Phylogenetic relationships among the Ithomiini (Lepidoptera: Nymphalidae) inferred from one mitochondrial and two nuclear gene regions

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Abstract. A phylogenetic hypothesis for the tribe Ithomiini (Lepidoptera: Nymphalidae: Danainae) is presented, based on sequences of the mitochondrial *cytochrome oxidase subunits I* and *II* (COI–COII) region and regions of the nuclear genes *wingless* and *Elongation factor 1-alpha*. Branch support for each clade is assessed, and a partition congruence index is used to explore conflict among gene regions. The monophyly of the clade is strongly supported, as are many of the traditionally recognized subtribes and genera. The data imply paraphyly of some genera and tribes, but largely support recent classifications and phylogenetic hypotheses based on morphological characters.

Introduction

The Ithomiini is a diverse neotropical butterfly clade comprising more than 300 species in some fifty genera (Motta, 2003; Lamas, 2004). The group has been treated historically as a subfamily of Nymphalidae or a family in its own right, but it is currently considered (and is treated here) as a tribe within the nymphalid subfamily Danainae (Ackery *et al.*, 1999), with concomitant reduction of traditional ithomiine and danaine tribes to subtribal status. The diagnostic synapomorphy for the group is the presence in adult males of patches of elongated androconial scales on the anterior margin of the hindwing. The monophyly of ithomiines with respect to other Nymphalidae was further supported by phylogenetic analysis of a short region of the *wingless* gene (Brower, 2000), and by three additional homoplastic morphological characters (extensive reflective areas on the pupa, relatively long antennae, saccus relatively long;

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Freitas & Brown, 2004). Danainae is also supported as monophyletic in the higher level molecular study of Nymphalidae by Wahlberg *et al.* (2003).

As far as is known, ithomiine butterflies are chemically defended; most species acquire pyrrolizidine alkaloids from the nectar of *Eupatorium* (Asteraceae), *Heliotropium* (Boraginaceae) and other flowers, and do not sequester toxins from larval food plants (Brower, 1984; Trigo *et al.*, 1996; but see Freitas *et al.*, 1996). Adult ithomiine butterflies are slow-flying and are generally considered to be aposematic (but see Kassarov, 2004). All members of the group are engaged in Müllerian mimicry rings with other ithomiines, danaines, heliconiines, day-flying moths and various other insects (Bates, 1862; Brown, 1979; Beccaloni, 1997). The remarkable convergence of appearances between unrelated forms at a given locality, combined with geographical polymorphism within individual species, has resulted in a complex and confusing taxonomy, with over 1200 ostensibly valid described and undescribed subspecies, not to mention hundreds of additional synonyms, infrasubspecific names and nomina nuda (Lamas, 2004).

The phylogenetic position of Ithomiini within the Nymphalidae has been controversial. Prior to Bates (1862), all long-winged neotropical nymphalids (and even some Pieridae) were usually placed in 'Heliconidae', a holdover from the Linnean subgeneric group 'Heliconii' (Linné,

1758; Godart, 1819; Boisduval, 1836; Doubleday; 1847). Recognizing that adaptive convergence and not phylogenetic affinity explained the similar wing patterns and body forms of 'danaoid Heliconidae' and 'acraeoid Heliconidae' (which included true *Heliconius*), Bates (1862) separated ithomiines from heliconiines and placed them in the 'Danaidae'. At the same time, Felder & Felder (1862) independently discovered that the anastomosis of the first and second anal veins of the forewing unites Danaini and Ithomiini, a character that may represent one of the few morphological synapomorphies uniting the Danainae, albeit with several convergent origins in other nymphalid clades (Ackery & Vane-Wright, 1984). Harvey (1991) made note of several larval chaetotaxy characters that suggested an affinity between Danaini and Ithomiini [including one discovered by Müller (1886)], but had very limited material available for comparison. Ackery *et al.* (1999) listed a behavioural trait, 'adults imbibe pyrrolizidine alkaloids from damaged or withered plants, or from nectar, and use them to make sex pheromones and/or for defence', as the synapomorphy uniting Danainae in their analysis. Freitas & Brown (2004) improved significantly on this, listing four adult and ten larval/pupal morphological characters that define the Danainae (Ithomiini + Tellervini + Danaini).

Fox (1956) considered Ithomiini to be one of the most primitive nymphalid groups, and to be more closely related to Satyrinae than Danaini, with particular affinity to Haeterini. Fox established the basis for the currently accepted classification of Ithomiini, and engaged in comprehensive monography of a number of genera (Fox, 1949, 1956, 1960, 1967; Fox & Real, 1971). Table 1 shows Fox's (1956) and subsequent classifications of ithomiine genera. A few new genera have been split out of old larger genera in recent times (e.g. Brown & Freitas, 1994; Constantino, 1999), but the generic classification has remained relatively stable. Relationships among genera, implied by these classifications, are much less stable, with some genera, such as *Methona*, *Placidina*, *Epityches* and *Aeria*, assigned to different tribes in almost every different scheme.

There have been several recent hypotheses of relationships among ithomiine genera based on phylogenetic analyses of morphological features. Brown & Freitas (1994) published an exemplar study of forty-one genera represented by characters from all life stages, rooted with the Australasian genus *Tellervo*. They obtained a well-resolved tree (Fig. 1A) that largely corroborates traditional tribal classification. An analysis by Motta (2003) (Fig. 1B), based on the morphology of first instar larvae, bears some similarity to the hypothesis of Brown & Freitas (1994) and to traditional classifications, but close scrutiny reveals that the two topologies share only three of a possible twenty-two components in common. Again, among the peripatetic taxa are *Methona*, *Placidula* and *Aeria*.

Molecular data may provide a valuable complement to morphological evidence, particularly in situations in which groups are weakly supported or unstable in traditional analyses (Miller *et al.*, 1997). In this paper, we analyse sequences of three gene regions to assess the relationships among ithomiine genera and to test the monophyly of the various hypothesized subtribes. Sampling is quite complete, with only three small and rarely collected genera (*Eutresis*, *Aremfoxia*, *Haenschia*) not represented. The resultant cladogram is well supported and provides a robust framework for studying evolutionary patterns and processes in this intriguing group of butterflies. The relationship among Ithomiini, Danaini and Tellervini and the position of Danainae within Nymphalidae will be addressed in a separate paper.

Materials and methods

Taxon sampling

The taxa examined in the current analysis are listed in Table 2. Ninety-nine exemplars representing forty-one ithomiine genera (and several recently synonymized genera, such as *Rhodussa*, *Hypomenitis* and *Prittwitzia*) and eighty-one species are included, as well as five outgroup genera representing the other two danaine tribes, Tellervini and Danaini. The three unsampled genera, *Eutresis*, *Aremfoxia* and *Haenschia*, are all small and considered to be closely related to sampled genera (*Athesis*, *Hypothyris* and *Episcada*, respectively). It is doubtful that their absence from the current analysis will have a major impact on the implied pattern of relationships, but we are sustaining our efforts to obtain representatives of as many taxa as possible.

Adult butterflies were netted in the field by the authors and numerous colleagues. Specimens were preserved either dry or in 95–100% ethanol (with wings removed and preserved dry to preserve pigmentation). Voucher wings and abdomens were prepared as in Brower (1996) and are maintained by the first author [for ultimate deposition in the American Museum of Natural History (AMNH)], except as noted in Table 2. Sequences for six samples published by Wahlberg *et al.* (2003) and Mallarino *et al.* (2005) were downloaded from GenBank.

DNA extraction, polymerase chain reaction (PCR) and sequencing

DNA was extracted from individual butterflies. In most cases, the head and thorax were ground, while the abdomen and appendages were preserved as voucher material. In a few instances (rare species that are poorly represented in collections), DNA was extracted from two dried legs so that the voucher specimen could be retained as a relatively complete pinned specimen. If the opportunity is available, we prefer to extract as much DNA as possible and preserve it indefinitely (frozen at $-20\,^{\circ}\text{C}$). The first author retains DNA samples, but they will ultimately be deposited at the AMNH. DNA was purified using either sodium dodecylsulphate (SDS)–phenol–chloroform extraction (see Brower, 1994) or DNAeasy Kits (Qiagen, Venlo, Netherlands), according to the manufacturer's instructions, with an initial 3-h incubation at $55\,^{\circ}\text{C}$ and a final elution volume of $300\,\mu\text{l}$.

For each specimen, DNA from three gene regions was amplified and sequenced: 2335 bp of the mitochondrial

Table 1. Representative classifications of ithomiine genera. In this paper, 'ithomiinae' is treated as a tribe of Danainae, and the tribes listed below are treated as subtribes. The order of each classification (when not alphabetical) presumably represents a hypothesized primitive–derived continuum. The author is listed after the first occurrence of each name. Synonymies are interpreted according to Lamas (2004).

Fox (1956)	Harvey (1991)	Motta (2003)	Lamas (2004)
Ithomiinae Kirby	Ithomiinae	Ithomiinae	Ithomiinae
Tithoreini Fox	Tithoreini	Tithoreini	Tithoreini
Roswellia Fox 1948[a]	*Elzunia*	*Aeria*	*Elzunia*
Athesis Doubleday 1847	*Tithorea*	*Elzunia*	*Tithorea*
Patricia Fox 1940	(new tribe)	*Tithorea*	*Aeria*
Eutresis Doubleday 1847	*Aeria*	Melinaeini	Melinaeini
Olyras Doubleday 1847	(new tribe)	*Athesis*	*Athesis*
Athyrtis C & R Felder 1862	*Roswellia*	*Athyrtis*	*Eutresis*
Tithorea Doubleday 1847	*Athesis*	*Eutresis*	*Athyrtis*
Elzunia Bryk 1937	*Patricia*	*Melinaea*	*Paititia*
Melinaeini Clark	Methonini	*Olyras*	*Olyras*
Melinaea Hübner 1816	*Methona* Doubleday 1847	*Paititia*	*Patricia*
Mechanitini Fox	(new tribe)	*Patricia*	*Melinaea*
Xanthocleis Boisduval 1870[b]	*Placidula*[f]	*Roswellia*	Mechanitini
Mechanitis Fabricius 1807	Melinaeini	Mechanitini	*Methona*
Sais Hübner 1816	*Athyrtis*	*Forbestra*	*Thyridia*
Scada Kirby 1871	*Olyras*	*Mechanitis*	*Scada*
Napeogenini Fox	*Eutresis*	*Methona*	*Sais*
Hyalyris Boisduval 1870	*Melinaea*	*Sais*	*Forbestra*
Hypothyris Hübner 1821	Mechanitini	*Scada*	*Mechanitis*
Rhodussa d'Almeida 1939[c]	*Paititia* Lamas	*Thyridia*	Napeogenini
Napeogenes Bates 1862	*Thyridia*	Ithomiini	*Aremfoxia*
Garsauritis d'Almeida 1938[d]	*Sais*	*Aremfoxia*	*Epityches*
Ithomiini Zerny and Beier	*Scada*	*Garsauritis*	*Hyalyris*
Ithomia Hübner 1816	*Forbestra* Fox 1967	*Megoleria*	*Napeogenes*
Pagyris Boisduval 1870	*Mechanitis*	*Hyalyris*	*Hypothyris*
Miraleria Haensch 1903[e]	Oleriini	*Hyposcada*	Ithomiini
Placidula d'Almeida 1922[f]	*Hyposcada*	*Hypothyris*	*Placidina* d'Almeida 1928
Oleriini Fox	*Ollantaya* Brown & Freitas 1994[g]	*Ithomia*	*Pagyris*
Hyposcada Godman & Salvin 1879	*Oleria*	*Napeogenes*	*Ithomia*
Oleria Hübner 1816	*Megoleria* Constantino 1999	*Oleria*	Oleriini
Aeria Hübner 1816	Napeogenini	*Ollantaya*[g]	*Megoleria*
n. gen.	*Epityches* d'Almeida 1938	*Pagyris*	*Hyposcada*
Dircennini d'Almeida	*Rhodussa*[c]	*Rhodussa*[c]	*Oleria*
Callithomia Bates 1862	*Aremfoxia* Real 1971	Dircennini	*Ollantaya*
Dircenna Doubleday 1847	*Napeogenes*	*Callithomia*	Dircennini
Velamysta Haensch 1909	*Garsauritis*	*Ceratinia*	*Ceratinia*
Ceratinia Hübner 1816	*Hyalyris*	*Ceratiscada*[d]	*Callithomia*
Hyalema Forbes 1942	*Hypothyris*	*Dircenna*	*Dircenna*
Episcada Godman & Salvin 1879	Ithomiini	*Dygoris*[i]	*Hyalema*
Pteronymia Butler & Druce 1872	*Miraleria*[e]	*Episcada*	*Episcada*

Table 1. Continued.

Fox (1956)

- Godyridini d'Almeida
- *Thyridia* Hübner 1816[h]
- *Epityches* d'Almeida 1938
- *Godyris* Boisduval 1870
- *Dygoris* Fox 1945[i]
- *Pseudoscada* Godman & Salvin 1879
- *Hymenitis* Hübner 1816[l]
- *Hypoleria* Godman & Salvin 1879
- *Mcclungia* Fox 1940
- *Hypomenitis* Fox 1945[m]
- *Veladyris* Fox 1945
- *Heterosais* Godman & Salvin 1880

Harvey (1991)

- *Pagyris*
- *Ithomia*
- Dircennini
- *Callithomia*
- *Velamysta*
- (new genus)
- *Dircenna*
- *Hyalenna*
- (new tribe)
- *Ceratinia*
- *Ceratiscada* Brown & d'Almeida 1970[j]
- *Prittwitzia* Brown & Ebert 1970[k]
- *Episcada*
- *Pteronymia*
- *Mcclungia*
- Godyridini
- *Godyris*
- *Dygoris*[i]
- *Pseudoscada*
- *Hypomenitis*[m]
- *Greta* Hemming
- *Hypoleria*
- *Mcclungia*
- *Veladyris*
- *Heterosais*

Motta (2003)

- *Epityches*
- (new genus)
- *Godyris*
- *Greta*
- *Heterosais*
- *Hyalenna*
- *Hypoleria*
- *Hypomenitis*[m]
- *Mcclungia*
- *Miraleria*[e]
- *Placidula*[f]
- *Prittwitzia*[k]
- *Pseudoscada*
- *Pteronymia*
- *Talamancana* Haber, Brown & Freitas 1994[n]
- *Veladyris*
- *Velamysta*

Lamas (2004)

- *Haenschia* Lamas 2004
- *Pteronymia*
- Godyridini
- *Velamysta*
- *Godyris*
- *Veladyris*
- *Hypoleria*
- *Brevioleria* Lamas 2004
- *Mcclungia*
- *Greta*
- *Heterosais*
- *Pseudoscada*

[a]*Roswellita* is a junior subjective synonym of *Athesis* Doubleday.
[b]*Xanthocleis* is a junior subjective synonym of *Thyridia* Hübner.
[c]*Rhodussa* is a junior subjective synonym of *Hypothyris* Hübner.
[d]*Garsauritis* is a junior subjective synonym of *Hypothyris* Hübner.
[e]*Miraleria* is a junior subjective synonym of *Pagyris* Boisduval.
[f]*Placidula* is not a valid name under the rules of nomenclature and was replaced by *Placidina* d'Almeida.
[g]*Ollantaya* is a junior subjective synonym of *Oleria* Hübner.
[h]*Thyridia* is here used incorrectly for *Methona* Doubleday.
[i]*Dygoris* is a junior subjective synonym of *Godyris*.
[j]*Ceratiscada* is a junior subjective synonym of *Episcada* Godman & Salvin.
[k]*Prittwitzia* is a junior subjective synonym of *Episcada* Godman & Salvin.
[l]*Hymenitis* Hübner is a preoccupied name and was replaced by *Greta* Hemming.
[m]*Hypomenitis* is a junior subjective synonym of *Greta* Hemming.
[n]*Talamancana* is a junior subjective synonym of *Pteronymia* Butler & Druce.
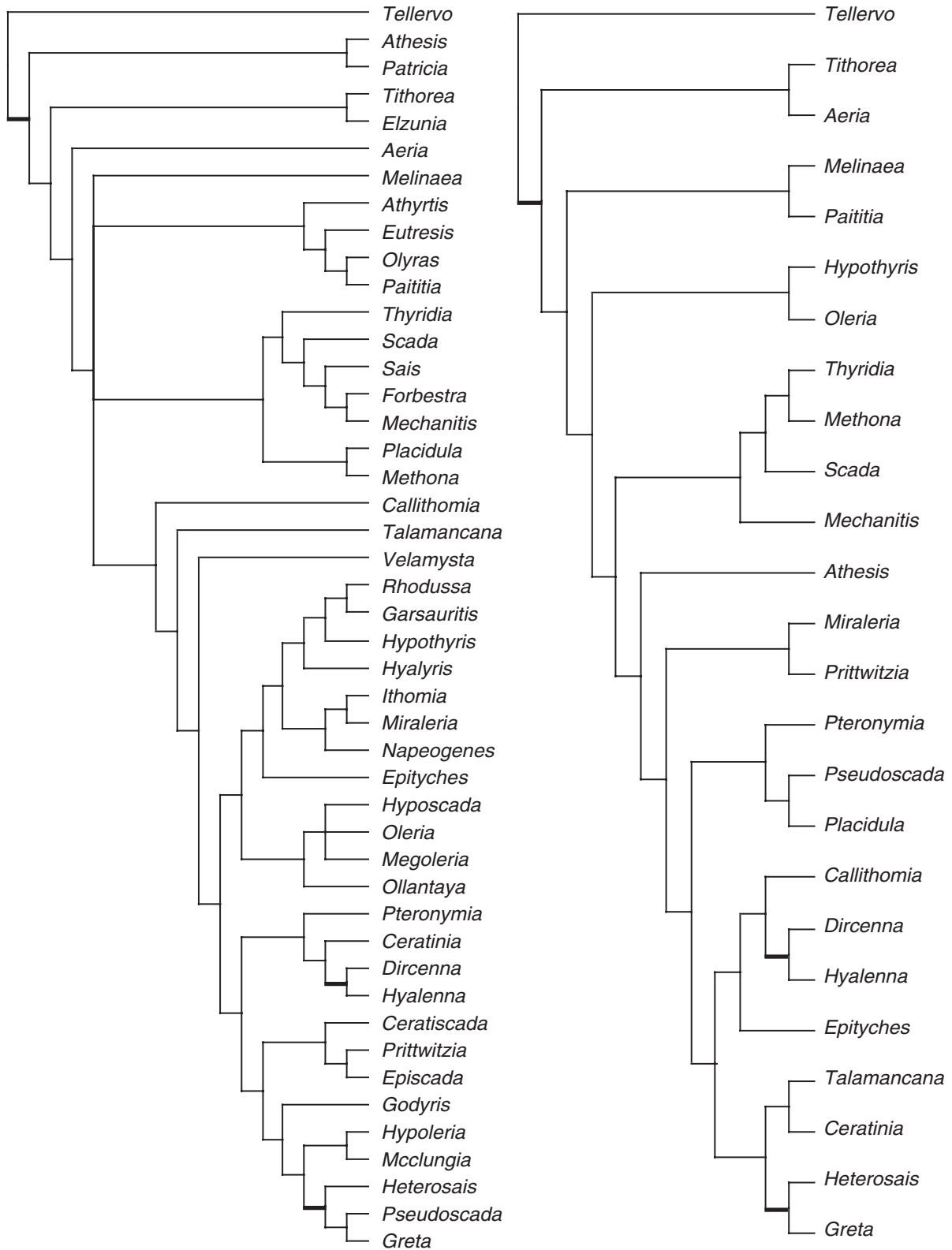
Fig. 1. Phylogenetic hypotheses for Ithomiini redrawn from Brown & Freitas (1994; fig. 1c) (A) and Motta (2003; fig. 19.5) (B). The thick branches are the components shared in common between the two trees.

Table 2. Ithomiini and outgroup taxa examined in this study. Voucher specimens have been retained by the first author and will be deposited in the American Museum of Natural History, except as noted. Abbreviated locality data are presented here. Full data are preserved with the voucher specimens and are available upon request. Missing sequences are indicated by "--"

Taxon	Collection locality	Sample code	GenBank Accession number COI–COII	wg	EF-1α
ITHOMIINI					
Aeria eurimedia (Cramer)	Ecuador: Esmereldas, Finca Durán	E-24-2	DQ157474	--	DQ177921
Aeria eurimedia (Cramer)	Ecuador: Esmereldas, Esmereldas	E-26-2	DQ157475	DQ175478	DQ177922
Athesis clearista Doubleday	Colombia: Antioquia, Venecia	C-24-1[a]	DQ157476	DQ175479	DQ177923
Athyrtis mechanitis C. & R. Felder	Brazil: Rondônia, Ariquemes	RB359	DQ069230	DQ071873	DQ073022
Brevioleria aelia (Hewitson)	Ecuador: Sucumbios, El Recodo	E-44-4	DQ157477	DQ175480	DQ177924
Callithomia hezia (Hewitson)	Ecuador: Esmereldas, Finca Durán	E-24-4	DQ157478	DQ175481	DQ177925
Callithomia lenea (Cramer)	Peru: Madre de Dios, Tambopata	PE-18-02	DQ069232	DQ071874	DQ073024
Callithomia lenea (Cramer)	Brazil: Rondônia, Ariquemes	RB380	DQ069231	--	DQ073023
Ceratinia neso (Hübner)	Brazil: Rondônia, Ariquemes	RB324	DQ157479	AF246559	DQ177926
Ceratinia neso (Hübner)	Brazil: Rondônia, Ariquemes	RB325	DQ157480	DQ175482	DQ177927
Ceratinia tutia (Hewitson)	Ecuador: Napo, Tena	E-02-08	DQ157481	DQ175483	DQ177928
Ceratinia tutia (Hewitson)	Ecuador: Carchi, Lita	E-17-6	DQ157482	--	DQ177929
Dircenna dero (Hübner)	Brazil: São Paulo, Jundiai	B-16-2	DQ157483	DQ175484	DQ177930
Dircenna dero (Hübner)	Ecuador: Sucumbios, El Recodo	E-44-3	DQ069233	DQ073010	DQ073025
Dircenna dero (Hübner)	Peru: Huanuco, Tingo Maria	PE-19-17	DQ157484	DQ175485	DQ177931
Dircenna dero (Hübner)	Brazil: Rondônia, Ariquemes	RB305	DQ157485	AF246571	--
Dircenna paradoxa (Staudinger)	Ecuador: Sucumbios, La Bonita	E-39-59	DQ157486	DQ175486	DQ177932
Elzunia pavonii (Butler)	Ecuador: Loja, Macara	E-12-1	DQ069234	AF246562	DQ073026
Episcada apuleia (Hewitson)	Ecuador: Loja, Rio Chiriguana	E-29-3	DQ157487	DQ175487	DQ177933
Episcada carcinia Schaus	Brazil: São Paulo, Campinas	B-15-5	DQ157488	DQ175488	DQ177934
Episcada carcinia Schaus	Brazil: São Paulo, Atibaia	B-17-1	DQ157489	DQ175489	DQ177935
Episcada clausina (Hewitson)	Brazil: São Paulo, Campinas	B-15-6	DQ157490	DQ175490	DQ177936
Episcada hymenaea (Prittwitz)	Brazil: São Paulo, Cotia	B-12-3	DQ157491	DQ175491	DQ177937
Epityches eupompe (Geyer)	Brazil: São Paulo, Cotia	B-12-2	DQ157492	DQ175492	DQ177938
Forbestra equicola (Cramer)	French Guiana: Kaw	G-36-2	DQ157493	DQ175493	DQ177939
Forbestra olivencia (Bates)	Peru: Madre de Dios, Tambopata	PE-18-3	DQ157494	--	DQ177940
Godyris duillia (Hewitson)	Ecuador: Zamora-Loja hwy km 40	E-37-1	DQ157495	DQ175494	DQ177941
Godyris zavaleta (Hewitson)	Ecuador: Sucumbios, El Recodo	E-44-1	DQ069235	DQ073011	DQ073027
Greta andromica (Hewitson)	Ecuador: Sucumbios, La Bonita	E-39-29	DQ157496	DQ175495	DQ177942
Greta annette (Guérin)	Costa Rica: Monte Verde	CR-1-5	DQ157497	DQ175496	DQ177943
Greta hermana (Haensch)	Ecuador: Sucumbios, La Bonita	E-39-46	DQ069236	DQ073012	DQ073028
Greta morgane (Geyer)	(captive-reared specimen)	70-9[b]	AY090206	AY090139	AY090172
Greta polissena (Hewitson)	Ecuador: Carchi, Lita	E-17-9	DQ157498	DQ175497	DQ177944
Greta theudelinda (Hew.)	Ecuador: Loja, S. Pedro de Vilcab.	E-28-4	DQ157499	--	DQ177945
Heterosais guilia (Hewitson)	Peru: Huanuco, Tingo Maria	PE-19-14	DQ157500	DQ175498	DQ177946
Hyalenna sp.	Ecuador: Zamora, Q. Chorillos	E-30-5	DQ157501	DQ175499	DQ177947
Hyalyris antea (Hewitson)	Ecuador: Zamora, Q. Chorillos	E-30-4	DQ069237	DQ073013	DQ073029
Hyalyris antea (Salvin)	Peru: Cuzco, Q. Chaupimayo	PE-10-14	DQ157502	AF246589	DQ177948
Hypoleria sp.	Brazil: Rondônia, Ariquemes	RB358	DQ157503	AF246576	--
Hypoleria lavinia (Hewitson)	Brazil: São Paulo, Jundiai	B-16-8	DQ157504	DQ175500	DQ177949
Hyposcada anchiala (Hewitson)	Peru: San Martin, Pte. Serranayacu	AW-02-0716[c]	DQ078359	DQ175501	DQ177950
Hyposcada illinissa (Hewitson)	Ecuador: Esmereldas, R. Chuchuvi	E-20-1	DQ157505	--	DQ177951
Hypothyris cantobrica (Hewitson)	Brazil: Rondônia, Ariquemes	RB360	DQ157506	DQ175502	DQ177952
Hypothyris daphnis d'Almeida	Brazil: Rondônia, Ariquemes	RB237	DQ157507	AF246591	DQ177953
Ithomia avella Hewitson	Ecuador: Sucumbios, El Higueron	E-35-5	DQ157508	DQ175503	DQ177954
Ithomia drymo Hübner	Brazil: São Paulo, Jundiai	B-16-5	DQ069238	DQ073014	DQ073030
Ithomia eleonora Haensch	Peru: Jun'n, Qbda. Siete Jeringas	9117[d]	AY713053	AY704113	AY704027
Ithomia iphianassa Doubleday	Panama: Darién, Cana	8804[d]	AY713064	AY704119	AY704033
Ithomia lagusa Hewitson	Ecuador: Zamora, Q. Chorillos	E-30-6	AY713086	AY704136	AY704050
Ithomia salapia Hewitson	Ecuador: Sucumbios, El Recodo	E-44-2	DQ157509	DQ175504	DQ177955
Ithomia terra Hewitson	Ecuador: Pichincha, Tandapi	E-15-2	DQ157510	DQ175505	DQ177956
Mcclungia cymo (Hübner)	Brazil: São Paulo, Campinas	B-15-1	DQ157511	DQ175506	DQ177957
Mcclungia cymo (Hübner)	Brazil: São Paulo, Campinas	B-19-3	DQ157512	DQ175507	DQ177958
Mechanitis polymnia (Linnaeus)	Brazil: São Paulo, Campinas	B-15-2	DQ157513	--	DQ177959
Mechanitis polymnia (Linnaeus)	Panama: Gamboa	P4	DQ157514	DQ175508	DQ177960

Mechanitis polymnia (Linnaeus)	Brazil: Rondônia, Ariquemes	RB233	DQ157515	AF246560	DQ177961
Megoleria susiana (C. & R. Felder)	Peru: San Martin, La Antena	AW-02-0402[c]	DQ157516	DQ175509	DQ177962
Melinaea menophilus (Hewitson)	Brazil: Rondônia, Ariquemes	RB288	DQ069240	AF014146	DQ073032
Melinaea isocomma Forbes	Ecuador: Sucumbios, La Bonita	E-39-52	DQ157517	DQ175510	DQ177963
Methona confusa Butler	Peru: San Martin, Tarapoto	AW-02-0622	DQ157518	DQ175511	DQ177964
Methona themisto (Hübner)	Brazil: São Paulo, M. Guaçu	B-20-1	DQ157519	DQ175512	DQ177965
Methona sp.	Brazil: Rondônia, Ariquemes	RB296	DQ069239	DQ073015	DQ073031
Napeogenes apulia (Hewitson)	Ecuador: Sucumbios, La Bonita	E-39-58	DQ157520	--	DQ177966
Napeogenes cranto C. & R. Felder	Panama: Chiriquî, Qbda. Hornito	8313[d]	AY713036	AY704098	AY704010
Napeogenes duessa (Hewitson)	Ecuador: Napo, Rio Tiputini	E-46-2	DQ157521	--	DQ177967
Napeogenes larilla (Hewitson)	Ecuador: Sucumbios, La Bonita	E-39-47	DQ069241	DQ073016	DQ073033
Napeogenes pharo (C. & R. Felder)	Brazil: Rondônia, Ariquemes	RB235	DQ157522	AF246601	DQ177968
Napeogenes sylphis (Guérin-Méneville)	Ecuador: Napo, Rio Tiputini	E-46-1	DQ157523	DQ175513	DQ177969
Oleria aquata (Weymer)	Brazil: Rondônia, Ariquemes	RB321	DQ157524	AF246558	DQ177970
Oleria onega (Hewitson)	Peru: S. Martin, Pongo-Barranquita	AW-02-0515[c]	DQ078390	DQ085441	DQ085453
Oleria santineza (Haensch)	Ecuador: Sucumbios, La Bonita	E-39-25	DQ157525	DQ175514	DQ177971
Ollantaya aegineta (Hewitson)	Ecuador: Zamora, Loja-Zamora rd.	E-32-1	DQ157526	DQ175515	DQ177972
Olyras insignis Salvin	Ecuador: Esmereldas, Finca Durán	E-24-1	DQ157527	--	DQ177973
Pagyris cymothoe (Hewitson)	Ecuador: Pichincha, San Antonio	E-16-2	DQ157528	--	DQ073035
Pagyris cymothoe (Hewitson)	Ecuador: Loja, S. Pedro de Vilcab.	E-28-3	DQ157529	--	DQ177974
Pagyris ulla (Hewitson)	Ecuador: Sucum., La Bonita-Tulcan	E-43-18	DQ157530	DQ175516	DQ177975
Paititia neglecta Lamas	Peru: San Martin	AW-02-1244[c]	DQ073038	DQ073017	DQ073034
Patricia dercyllidas (Hewitson)	Ecuador: Sucumbios, El Higueron	E-35-7	DQ157531	DQ175517	DQ177976
Placidina euryanassa (C. & R. Felder)	Brazil: São Paulo, Jundiai	B-16-1	DQ157532	DQ175518	DQ177977
Placidina euryanassa (C. & R. Felder)	Brazil: São Paulo, Atibaia	B-17-2	DQ157533	DQ175519	DQ177978
Pseudoscada erruca (Hewitson)	Brazil: S. Paulo, Campos do Jordão	B-13-3	DQ157534	--	DQ177979
Pseudoscada timna (Hewitson)	Brazil: São Paulo, M. Guaçu	B-20-3	DQ157535	DQ175520	DQ177980
Pseudoscada timna (Hewitson)	Ecuador: Carchi, Lita	E-17-4	DQ157536	DQ175521	DQ177981
Pteronymia aletta (Hewitson)	Ecuador: Esmereldas, Esmereldas	E-26-1	DQ157537	DQ175522	DQ177982
Pteronymia hara (Hewitson)	Ecuador: Sucumbios, La Bonita	E-39-43	DQ157538	DQ175523	DQ177983
Pteronymia oneida (Hewitson)	Ecuador: Sucum., La Bonita-Tulcan	E-43-1	DQ157539	DQ175524	DQ177984
Pteronymia teresita (Hewitson)	Ecuador: Loja, R. Chiriguana	E-29-1	DQ157540	DQ175525	DQ177985
Pteronymia ticida (Hewitson)	Ecuador: Sucumbios, La Bonita	E-39-34	DQ157541	DQ175526	DQ177986
Pteronymia tucuna (Bates)	Peru: Cuzco, Q. Chaupimayo	PE-10-12	DQ157542	AF246606	DQ177987
Pteronymia veia (Hewitson)	Ecuador: Sucum., La Bonita-Tulcan	E-43-16	DQ069042	DQ073018	DQ069242
Sais rosalia (Cramer)	Peru: Madre de Dios, Tambopata	PE-18-5	DQ157543	DQ175527	DQ177988
Scada reckia (Hübner)	Peru: Cuzco, Q. Chaupimayo	PE-10-15	DQ157544	DQ175528	DQ177989
Thyridia psidii (Linnaeus)	Peru: Cuzco, Q. Chaupimayo	PE-10-11	DQ157545	--	DQ177990
Thyridia psidii (Linnaeus)	Brazil: Rondônia, Ariquemes	RB314	AF014157*	AF014147	DQ177991
Tithorea harmonia (Cramer)	Peru: Cuzco, Rosalina	PE-12-3	--	--	DQ073037
Tithorea harmonia (Cramer)	Peru: Huanuco, Tingo Maria	PE-19-19	DQ069043	DQ073019	--
Tithorea harmonia (Cramer)	Venezuela: Barran., Delta Amacuro	V20	DQ157546	AF246561	DQ177992
Veladyris pardalis (Salvin)	Ecuador: Sucum., La Bonita–Tulcan	E-45-1	DQ157547	DQ175529	DQ177993
Velamysta phengites Fox	Ecuador: Sucumbios, La Bonita	E-39-32	DQ157548	DQ175530	DQ177994
Velamysta pupilla (Hewitson)	Ecuador: Sucum., La Bonita–Tulcan	E-43-3[e]	DQ071864	DQ073020	DQ073021
TELLERVINI					
Tellervo zoilus (Fabricius)	Australia: Queensland, Kirrana S. F.	QL1	DQ157549	AF246563	DQ177995
DANAINI					
Amauris ellioti Butler	Tanzania: Mbeya Range	(Wahlberg)[b]	AY218234	AY218272	AY218253
Danaus erippus (Cramer)	Argentina: Buenos Aires	DE-1	AY569158	--	--
Euploea camaralzeman Butler	(captive-reared specimen)	70-8[b]	AY090205	AY090138	AY090171
Lycorea cleobaea (Godart)	Brazil: Rondônia, Ariquemes	RB241	DQ157550	AF246567	DQ177996

COI–COII, *cytochrome oxidase subunits I and II*; EF-1α, *Elongation factor 1-alpha*; wg, *wingless*.
[a]Voucher retained at UNICAMP, Campinas, Brazil.
[b]Voucher retained by N. Wahlberg (sequences from GenBank).
[c]Voucher retained at the Natural History Museum, London.
[d]Voucher retained by C. D. Jiggins. (sequences from GenBank)
[e]Voucher retained by K. R. Willmott.

cytochrome oxidase subunits I and II (COI–COII), 1260 bp of the *Elongation factor 1-alpha* gene (EF-1α) and 382 bp of the *wingless* gene. One taxon, the Peruvian Tithorea harmonia, is a combination of two specimens: EF-1α from PE-12-3 and COI-COII + wg from PE-19-19.

Primers for COI–COII include those listed in Brower & Jeansonne (2004) and Table 3, for EF-1α from Cho *et al.* (1995) and Table 3, and for *wingless* from Brower & DeSalle (1998). PCR amplifications were performed in a 50 µl reaction volume, with 3.1 µl of template, 6 µl of 25 µM MgCl$_2$, 5 µl of 10× buffer (0.1 M Tris-HCl, 0.1 M KCl, 1% Triton X-100, pH 8.3), 1 µl of 10 µM dNTPs, 0.4 µl of Taq polymerase, 1 µl of each primer (10 mM) (2 µl of each for the nuclear genes) and 32.5 µl of H$_2$O (30.5 µl for nuclear genes). The cycling profile for COI–COII was initial annealing at 95 °C for 3 min, followed by thirty-three cycles of 95 °C for 1 min, 45 °C for 1 min and 72 °C for 90 s, and a final extension period of 72 °C for 5 min. The cycling profile for *wingless* was the same as that for COI–COII, except that the annealing temperature was 62 °C. The cycling profile for EF-1α was 94 °C for 2 min, followed by thirty-seven cycles of 94 °C for 1 min, 62 °C for 1 min and 72 °C for 90 s, and a final extension period of 72 °C for 10 min. For all three gene regions, the PCR primers and additional internal primers were used for sequencing. Amplified DNA fragments were gene-cleaned with silica beads (Bio101, Qbiogene, irvine, CA) or Qiaquick PCR purification kits (Qiagen), cycle-sequenced in an MJ Research DNA Engine (Biorad, Hercules, CA), and run on an ABI 373 automated sequencer (Applied Biosystems, Foster City, CA) from sense and antisense strands. Some of the sequencing was outsourced to a commercial firm (Macrogen, Seoul, South Korea). Automated sequence outputs were edited manually and aligned by eye. The aligned data matrix is available on the web at http://wwwsciencet. regonstate. edu/systematics/browera/datasets/pubdata/ithomiini. html, and individual sequences have been submitted to GenBank (see Table 2 for accession codes). Some of the sequences were concurrently published in Whinnett et al. (2005).

Phylogenetic analysis

The data were concatenated and analysed as a single matrix under the parsimony criterion using PAUP*4.0b10 (Swofford,

2002). Characters were treated as unordered and weighted equally, with inferred gaps encoded as 'missing'. A tree bisection and reconnection (TBR) heuristic search with 1000 random addition sequences was performed, and two most parsimonious cladograms were discovered. The tree was rooted with *Tellervo*, and other non-ithomiine taxa were included in the ingroup to test the monophyly of Ithomiini. The structure of the data was explored with separate analyses of each gene region, using the same procedures as the simultaneous analysis. Branch support (BS; Bremer, 1988, 1994) and partitioned branch support (PBS; Baker & DeSalle, 1997; Baker *et al.*, 1998) parameters were calculated by the anticonstraint method with ten random addition sequences per node. Fractional PBS values were rounded to two decimal places.

Incongruence amongst gene regions was assessed at each node by comparison of PBS values. Because a BS value of '1' is a summary that does not reveal conflict amongst partitions, a new parameter, the partition congruence index (PCI; A. V. Z. Brower, in prep.), was used to measure the degree of conflict amongst partitions relative to PBS. The PCI for a given branch is calculated as follows:

$$PCI = BS - ((\sum_{i=1}^{n} |PBS_j| - BS)/BS)$$

In words, the partition congruence index for a given branch equals the branch support minus (the difference between the sum of the absolute values of the branch support for each partition and the branch support, divided by the branch support). PCI is always equal to or less than BS for a given branch. It is equal to BS for a branch that is not contradicted by any partition, and becomes negative for nodes with low BS and one or more partitions with strongly negative PBS. Branches with a negative PCI can be considered weakly supported, and branches with highly negative values, indicating low overall support underlain by partitions with strong disagreement, should be re-examined. The PCI is discussed further in A.V.Z. Brower (in prep.).

The classification of Ithomiini discussed below is based on the results of the cladistic analysis. The nomenclatorial

Table 3. New polymerase chain reaction (PCR) and sequencing primers employed in this study. Additional primers are listed in the references cited in the 'Materials and methods' section. 'Strand' refers to the sense/antisense strand of the target gene region.

Name	Strand	3′ position	Sequence (5′–3′)
mtDNA COI–COII			
RUSH	S	1459[a]	TAC AAT TTA TCG CCT AAA CTT CAG CC
RON	S	1751[a]	GGA TCA CCT GAT ATA GCA TTC CC
JERRY	S	2183[a]	CAA CAT TTA TTT TGA TTT TTT GG
WYMAN	A	2317[a]	GYT GAG CTC AWA CAA TAA ATC CTA
NANCY	A	2191[a]	CCC GGT AAA ATT AAA ATA TAA ACT TC
JANE	A	2518[a]	TAA AAT TAC TCC TGT TAA TCC TCC
IMELDA	A	3812[a]	CAT TAG AAG TAA TTG CTA ATT TAC TA
EF-1α			
GENNIFER	A	3320[b]	CGC ACG GCA AAA CGA CCG AGR GG

COI–COII, *cytochrome oxidase subunits I and II*; EF-1α, *Elongation factor 1-alpha*; mtDNA, mitochondrial DNA.
[a]Primer positions based on *Drosophila yakuba* sequence (Clary & Wolstenholme, 1985).
[b]Primer positions based on *Drosophila melanogaster* sequence (Hovemann *et al.*, 1988).

philosophy is that all named superspecific taxa should be monophyletic. We provide names for subtribes representing monophyletic groups of genera, and recommend that a few small or monotypic genera should be synonymized (because recognizing them results in paraphyly of a larger genus). To preserve clarity with respect to current literature (e.g. Lamas, 2004), we do not formally alter generic affiliations here.

Results

The aligned data matrix consists of 3957 characters, 2250 of which are invariant and 1290 of which are parsimony informative. Some sequences are incomplete or missing for a few taxa (see Table 2), but these apparently do not impact on the ability of the data to provide relatively unambiguous resolution. The analysis of the entire data set yielded two most parsimonious trees with a length of 10 366 steps, the strict consensus of which is presented in Fig. 2.

Separate analyses of the three gene regions were conducted to investigate incongruence (Mickevich & Farris, 1981; Farris *et al.*, 1994). Although the data set as a whole is highly homoplastic, almost none of this (< 2%) is due to incongruence amongst gene regions (Table 4). Table 4 also shows that each gene region contributes positively to the overall support (sum of all branch support values) for the most parsimonious topology, with the mitochondrial DNA (mtDNA) yielding the most (77.2%), followed by EF-1α (17.9%) and *wingless* (4.9%). The mtDNA region provides the most support per nucleotide sequenced (0.59), followed by EF-1α (0.26) and wingless (0.23). The mtDNA region also provides the most support per informative site (1.64), followed by EF-1α (1.01) and *wingless* (0.61). Of the 100 resolved branches in the tree, mtDNA provides positive support to eighty-nine and contradicts eight, EF-1α supports sixty-four and contradicts twenty-seven, and *wingless* supports forty-four and contradicts thirty-seven (fifteen taxa are missing the *wingless* sequence). Twenty-eight branches are supported by all three gene regions, twenty-seven by mtDNA + EF-1α, nine by mtDNA + *wingless*, four by EF-1α + *wingless*, twenty-four by mtDNA only, five by EF-1α only and three by *wingless* only. Thus, although the mtDNA provides most of the phylogenetically informative characters, no single gene region drives the topology of the combined hypothesis of relationships, which is different from any of the trees implied by single genes analysed separately (results from separate analyses not shown). Support and partition congruence for individual clades are discussed below. In the following paragraphs, the numbers in parentheses after the names of taxa refer to numbered clades in Fig. 2.

Discussion

Implications of this analysis for the phylogeny and classification of Ithomiinae

Although Ithomiini (97) and Danaini (100) have long been considered close relatives (Bates, 1862), few unambiguous characters have been discovered to support the grouping (de Jong *et al.*, 1996; Freitas & Brown, 2004). Brower's (2000) analysis based on *wingless* sequences was the first study to provide explicit empirical support for a sister group relationship between Ithomiini and Danaini, although the result was not stable under successive approximations weighting. The monophyly of Ithomiini with respect to Danaini is strongly corroborated by this analysis (BS 7; PCI 6.86) [the monophyly of Danainae and the close relationship of Tellervini, Danaini and Ithomiini were supported by molecular data in Brower (2000) and morphological characters in Freitas & Brown (2004) and will be addressed in greater detail elsewhere].

In general, the results presented here imply a very similar pattern of relationships to those presented by Brown (1985, 1987) and Brown & Freitas (1994), which were based on morphology of adult and immature stages. However, there are some notable differences, particularly in the branching order near the base of the cladogram.

Previous classifications have implied that the subtribe Tithoreina is the sister group to all other ithomiines, based on larval host preference for Apocynaceae and aposematic larvae with 'danaoid' thoracic filaments (Brown, 1985, 1987; Ackery, 1988; Motta, 2003; Freitas & Brown, 2004). Brown & Freitas (1994) found *Athesis* + *Patricia* (and then Tithoreina) to be sister taxa to the remaining ithomiines. The molecular data imply that Melinaeina (96), composed of the genera *Melinaea*, *Athyrtis*, *Olyras* and *Paititia*, is the sister group to all other ithomiines. Known larval stages (from *Melinaea*, *Athyrtis* and *Olyras*) share with Tithoreina the putatively plesiomorphic 'danaoid' features, but feed on Solanaceae (Brown & Freitas, 1994).

The composition of the subtribe Melinaeina (96) implied by the molecular data is the same as that found by the combined morphological analysis of Brown & Freitas (1994) (see Fig. 1A), with *Paititia* as the sister taxon of *Olyras* (95: BS 17; PCI 16.94). This result differs from the suggestion of Lamas (1979), who described *Paititia* as 'very close to *Thyridia*' and placed the genus in Mechanitina, although he noted its similarity in a number of morphological structures to *Olyras*.

Apart from the exclusion of *Thyridia* + *Methona* (see below), the constitution of the well-supported subtribe Mechanitina (91: BS 13; PCI 12.92) implied by the molecular data (*Mechanitis*, *Forbestra*, *Scada*, *Sais*) is fairly traditional. *Forbestra* (86: BS 17; PCI 16.54) is strongly supported as sister group to *Mechanitis*, but the support for the monophyly of *Mechanitis* (88: BS 1; PCI 1) is weak. This may represent an artefact of missing data or insufficient sampling (all *Mechanitis* sampled are *M. polymnia*), although Fox (1967) stated that *Mechanitis* and *Forbestra* were closely related when he removed the latter from the former. The subtribe Mechanitini, including *Thyridia*, is easily recognized by morphological characters of both adults and immatures, and the exclusion of *Thyridia* based on the present results should be examined in further detail.

Tithoreina is a well-supported clade (4: BS 8; PCI 8) composed of the genera *Tithorea*, *Elzunia* and *Aeria*. Fox
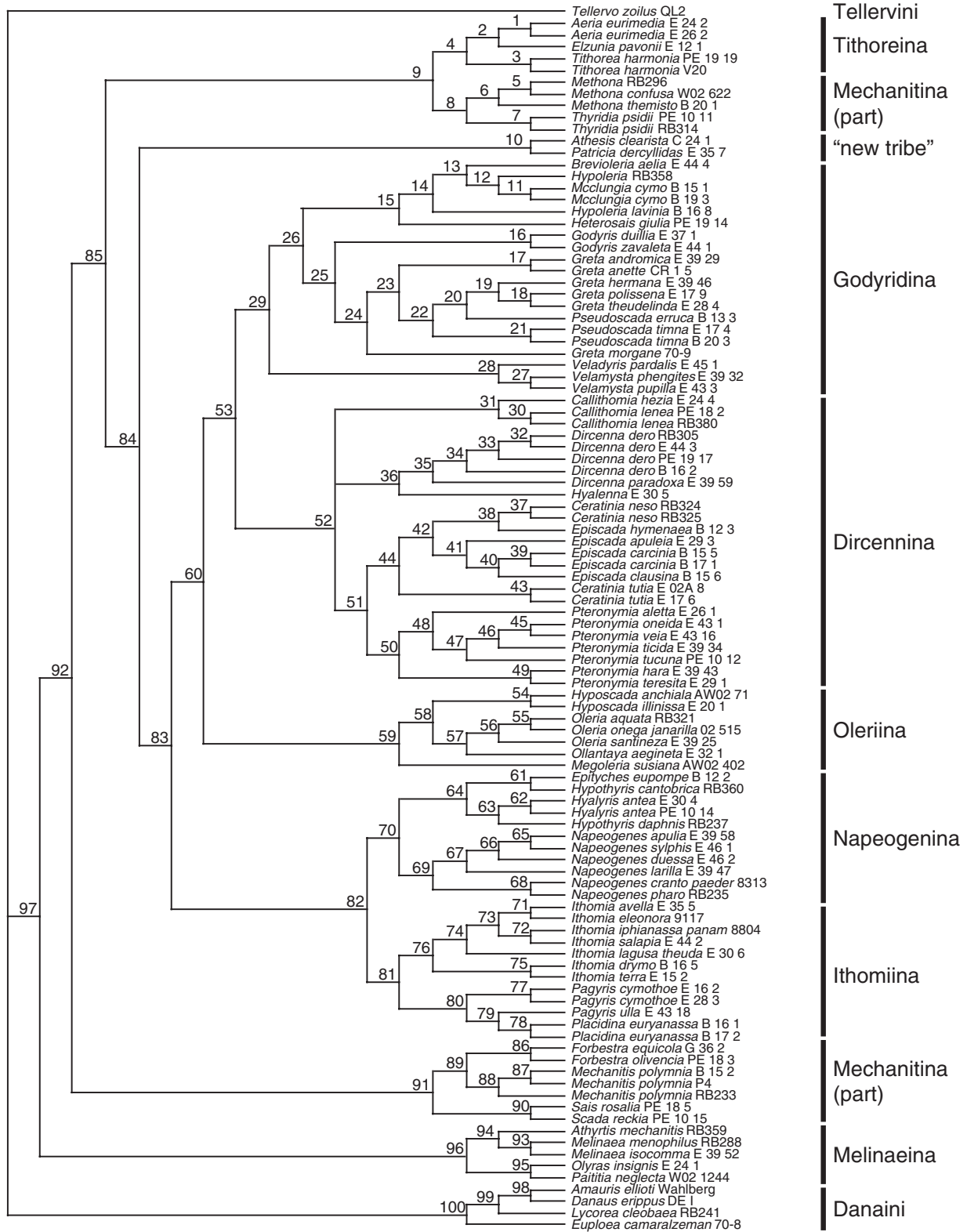
Fig. 2. Strict consensus of two most parsimonious trees, length 10 366 steps (CI × = 0.2100; RI = 0.5079). Clade numbers are indicated above the branches. Corresponding branch support values, partitioned branch support values and partition congruence indices are presented in Table 5. Taxa are identified by name and voucher code. The tree is rooted with *Tellervo*.

Table 4. Parameters of the data for individual gene regions and the entire matrix.

Gene region	No. of bases	Informative sites	Min. steps	No. of trees	Shortest tree	Intrinsic homoplasy	D homoplasy	Total support
COI–COII	2335	834	1606	79	7556	5950 (78.7%)	–	1371.3
EF-1α[a]	1240	315	618	797	1832	1196 (65.3%)	–	318.2
Wingless[b]	382	141	293	Thousands	809	516 (63.8)%	–	86.5
All data	3957	1290	2517	2	10366	–	169 (1.6%)	1776

COI–COII, *cytochrome oxidase subunits I and II*; EF-1α, *Elongation factor 1-alpha*.
[a]Two taxa missing EF-1α sequences were deleted from the analysis.
[b]Fifteen taxa missing *wingless* sequences were deleted from the analysis.

(1956) placed *Aeria* in Oleriini, but it has since been recognized as belonging in a separate tribe based on larval morphology and host associations (Ackery, 1988; Brown & Freitas, 1994). Keith Brown (UNICAMP, Campinas, pers. comm.) has described *Aeria eurimedia* as 'just a tiny *Tithorea*', and the molecular results support his view.

The sister relationship of *Methona* and *Thyridia* has not been proposed before, although Brown & Freitas (1994), Motta (2003) and Lamas (2004) placed both genera in the Mechanitina clade. The two genera are so superficially similar in appearance that their nomenclature has been confused in the past [Fox (1956) and others called *Methona* '*Thyridia*' and *Thyridia* '*Xanthocleis*']. The sister relationship implied by these data (8) is fairly well supported by the mtDNA (BS 5; PCI 0.66), although quite strongly contradicted by the nuclear genes. At present, no morphological characters are known to support this clade. The pupae of *Methona* and *Thyridia* illustrated in Brown & Freitas (1994) appear to be dorsoventrally longer and less curvaceous than many other ithomiine pupae, which tend to have compressed abdomens and be bent at the thorax–abdomen junction. However, the pupae of *Thyridia* and *Mechanitis* are almost identical (A. V. L. Freitas, pers. obs.), suggesting that the position of *Thyridia* may change with the addition of morphological characters. Another novel pattern suggested by these data, but not supported by morphological evidence to date, is the association of *Methona* + *Thyridia* with Tithoreina (9: BS 5; PCI 1.44), which is moderately well supported, but again driven by the mtDNA and opposed by the nuclear genes.

Athesis + *Patricia*, hypothesized as sister taxa by Brown & Freitas (1994), are strongly supported by the molecular data (10: BS 19; PCI 19), and have been regarded as a 'new tribe' by several authors. The position of this clade with respect to Tithoreina + Methonina (9) and Mechanitina (92) is not well resolved, although the branch between these clades and the remainder of Ithomiini (83: BS 13; PCI 13) is strongly supported by all three gene regions. Branches 84 and 85 both have negative PCI scores, with the mtDNA strongly conflicting with the other two gene regions.

Ithomiina is also a well-supported monophyletic group (81: BS 19; PCI 18.32), and is well supported as the sister group of Napeogenina (82: BS 8; PCI 6.0), which is also well supported (70: BS 5; PCI 3.52). Ithomiina consists of the genera *Ithomia*, *Pagyris* and *Placidina*. The last two

genera are strongly supported as sister taxa (80: BS 19; PCI 18.32), with *Pagyris* weakly paraphyletic with respect to *Placidina*. Thus, despite its morphological autapomorphies, the separation of *Placidina* into its own tribe does not seem warranted. A recent study of *Ithomia* (Mallarino *et al.*, 2005; from which *Ithomia eleonora*, *I. lagusa* and *I. iphianassa* were drawn for this study) provides much more detailed sampling within the genus, but does not address the relationships between *Ithomia* and other members of the subtribe. Napeogenina is moderately well supported: branches 61 (*Epityches* + '*Rhodussa*' *cantobrica*) and 64 (*Epityches* + *Hyalyris* + *Hypothyris* + '*Rhodussa*') have amongst the lowest PCI scores (− 8.59 and − 7.81, respectively) of any in the entire tree, with the signal from mtDNA contradicted strongly by both nuclear genes. If *Rhodussa* is included in *Hypothyris*, as preferred by Lamas (2004), this cladogram implies that *Hypothyris* is paraphyletic with respect to both *Hyalyris* and *Epityches*. Future work will increase sampling in this part of the tree with the hope that a denser representation of taxa will provide better support for the subtribe and its constituent genera.

Oleriina is a well-supported monophyletic group (59: BS 14; PCI 13.62) composed of the genera *Hyposcada*, *Oleria* and the recently described *Megoleria* and *Ollantaya* (even more recently synonymized; Lamas, 2004). Species of both of these newer genera were formerly placed in *Oleria*, but the current analysis implies that monophyly of *Oleria* is achieved by the removal, at least, of *Megoleria*. A more intensive exemplar study of Oleriina is presented elsewhere (Whinnett *et al.*, 2005).

Dircennina in the broad sense is well supported (52: BS 12; PCI 12) and, although there is a basal polytomy in the strict consensus tree reflecting the separation of tribes (Callithomia, Dircennina in the narrow sense and Ceratiniina) hypothesized by Harvey (1991), Ceratiniina is only weakly supported as distinct from a paraphyletic Dircennina s.s. (51: BS 1; PCI 0.66). All genera with more than one representative sampled are recovered as monophyletic, except *Ceratinia* and *Episcada*, which are paraphyletic with respect to one another. Dircennina was not recovered as a monophyletic group in the morphological analyses of Brown & Freitas (1994).

Fox (1945) erected the genus *Veladyris*, which he placed in Godyridina, by removing a single species from its former

Table 5. Support indices for the branches in Fig. 2.

Clade number	Partitioned branch support			Branch support	Partition congruence index
	COI–COII	wg	EF-1α		
1	88	−3	15	100	99.94
2	3	0	0	3	3
3	20	4.5	10.5	35	35
4	0	1	7	8	8
5	46	0	2	48	48
6	56	21	38	115	115
7	47.5	18.5	36	102	102
8	15.86	−5.64	−5.21	5	0.66
9	13.91	−4.41	−4.5	5	1.44
10	11.5	2.5	5	19	19
11	42	3	0	45	45
12	6	0	0	6	6
13	5	2	3	10	10
14	15.33	0.17	5.5	21	21
15	0	0	8	8	8
16	19	3	3	25	25
17	13.5	1	4.5	19	19
18	7	0	0	7	7
19	11	−1	5	15	14.87
20	6.5	0	−2.5	4	2.75
21	3.88	0.38	1.75	6	6
22	2	1	2	5	5
23	−1	0	3	2	1
24	5.5	0.5	1	7	7
25	0.5	0	0.5	1	1
26	6.67	−2.83	2.17	6	5.06
27	28	8.5	8.5	45	45
28	1	−2	8	7	6.43
29	−1	2.75	2.25	4	3.5
30	43	−0.5	3.5	46	45.98
31	49	10	13	72	72
32	3	0	0	3	3
33	3	1	0	4	4
34	34	7.5	−7.5	35	33.56
35	−3.5	6.5	−1	2	−2.5
36	2	3.5	7.5	13	13
37	58.33	7.06	8.61	74	74
38	0.67	−0.17	0.5	1	0.67
39	10	0	1	11	11
40	17.75	0	4.25	22	22
41	1.14	1.07	7.79	10	10
42	2	−0.5	−0.5	1	−1
43	35.22	−0.17	9.94	45	44.99
44	15	6.25	1.75	23	23
45	7	1	0	8	8
46	5.5	0.5	2	8	8
47	21	2	2	25	25
48	3	4	3	10	10
49	6	0	−3	3	1
50	8	−0.5	4.5	12	11.92
51	0.33	−0.17	0.83	1	0.66
52	11	0.5	0.5	12	12
53	0.5	0.5	2	3	3
54	2.83	−3	3.17	3	1
55	14	9	6	29	29
56	−4	2	3	1	−7
57	0	2.17	−0.17	2	1.83

Table 5. Continued.

Clade number	COI–COII	wg	EF-1α	Branch support	Partition congruence index
58	5.33	−3.61	0.28	2	−1.62
59	7.66	−2.64	8.98	14	13.62
60	8.3	−4.65	−1.65	2	−4.3
61	12.58	−4.67	−5.69	2	−8.59
62	43.75	−5.75	−4	34	33.43
63	29.83	1.5	3.67	35	35
64	11.81	−4.13	−5.69	2	−7.81
65	0.5	−0.5	5	5	4.8
66	13	−0.5	14.5	27	26.96
67	−3	−0.5	4.5	1	−6
68	−3	−0.5	4.5	1	−6
69	13	1	1	15	15
70	8.7	−2.4	−1.3	5	3.52
71	6	0	−4	2	−2
72	10.67	0.83	7.5	19	19
73	−4.33	−0.17	11.5	7	5.71
74	23	−2.54	2.54	23	22.78
75	25.87	0.9	−1.77	25	24.86
76	−1	3	3	5	4.6
77	14.29	−1.07	3.79	17	16.87
78	73	0	29	102	102
79	7	0	−6	1	−11
80	20.33	−6.5	5.17	19	18.32
81	23.07	−3.97	−0.1	19	18.57
82	16	−5.17	−2.83	8	6
83	7.33	0.67	5	13	13
84	4	−2	−1	1	−5
85	10.72	−3.46	−5.26	2	−6.72
86	17.4	−3.9	3.5	17	16.54
87	1	0	0	1	1
88	1	0	0	1	1
89	15	0	16	31	31
90	6.5	−4	1.5	4	2
91	13.33	−0.5	0.17	13	12.92
92	4	−2	−1	1	−5
93	72	5.5	12.5	90	90
94	4	−2	−1	1	−5
95	14	3.5	−0.5	17	16.94
96	11	2.5	−5.5	11	6.63
97	4	3.5	−0.5	7	6.86
98	1	0	2	3	3
99	1	6	−4	3	0.33
100	−1.75	14.25	−0.5	12	11.63

COI–COII, *cytochrome oxidase subunits I and II*; EF-1α, *Elongation factor 1-alpha*; wg, *wingless*.

position in the dircennine genus *Velamysta*. The current analysis supports Lamas' recent classification (Lamas, 2004), implying that these two genera are sister taxa (28) and that both form a sister taxon to the remaining Godyridina (26). The latter divides subsequently into three well-supported groups: *Heterosais + Hypoleria + Brevioleria + Mcclungia* (15: BS 8, PCI 8), *Godyris* (16: BS 25, PCI 25.0) and the *Greta* (including 'Hypomenitis') + *Pseudoscada* complex (24: BS 7, PCI 7). *Mcclungia* is a genus comprising a single geographically variable species that our analysis implies is nested within a paraphyletic *Hypoleria* and should be synonymized. *Brevioleria*, another small genus recently erected by Lamas (2004) for species

removed from *Hypoleria*, also implies that the residual *Hypoleria* is paraphyletic. The relationships among members of *Greta* and *Pseudoscada* in Fig. 2 are complex, with neither genus appearing as monophyletic with respect to the other. Lamas (2004) has synonymized *Hypomenitis* into *Greta*, and this is another component of the tree that we intend to assess with increased taxon sampling to attempt to resolve relationships in greater detail. The genotype of *Greta*, *G. diaphana* (Drury), which is the only ithomiine species found in the Greater Antilles, has not yet been sampled.

Evolution of larval host plant affinities

The molecular data presented here provide a quite robust hypothesis of relationships for Ithomiini that provokes the reconsideration of previous hypotheses of host shifts in ithomiines. Traditionally, Apocynaceae-feeding Tithoreina have been considered the sister taxon to the rest of Ithomiini, suggesting that the host shift from Apocynaceae to Solanaceae occurred once within the tribe (Brown, 1987; Ackery, 1988; Brown *et al.*, 1991; Brown & Freitas, 1994). The current results imply that the use of Apocynaceae by Tithoreina is an independent shift back to the danaine host family, which is a hypothesis that warrants additional scrutiny in comparison with morphological evidence. By contrast, the aposematic coloration of larvae in Melinaeina, the 'new tribe' (known from *Athesis* and *Patricia*; Brown & Freitas, 1994; A. V. L. Freitas, unpublished results) and Tithoreina appears to be a plesiomorphy shared with Danaini and Tellervini, and the shift to cryptic larvae lacking thoracic tubercles appears to be a synapomorphy for the 'derived' subtribes (branch 84). We predict that the larvae of *Eutresis* and *Athyrtis*, when discovered, will also display aposematic coloration.

There are numerous other ecological hypotheses involving larval host plants and mimicry (cf. Willmott & Mallet, 2004) that the current molecular data bear upon, but discussion of these topics will be reserved for a subsequent publication that combines these data with a complementary morphological data set generated by coauthors A. V. L. Freitas and K. R. Willmott.

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