upward
Clade 4. *Panacea procilla*, *Panacea bleuzeni*, *Panacea divalis* and *Panacea regina*
(8:0) Males: Ventral surface of forewing apex uniformly dirty red-orange
(11:0) Ventral surface of hindwing with prominent dark line across basal half of cell Sc + R_1
(12:0) Ventral surface of hindwing with prominent dark line across discal cell
(13:0) Ventral surface of hindwing discal cell with two black dots in basal half
(14:0) Ventral surface of hindwing with nearly continuous line through medial area that crosses cells Sc + R_1, Rs, M_1, M_2, M_3, Cu_1 and Cu_2
(15:1) Ventral surface of hindwing with dark line not contiguous and line in cell Cu_2 more apical than line in cell Cu_1
(18:0) Female: ventral surface of hindwing with white patch of scales in medial area of cell M_2
Clade 5. *Panacea procilla*, *Panacea bleuzeni* and *Panacea divalis*
(5:0) In dorsal view, forewing subapical white band well developed
(6:0) In ventral view, forewing discal cell with two red-orange spots, one at base and one at mid-length
Clade 6. *Panacea procilla* and *Panacea bleuzeni*
(3: 0) In dorsal view, male forewing with oblique, diffuse black band encroaching on postmedial blue/green band.

face with reddish apex, white subapical band and distinct red spots outlined by black in discal cell (see *P. regina*). Hindwing ventral surface brownish red with a faint purple sheen; broken transversal black medial lines, the most distal starting at Sc + Rs and ending at 1A; postmedial ocelli (black "rings") on almost all cells; conspicuous black submarginal line. Females with a short, white longitudinal stripe in ventral hindwing cell M_2–M_3, nearly at the center of wing. Incomplete ocelli on dorsal surface of hindwing vary in size, and may be absent in some specimens.

Distribution. Upper Amazon (Seitz 1916), Colombia to Peru (D'Abrera 1987) and western Brazil (Emmel & Austin 1990).

Variation. In males the dorsal hindwing marginal band varies among samples from Brazil and Ecuador; the dorsal hindwing ocelli vary from diffuse to sharp; a short, ventral longitudinal stripe may occur in ventral hindwing cell M_2–M_3. In females the white, ventral longitudinal stripe in hindwing cell M_2–M_3 may be diffuse or faintly expanded into the two cells above.

Subspecies. None.

Panacea procilla (Hewitson, 1852)
(Figs. 9, 12, 13)

Pandora procilla Hewitson, 1852. Exot. Butt. 1.
Panacea lysimache Godman and Salvin 1883. Biol. Centr. Americana p. 275.
P. procilla ocana Fruhstorfer, 1912. Ent. Rundschau 29(6):46.

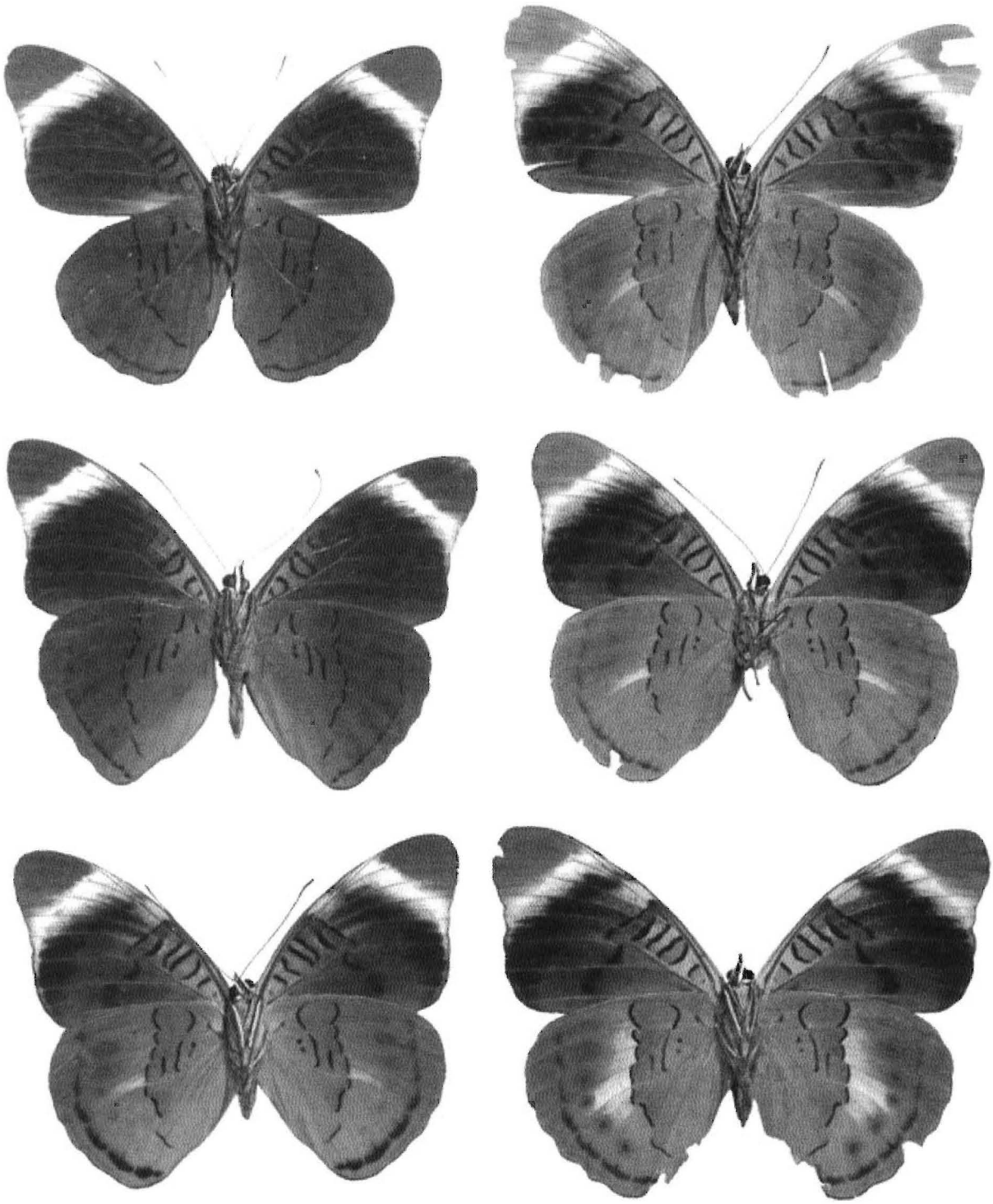

FIG. 8. *Panacea divalis*, ventral. Left column, males; right column, females. Top row, Rondonia, Brazil; middle and bottom rows, Garza Cocha, Ecuador. Note variation in white stripe centered in cell M_2–M_3.

P. procilla salacia Fruhstorfer, 1915. Soc. Ent. 30(12):66.
P. procilla lysimache Seitz, 1916. Die Gross Schmetterlinge der Erde p. 537.
P. procilla var. *marmorensis* Hall, 1917. Entomologist 50(651):171–174.

Species characters. Dorsal surface with broken blue-green iridescent bands. Forewing ventral surface with distinct red outlined by black in discal cell, reddish apex and white subapical band. Hindwing ventral surface brownish red with a faint purple

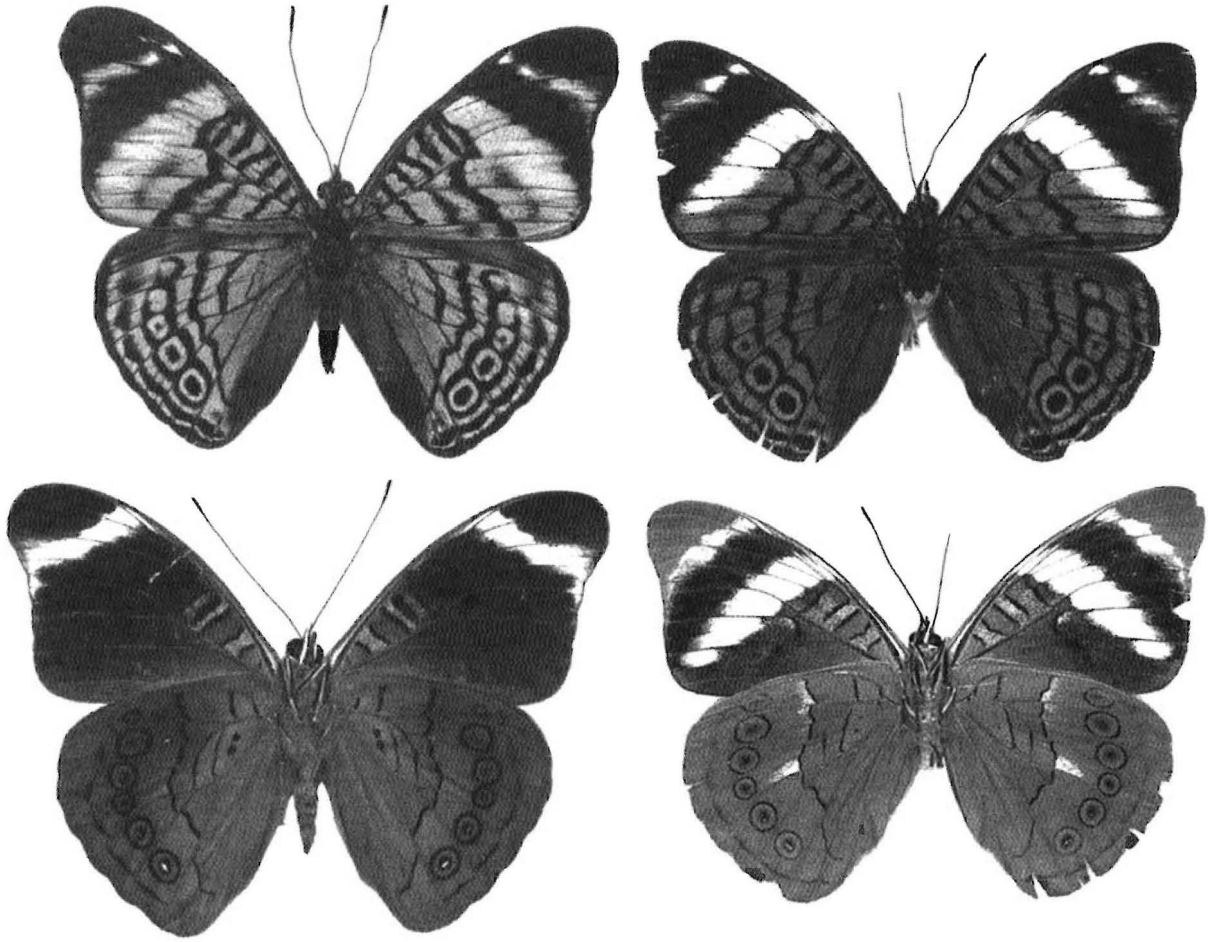

FIG. 9. *Panacea procilla*, dorsal and ventral. Left column, male; right column, female. Specimens from Cali, Colombia.

sheen; broken transverse medial black lines, the most distal starting at Sc + Rs and ending at 1A; complete postmedial ocelli on almost all cells, those on cells M_3–Cu_1 and Cu_1–Cu_2 with iridescent pupil; conspicuous black submarginal line. Dorsal surface of hindwing with a medial blue band adorned with black ocelli; conspicuous submarginal wavy line. Females with white medial band on ventral forewing, and also with a white band on ventral hindwing from cell Sc + R_1–Rs to M_2–M_3, sometimes interrupted on M_1–M_2.

Distribution. Costa Rica south to Colombia and throughout the upper Amazon basin and the Guianas (Kretzschmar 1894, Apolinar 1926).

Variation. We observed some males that have a short, white longitudinal stripe in ventral hindwing cell M_2–M_3, nearly at the center of wing—a pattern similar to females of *P. regina* and *P. divalis*.

Subspecies. *Panacea procilla procilla*, western Venezuela (Neild 1996), *P. p. ocana*, from lower Magdalena River, Colombia (Seitz 1916, D'Abrera 1987); *P. p. salacia*, from Colombia (Seitz 1916, D'Abrera 1987); *P. p. lysimache* from Volcan Chiriqui, Panama, Finca la Selva, Costa Rica (DeVries 1987, 1989).

Panacea bleuzeni Plantrou and Attal, 1986
(Figs. 10, 12, 13)

Panacea bleuzeni Plantrou and Attal, 1986. Bull. Société Sciences Nat. 50:23.

Panacea bella D'Abrera, 1987. Butterflies of the Neotropical Region, part III: p. 487, **new synonoym**

Species characters. Dorsal surface distinctively blue or blue-green. Dorsal surface of hindwing with a blue medial band adorned with large black ocelli; wavy iridescent submarginal line conspicuous. Ventral forewing with distinct red outlined by black in discal cell, reddish apex and white subapical band (similar to *procilla*). Ventral hindwing with transverse medial black line continuous from cell Sc + Rs to vein 1A; ocelli faint. Females with white marking extending distally along black medial line from cell Sc + Rs to Cu_2–1A.

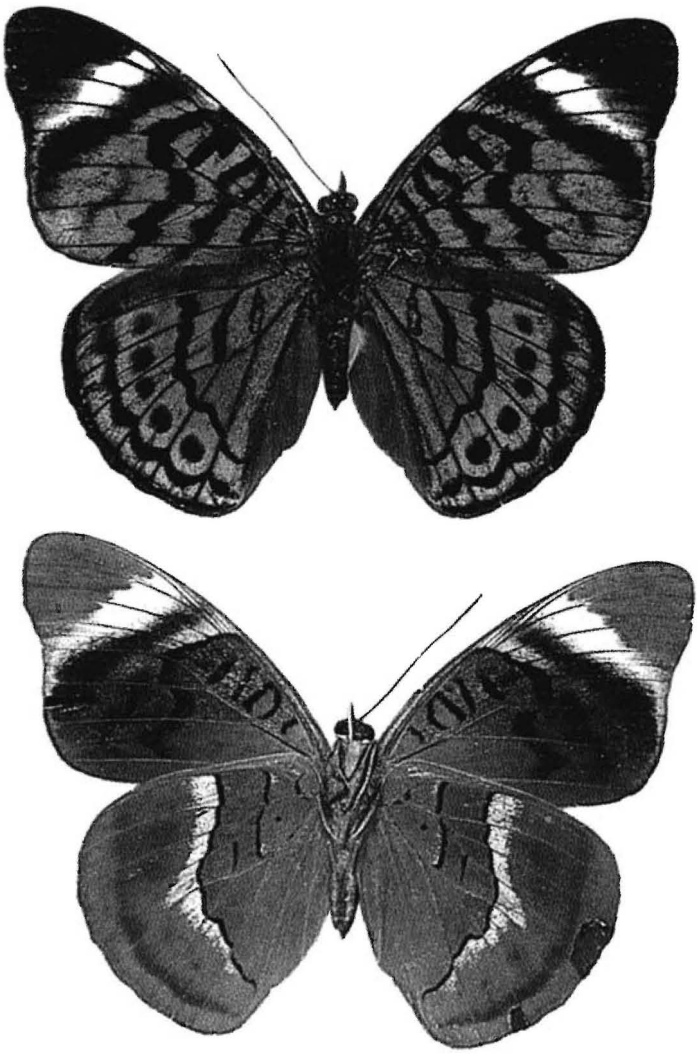

FIG. 10. *Panacea bleuzeni*, female, dorsal and ventral. This figure is reproduced through the kind permission of B. d'Abrera [Butterflies of the Neotropical Region, part III:487]. It is the type of *Panacea bella* D'Abrera, 1987

Distribution. Apparently endemic to the Guianas (Plantrou & Attal 1986). However, it's overlapping range with *procilla* and close relationship to it (Table 2, clade 6) suggest the possibility that this taxon may be a subspecies of *procilla*. This point needs critical evaluation.

Synonymic notes. Examination of the collection of the BMNH by A. Neild (pers. com.) revealed that the single female holotype of *P. bella* is also a paratype of *P. bleuzeni*. This, therefore, indicates that *P. bella* and *P. bleuzeni* represent a single species with *bella* as a junior synonym of *bleuzeni*. Comparing the illustration of the type specimen of *bella* (in D'Abrera 1987) with photographs of male and female *P. bleuzeni* provided by G. Attal confirms this assessment.

DISCUSSION

Our analysis showed that *Batesia* and *Panacea* form a monophyletic group, with *B. hypochlora* basal to *Panacea*. Therefore, despite similarities in early stage morphology and host plant use, we reject the hypothesis that *B. hypochlora* is a derived species from within *Panacea*. Our study confirms the maintenance of *Batesia* and *Panacea* as separate taxa (e.g., Godman & Salvin 1883, Seitz 1916), and serves as a framework for future systematic work on both genera. We note that, without examining material firsthand, *P. chalcothea* is presumed to be the sister taxon of *P. regina*. However, the phylogenetic position of *chalcothea* requires confirmation, including its taxonomic rank.

Insect genitalia are widely used for phylogenetic

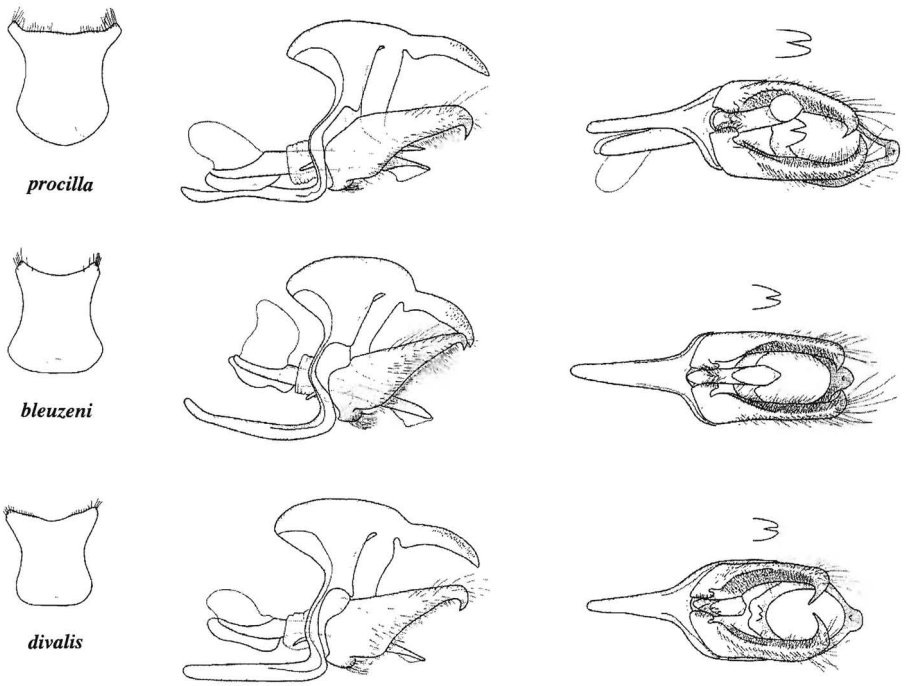

FIG. 11. Male genitalia: hypandrium, lateral view, ventral view (inset: tip of gnathos in ventral view). *Panacea procila*, *P. bleuzeni*, and *P. divalis*.

reconstruction and delimiting species boundaries because their morphology may diverge rapidly, and therefore provide informative characters (Eberhard 1985, Porter & Shapiro 1990, Arnqvist 1998). In *Panacea*, however, we found that the genitalia were highly conserved and provided no informative characters for phylogeny reconstruction, or discrimination among species. Rather, the species-level rela-

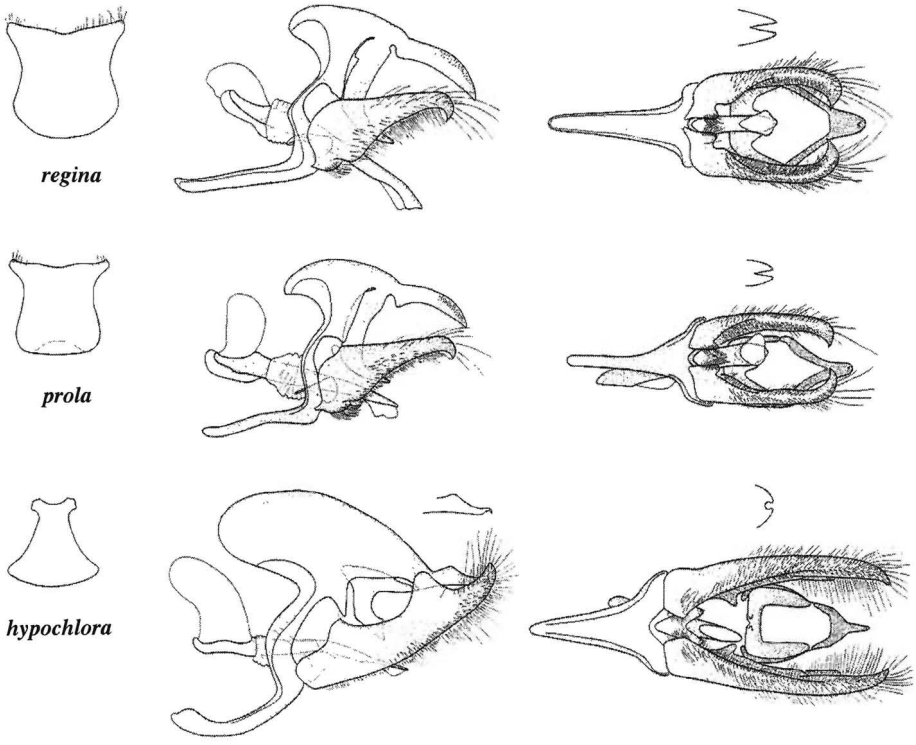

FIG. 12. Male genitalia: hypandrium, lateral view (inset: uncus in lateral view), ventral view (inset: tip of gnathos in ventral view). *Panacea regina*, *P. prola*, and *Batesia hypochlora*.

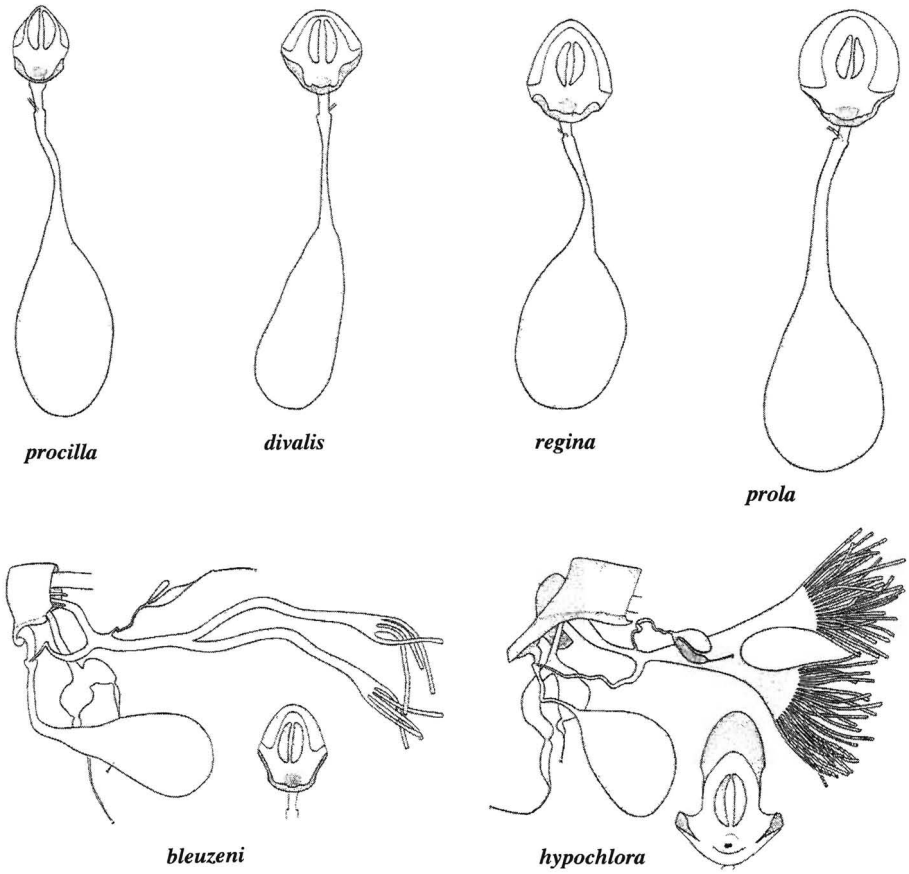

FIG. 13. Female genitalia: ventral view, *Panacea procila*, *P. divalis*, *P. regina*, and *P. prola*. Lateral view: *P. bleuzeni*, and *Batesia hypochlora* (insets: genitalia in ventral view). Note differences in the number of ovarioles between *P. bleuzeni* and *B. hypochlora*.

tionships proposed here were derived solely from characters of wing pattern (Fig. 14, Table 2). Our study suggests that the most distinctly colored species, *P. prola*, is basal to other congeners, with remaining species groupings justified by differences in wing patterns.

The distinctive behavior and coloration make *Panacea* easily recognizable in the field. However, in large samples from one Ecuadorian site we found considerable intraspecific variation in both genital morphology and wing color patterns. This concurs with Seitz (1916) who noted that in some *Panacea* species within population phenotypic variation may be greater than among population variation, indicating that there may be transitions among species with respect to color pattern. With the possible exception of *P. prola*, such phenotypic variation precludes the notion that sympatric *Panacea* species can be positively identified in nature without capturing them.

Batesia and *Panacea* are obvious and often abundant elements of many Neotropical butterfly faunas and museum collections. Nevertheless, some taxa are rare in collections, and this study points to several questions that will require a full taxonomic revision to resolve, particularly regarding the status of *P. chlacothea* and *P. bleuzeni*. Although potentially useful tools for conservation ecology, little has been reported on the natural history *Batesia* and *Panacea*. What we do know is that adults of both genera show significant flight height preference in some lowland rainforests, and that trees in the genus *Caryodendron* are larval hostplants (see DeVries 1989, Montoya 1991, DeVries et al. 1999, DeVries & Walla 2001). We do not know if all taxa exhibit vertical stratification, if these butterflies use other hostplant genera, or if some species are warningly colored (e.g., *P. prola, Batesia*) that represent models in mimicry complexes. We believe that field studies, in concert with phylogenetic analyses of *Hamadryas*, *Ectima*, *Eunica*, and related genera is the next step toward understanding the evolution of *Batesia* and *Panacea*, and the diversification of the Biblidini.

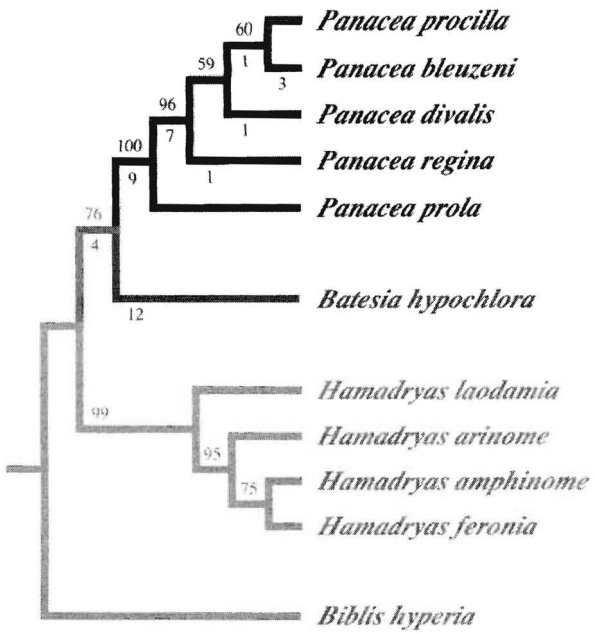

FIG. 14. Single most parsimonious tree obtained from the analysis of 53 characters for 11 species (tree length = 79, CI = 0.82, RI = 0.88). Numbers above and below tree branches represent bootstrap values and the number of unambiguous changes respectively.

ACKNOWLEDGMENTS

We thank Eric Schwartz and the staff of La Selva Jungle Lodge for supporting this and other aspects of our research. G. Attal (France), G. Austin (Nevada State Museum), and B. Brown (Natural History Museum of Los Angeles County) kindly facilitated our work by loaning specimens. We are sincerely grateful to P. Ackery (The British Natural History Museum), G. Lamas (Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Peru), G. McGavin (Oxford University Natural History Museum), A. Neild (London), and C. Smith (The British Natural History Museum) for providing historical information and comparing type specimens that helped unravel the taxonomic mysteries of *P. chalcothea* and *P. bleuzeni*. We thank B. d'Abrera for permission to reproduce his figures of *Panacea bella*. Comments by G. T. Austin and André Freitas improved the clarity of this manuscript. RIH thanks Lee Robertson for inspiration. This study was supported in part by the National Science Foundation (DEB 00-96241) and is dedicated to the memory of Sonny Clark, Bill Evans, Thelonius Sphere Monk, and Bud Powell.

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Received for publication 19 June 2002; revised and accepted 3 July 2002.

APPENDIX 1. Character list used in the phylogenetic analysis. Relevant figures are noted, and comments are included when needed. Definitions are in the Characters and Terminology section.

Wing Characters:

1. Forewing outer margin: concave (0), straight (1), convex (2).
2. Fringe of scales in the outer margin of wings: solid dark color (0), dark interspersed with white sections (1).
3. In dorsal view, male forewing with oblique, diffuse black band encroaching on postmedial blue-green band (0); devoid of such a pattern (1). Note: *P. bleuzeni* was scored using original description, illustration in D'Abrera and photos provided by G. Attal.
4. Forewing postmedial band expressed dorsally and ventrally (0); expressed dorsally only (1); absent or reduced (2). Note: *H. laodamia* and *P procilla* were polymorphic for this character because of differences between the sexes.
5. In dorsal view, forewing subapical white band well developed (0); reduced (1); absent (2).
6. In ventral view, red-orange spots on forewing discal cell: two spots present, one at base and one at mid-length (0), one spot present, at mid-length (1), absent (2).
7. Ventral surface of forewing with white subapical band (0); devoid of such pattern (1).
8. Males, ventral surface of forewing apex: uniformly dirty red-orange (0); dark, same color as medial area (1); dark, with a yellow band (2).
9. Dorsal and ventral sides of hindwing consistently with four complete ocelli (0); dorsal side of hindwing with five incomplete ocelli (lacking outer ring) and clearly separated from any black lines (1); ventral side of hindwing with four to six complete ocelli (2); devoid of such patterns (3). Note: To understand the variation in this character a large number of specimens were examined, and we found no exceptions to the patterns described here (see Methods, Species studied).
10. Ventral surface of hindwing largely colored red-orange, with or without purplish sheen (0); devoid of such a pattern (1). Note: although the presence of a purplish sheen has been used to separate *P. procilla* and *P. divalis*, we found this character to be present in both these species and variable within each of them.
11. Ventral surface of hindwing with prominent dark line across basal half of cell Sc + R_1 (0); devoid of such a pattern (1).
12. Ventral surface of hindwing with prominent dark line across discal cell (0); devoid of such a pattern (1).
13. Ventral surface of hindwing: discal cell with two black dots in basal half (0); devoid of such a pattern (1). Note: of the 57 *P. prola* specimens examined, three had two dots, 22 had one dot, and 32 lacked dots; in *P. divalis*, four of the 53 specimens had dots merged into a single marking.
14. Ventral surface of hindwing with: nearly continuous line through medial area that crosses cells Sc + R_1, Rs, M_1, M_2, M_3, Cu_1 and Cu_2 (0); devoid of such a pattern (1).
15. Ventral surface of hindwing with: dark line in cell Cu_2 and cell Cu_1 contiguous (0); dark line not contiguous and line in cell Cu_2 more apical than line in cell Cu_1 (1); dark line not contiguous and line in cell Cu_2 more basal than cell Cu_1 (2); dark line absent from cell Cu_2 (3).
16. Ventral surface of hindwing with black submarginal line which is discrete in anal area and becomes more diffuse toward costal area (0); devoid of such a pattern (1). Note: *P. bleuzeni* was scored using the illustrations in D'Abrera (1987) and photos from the collection of G. Attal.
17. Ventral surface of hindwing with dark line imposed upon cross-vein m_2–m_3 (at distal edge of discal cell) (0), devoid of such a dark line (1). Note: in *P. prola*, three of 53 specimens lacked the dark line.
18. Female, ventral surface of hindwing with white patch of scales in medial area of cell M_2 (0); devoid of white patch (1). Note: two males of *P. procilla* had similar white patch. In *P. divalis* one of 12 lacked the patch, and in *P. regina* two of 14 lacked the patch.
19. Forewing venation: M_1 arched toward anal margin (0); devoid of such a pattern (1).
20. Forewing venation: M_2 arched toward anal margin (0); devoid of such a pattern (1).
21. Forewing cross-vein m_2–m_3 + cu_1: joins M_3 + Cu_1 at or distal to the fork M_3 and Cu_1 (0); proximally to the fork M_3 and Cu_1 (1); absent (2). Note: M_3 + Cu_1 denotes the combination of vein M_3 and Cu_1 proximal to the fork where they split.
22. Forewing cross-vein r–m_1, and the base of M_1 and M_2: inflated (0); not inflated (1).

Body Characters:

23. Foreleg with white scales laterally (0); devoid of white scales (1).
24. Thorax: ventral portion completely covered with red-orange scales (0); devoid of such a pattern (1).

Male Genitalia Characters:

25. Hypandrium: narrow, plate like, with obvious constriction near the middle of its long axis (0); broad, curling laterally, without a constriction (1).
26. In lateral view, hypandrium with long ramus projecting posteriorly (0); devoid of projections (1).
27. In lateral view, hypandrium with anterior rod-like projections (0); devoid of such a pattern (1).
28. In lateral view, posterior corner of hypandrium extended into an obvious lobe-like process that projects dorsally (0); less lobe-like and not as projected dorsally (1).
29. In lateral view, anterior portion of tegumen extremely projected (0); devoid of such a pattern (1).
30. In lateral view, uncus tip: pointed (0); sharply hooked (1).

APPENDIX 1. Continued.

31. Uncus: bifid (0); entire (1).
32. Uncus: elongate (0); short (1).
33. In lateral/ dorso-lateral view, base of uncus with obvious large dorsal ridges (0); with small ridges (1); devoid of such a pattern (2).
34. In lateral view, tip of uncus reaching or extending beyond tip of valva (0); devoid of such a pattern (1).
35. Uncus with obvious, long setae dorsally (0); devoid of setae (1).
36. Distal portion of gnathos: completely fused (0); bifid (1).
37. Distal portion of gnathos: small and projected ventrally (0); large and projected posteriorly (1).
38. In ventral view, distal portion of gnathos: with a rounded invagination (0); invaginated in a perfect "V" (1).
39. Valva: with dentate process approximately 2/3 from its base (0); without such a process (1).
40. Process of valva: projecting dorsally (0); projecting medially (1).
41. Process of valva: with setae (0); without setae (1).
42. Distal portion of valva: curving ventrally (0); curving dorsally or straight (1).
43. Distal portion of valva with large bare chitinous tip (0); with small bare chitinous tip (1); devoid of such patterns (2).
44. In lateral view, basal portion of valva: with large conspicuous ventrally produced rounded projection (0); devoid of such a pattern (1).
45. In lateral view, rod-like projections of juxta: large (0); small (1).
46. In lateral view, distal portion of saccus: strongly projected upward (0); straight to slightly projected upward (1).
47. In lateral view, vinculum with obvious dentate process along anterior margin (0); process shaped as a bump, not dentate (1).

Female Genitalia Characters:

48. Signa: present (0); absent (1).
49. Sterigma: present (0); absent (1).
50. Lamella antevaginalis: continuous across ventral surface (0); split (1).
51. Lamella antevaginalis: fused to edge of eighth sternite (0); not fused (1).
52. Ductus seminalis connecting to ductus bursa: very near corpus bursa (0); far from corpus bursa, and near ostium bursa (1).
53. Antrum: heavily sclerotized (0); mostly membranous (1).

APPENDIX 2. Character Matrix.

Ingroup						
Batesia hypochlora	1010221231	1111301101	0110011?01	1101110000	1111001111	111
Panacea prola	0011100130	1111300111	0100111010	101011111?	?000011110	110
Panacea procilla	000(0,1)000000	0000100011	0100111010	101011111?	?000011110	110
Panacea divalis	0011000030	0000100011	0100111110	101011111?	?000011110	110
Panacea regina	0011120020	0000100011	0100111010	101011111?	?000011110	110
Panacea bleuzeni	0002000010	0010000011	??00111010	101011111?	?000011110	110
Outgroups						
Biblis hyperia	2112221131	1111311111	2111110?10	0020101?1?	?101001100	110
Hamadryas laodamia	211(0,2)221131	1111311111	1011100?10	1010011?01	0121100011	000
Hamadryas arinome	1110221131	1111311110	1001100?10	1011011101	0121100011	000
Hamadryas amphinome	11102?1130	1111310110	1001100?10	10?1011101	0121100011	000
Hamadryas feronia	111?211?01	0110210?10	1001100?10	1011011101	0121110011	000