Monophyly, composition, and relationships within Saturniinae (Lepidoptera: Saturniidae): Evidence from two nuclear genes

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The approximately 1500 species of Saturniidae or wild silk moths, which include some of the largest of all lepidopterans, have provided important model systems for studies of ecology, developmental genetics, and behavior. Such studies would benefit from a robust comparative framework, but there has been little phylogenetic analysis of this family. To address this, we use nuclear gene sequences to test hypotheses about the monophyly and internal relationships of the large and geographically widespread subfamily Saturniinae (63 genera, 644 spp.). Extending our previous examination of the genera of Attacini, we analyze coding sequence from elongation factor-1α (1240 nt) and dopa decarboxylase (typically 1051 nt) in 64 species representing four of five tribes in Saturniinae, 11 of 16 genera in Saturniini, and outgroups in Saturniidae and other bombycoids. The results support a recent postulate that Saturniinae, largely Oriental and Palearctic in distribution, should include the African Micragonini. The alternative that Micragonini or some subgroup thereof constitute its own subfamily (previously called Ludiinae) is shown to result in a paraphyletic Saturniinae. Micragonini group strongly with the tribe Bunaeini, also African. Monophyly for Saturniinae, including Micragonini, is strongly supported, as is a basal split between Attacini + Saturniini and Bunaeini + Micragonini. As a consequence, a postulated affinity to the African tribes of two Madagascan endemic Saturniini, thus rendering Saturniini paraphyletic, is rejected. However, there is no strong evidence either way on monophyly of Saturniini versus paraphyly with respect to the clearly monophyletic Attacini (atlas moths and relatives). This result reflects generally weak resolution of deeper divergences in Saturnini. Several lower-level groupings within Saturniini are strongly corroborated, including the tailed-hindwinged 'moon moths' (*Argema, Actias, Graellsia*) that specialize on resinous hostplants, and *Saturnia* sensu lato, a consolidation of eight small, former genera.

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Introduction

The Saturniidae, or wild silk moths, are the most diverse family of Bombycoidea sensu stricto (Minet 1994), consisting of about 1500 species. Saturniids, which include some of the largest and most conspicuous of all moths, have provided important model systems for studies of insect/ plant interactions and caterpillar ecology (e.g., Johnson 1999, Scriber 1983), developmental genetics (e.g., see chapters in Goldsmith & Wilkins 1995), and mediation of insect behavior by pheromones (e.g., Baker & Vogt 1988, Capinera 1980, Riddiford & Williams 1971), among other subjects. Many of the resulting hypotheses are inherently compara-

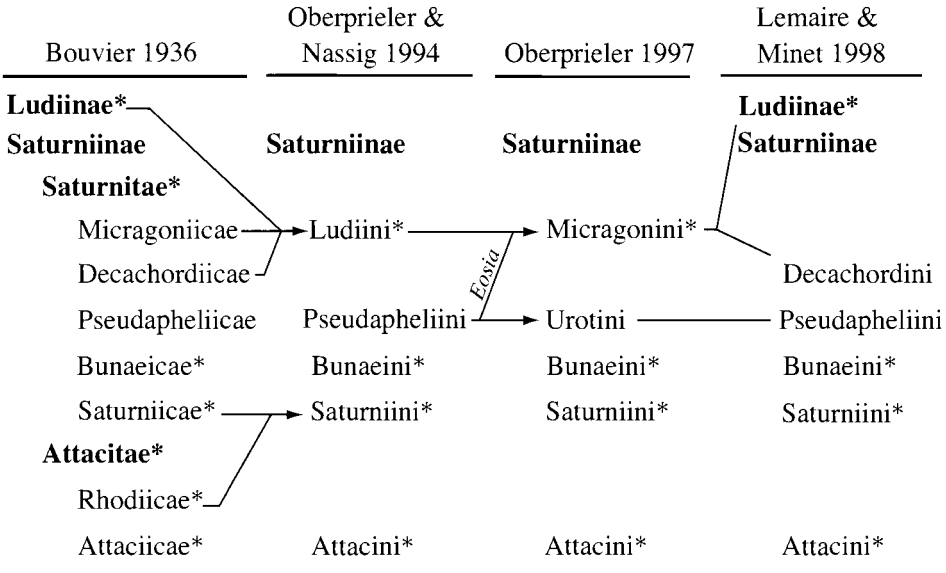

Figure 1. Changing concepts of tribes within Saturniinae. Asterisks identify groups sampled in the current study.

tive and will require an explicit phylogenetic framework for a rigorous modern test. Examples include a postulated role for heterochrony in the evolution of saturniid chorion (eggshell) morphology (Regier & Hatzopoulos 1988), and a putative adaptive linkage between variation in larval host-plant use and variation in adult longevity and associated traits, among and within bombycoid families including Saturniidae (Janzen 1984, Holloway 1987). However, there has been little modern study of saturniid phylogeny (but see, e.g., Peigler 1989, Balcázar & Wolfe 1997).

In this paper we extend our previous application of nuclear gene sequences to saturniid phylogenetics (Friedlander et al. 1998, Regier et al. 1998), taking another step toward the goal of a robust phylogeny for this family. We focus on the geographically most widespread and second-most-diverse subfamily, Saturniinae (63 genera, 644 spp.), which contains both the largest saturniid species (e.g., the atlas moths; Peigler 1989) and those most often used as experimental models. Using outgroups from other subfamilies and bombycoid families, we first test the monophyly of Saturniinae. We then estimate relationships among the major tribes of Saturniinae and extend our previous generic-level analysis of Attacini (Friedlander et al. 1998) to the more diverse Saturniini. The nuclear genes we use encode genes for elongation factor-1α (*EF-1*α) and dopa decarboxylase (*DDC*). They have previously been shown to provide strong phylogenetic signal, singly and (particularly) in combination, at a range of taxonomic depths and divergence levels, in Lepidoptera and other insects, that subsumes those sampled in this study (Cho et al. 1985, Fang et al. 2000; Friedlander et al. 1998; Mitchell et al. 2000; Regier et al. 2001).

Systematic background, taxon sampling, and hypotheses tested. – The monophyly of Saturniidae had been uncertain, particularly as regards the placement of the sometimes-segregated Cercophaninae and Oxyteninae (Packard 1914, Tutt 1902, Forbes 1923, Jordan 1924, Henke 1936, Michener 1952). However, Minet (1994) has identified seven synapomorphies for the family when Cercophaninae and Oxyteninae are included. Lemaire and Minet (1998) recognize nine subfamilies within Saturniidae, but a modern assessment of their monophyly and of their inter-relationships has not been published.

Two of these saturniid subfamilies, Saturniinae and Ludiinae, are of particular relevance to this report, as the monophyly of the former appears to hinge on its relationship to the latter (Fig. 1;

Table 1. Geographical distribution, diversity, and sampling density of higher taxa represented in this study.

Group (No. species sampled)*	Geographical distribution	No. species/ No. genera**
Saturniidae (48-49)	~ worldwide	1862/161
Oxyteninae	Neotropics	45/3
Cercophaninae	Neotropics	12/4
Arsenurinae	Neotropics	64/10
Ceratocampinae (2)	New World	225/28
Hemileucinae (1-2)	New World	860/51
Agliinae	Palearctic	3/1
Salassinae	Orient	9/1
Saturniinae (45)	~ worldwide	644/63
Bunaeini (4)	Africa	150/16
Decachordini	Africa, Orient	10/1
Urotini	Africa	45/12
Micragonini (1)	Africa	90/9
Attacini (18)	~ worldwide	88/9
Archaeoattacus (1)	Orient	2/
Attacus (3)	Orient	14/
Callosamia (3)	North America	3/
Coscinocera (1)	Australia	4/
Epiphora (1)	Africa	20/
Eupackardia (1)	USA to Central America	1/
Hyalophora (3)	North America	4/
Rothschildia (2)	USA to South America	25/
Samia (3)	eastern Asia	15/
Saturniini (22)	~ worldwide	261/16
Rhodinia (1)	Palearctic	5/
Pararhodia	Indo-Australia	5/
Antheraea (3)	mostly Orient, some Nearctic and Palearctic	67/
Antherina (1)	Madagascar	1/
Ceranchia (1)	Madagascar	2/
Opodiphthera (1)	Australia	24/
Lemaireia	Orient	4/
Syntherata	Australia	8+/
Saturnia (8)	Palearctic, Orient, Nearctic	38/
Perisomena (1)	western Palearctic	1/
Neoris (1)	Palearctic	5/
Caligula (1)	Orient, Palearctic	13/
Rinaca	Orient	1/
subgenera** *Eriogyna*	Orient	3/
Saturnia	southwestern Palearctic	2/
Eudia	Palearctic	3/
Calosaturnia (3)	western Nearctic	3/
Agapema (2)	western Nearctic	7/
Copaxa (1)	Neotropics	40/
Loepa (1)	Orient	20/
Cricula	Orient	17/
Solus	Orient	2/
Actias (3)	Orient, some Nearctic and Palearctic	23/
Graellsia (1)	western Palearctic	1/
Argema (1)	Africa	4/

* Subfamily names follow Lemaire & Minet (1998) except that Ludiinae is placed inside Saturniinae as Micragonini, in accordance with Oberprieler (1997) (see Fig. 1) and the results presented in this report (see Fig. 3 and 4). Tribal names within Saturniinae follow Oberprieler (1997) (see Fig. 1). Generic names within Saturniini and Attacini follow Oberprieler & Nässig (1994) except that *Graellsia* is a genus rather than a subgenus within *Actias* (see Fig. 2 and text).

** Information cited from Oberprieler & Nässig (1994), Heppner (1996), D'Abrera (1998), Lemaire & Minet (1998) and Peigler unpubl. obs.

** *Dictyoploca* is listed in the literature as a subgenus of *Saturnia* but is not an available name because the type species is the same as the previously named *Caligula*.

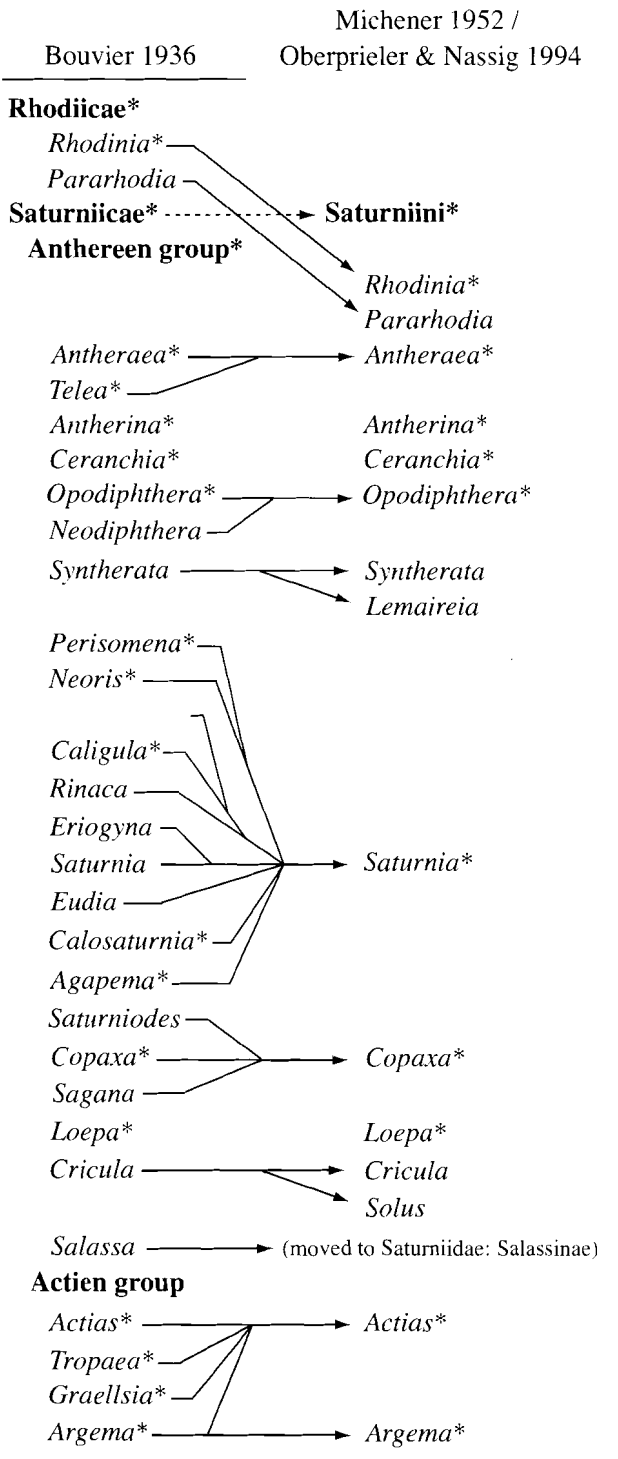

Figure 2. Changing concepts of genera within Saturniini. Asterisks identify groups sampled in the current study.

Lemaire & Minet 1998, Oberprieler & Nässig 1994, Oberprieler 1997). Oberprieler (1997) argues that Micragonini, expanded from the Ludiinae concept of Bouvier (1936) to include Decachordini (Oberprieler & Nässig 1994) and *Eosia* (formerly placed in Saturniinae: Urotini) constitutes one of five tribes within Saturniinae; whereas, Lemaire & Minet (1998) conclude, after expressing previous uncertainty (Minet 1994), that their Ludiinae (without Decachordini) warrants separate subfamily status. Our taxon sampling allows an initial molecular test of these conflicting hypotheses, although we lack exemplars for Decachordini.

Within Saturniinae, five tribes are recognized (Tab. 1, Fig. 1, Oberprieler 1997). Monophyly of Bunaeini has been questioned (Oberprieler 1997), particularly as regards the relationship of the 'higher' Bunaeini to the most primitive-looking taxa (e.g., *Eochroa, Melanocera, Bunaeopsis*). Sampling four species in two genera, we provide an initial molecular test of the uncertain monophyly of Bunaeini, although we lack the putatively primitive genera about which the greatest doubt has been expressed. Monophyly of Urotini also remains uncertain, particularly as regards the placement of *Usta* and *Parusta* (Oberprieler 1997). Our study lacks an exemplar from Urotini.

The monophyly of Attacini appears well established (Peigler 1989). Inter-generic relationships have been assessed by Peigler (1989), using morphology, and by Friedlander et al. (1998), using combined *EF-1α* and *DDC* sequences (2291 nt) for 15 species representing all 9 genera, plus six species of Saturniini as outgroups. All multiply sampled genera were strongly recovered (BP = 100%), as were five of the eight possible higher-level relationships (BP = 100%). Only the positions of *Archaeoattacus*, *Epiphora*, and *Hyalophora /Callosamia /Samia* were not strongly supported (BP = 54-70%). In this study we have increased our taxon sample to determine whether node support for these placements will increase.

Monophyly and composition are especially uncertain for the largest tribe, Saturniini, originally defined by Bouvier (1936). Several postulates of paraphyly for Saturniini have been offered. Our sample, which includes about two-thirds of the genera (Tab. 1, Fig. 2), permits tests of two of these. In Michener's (1952) influential revision (see Tab. 1, Fig. 2), the tribe formerly consisting of *Rhodinia* and *Pararhodia* was synonymized with Saturniini. Inclusion of *Rhodinia* in our sample permits a test of the hypothesis, advanced by several authors (Peigler 1989), that these genera are nonetheless phylogenetically closest to Attacini, as implied by the classification of Bouvier (1936; see Fig. 1). Second, we test the suggestion advanced by Oberprieler (1997) that the putatively primitive, Madagascan endemic genera *Antherina* and *Ceranchia* (both in our sample) are phylogenetically closer to the African tribe Bunaeini than to other Saturniini. A test is also needed to determine whether Michener's removal of *Salassa* to its own subfamily renders Saturniini (or Saturniinae) paraphyletic, but *Salassa* is not included here. Within Saturniini, we test Michener's synonymization of eight Bouvier genera, of which we sampled six, with *Saturnia*.

Materials and methods

Abbreviations. – BP, bootstrap percentage; *EF-1α*, elongation factor-1α; *DDC*, dopa decarboxylase; MP, maximum parsimony; ML, maximum likelihood.

Taxon and gene sampling and experimental design. – *EF-1α* (1240 nucleotides in length each) and *DDC* (up to 1051 nucleotides each) sequences were obtained from 48 species of Saturniidae, two of Brahmaeidae, and 14 of Sphingidae (Tab. 2). Seventy-six of these sequences were published previously, while 62, all from Saturniidae, are new (Tab. 2, Friedlander et al. 1998, Regier et al. 2000, Regier et al. 2001). GenBank accession numbers and specimen collection localities are listed in Tab. 2.

To uniquely represent the saturniid subfamily Hemileucinae, the *EF-1α* sequence of *Polythysana apollina* and the partial *DDC* sequence of *Automeris io* were combined to form a hybrid sequence called '*Polythysana* + *Automeris*.' Similarly, to uniquely represent the genus *Caligula* within the tribe Saturniini, the *EF-1α* sequence of *Caligula japonica* and the *DDC* sequence of either *Caligula japonica* or *C. jonasi* (origin uncertain) were combined and called *Caligula* sp. The *Darapsa* and *Ceratomia* specimens were not identified to species because they were too badly damaged.

Two overlapping data sets were analyzed phylogenetically, one for 64 taxa that included non-saturniid outgroups (i.e., Brahmaeidae, Sphingidae); the other, the subset of 48 saturniid-only taxa. The 64-taxon sample was designed to root the

Table 2. Species sampled, collection localities, and GenBank accession numbers.

Taxa	Collection locality	GenBank accession no. EF-1α*	DDC*
Saturniidae, Ceratocampinae			
Dryocampa rubicunda	USA	AF234564	AF234586 (423 nt) AF234587 (202 nt)
*Eacles imperialis***	USA	AF373933	AF373961 (423 nt) AF373962 (202 nt)
Saturniidae, Hemileucinae			
*Polythysana apollina***	Chile	AF373939	
*Automeris io***	USA		AF373956 (422 nt) AF373957 (200 nt)
Saturniidae, Saturniinae, Bunaeini			
*Imbrasia petiveri***	Congo	AF373935	AF373964
*Imbrasia tyrrhea***	South Africa	AF373937	AF373966
*Imbrasia macrothyris***	Zimbabwe	AF373936	AF373965
*Cirina forda***	South Africa	AF373931	AF373959
Saturniidae, Saturniinae, Micragonini			
*Holocerina smilax***	South Africa	AF373934	AF373963 (709 nt)
Saturniidae, Saturniinae, Attacini			
Archaeoattacus edwardsii	Malaysia	AF015067	AF015046 (909 nt)
Attacus atlas	Thailand	AF015066	AF015045
*Attacus caesar***	Philippines	AF373922	AF373948
*Attacus lorquinii***	Philippines	AF373924	AF373950
Callosamia angulifera	USA	AF015071	AF015050
Callosamia securifera	USA	AF015074	AF015053
Callosamia promethea	USA	AF015073	AF015052
Coscinocera hercules	New Guinea	AF015072	AF015051
Epiphora mythimnia	South Africa	AF015076	AF015055
Eupackardia calleta	USA	AF015075	AF015054
Hyalophora euryalus	USA	AF015078	AF015057
Hyalophora cecropia	USA	AF015077	AF015056
Hyalophora gloveri	USA	AF015079	AF015058
Rothschildia forbesi	USA	AF015081	AF015060
Rothschildia orizaba	Mexico	AF015083	AF015062
Samia ricini	Philippines	AF015086	AF015065
Samia cynthia	USA	AF015084	AF015063
*Samia luzonica***	Philippines	AF373944	AF373972
Saturniidae, Saturniinae, Saturniini			
Rhodinia fugax	Japan	AF015082	AF015061
*Antheraea paphia***	India	AF373926	AF373952
Antheraea pernyi	USA (in culture)	AF015070	AF015049
*Antheraea polyphemus***	USA	AF373927	AF373953
*Antherina suraka***	Madagascar	AF373929	AF373955 (709 nt)
*Ceranchia apollina***	Madagascar	AF373930	AF373958 (709 nt)
*Opodiphthera eucalypti***	New Zealand	AF373938	AF373967
*Saturnia (Calosaturnia) mendocino***	USA	AF373945	AF373973
*Saturnia (Calosaturnia) walterorum***	USA	AF373947	AF373975
*Saturnia (Calosaturnia) albofasciata***	USA	AF373940	AF373968
*Saturnia (Agapema) anona***	USA	AF373941	AF373969
*Saturnia (Agapema) galbina***	Mexico	AF373943	AF373971
*Saturnia (Perisomena) caecigena***	Yugoslavia	AF373942	AF373970
Saturnia (Caligula) sp.	Japan	AF015085	AF015064
*Saturnia (Neoris) naessigi***	Turkey	AF373946	AF373974
*Copaxa multifenestrata***	Mexico	AF373932	AF373960
Loepa sikkima	India	AF015080	AF015059
*Actias isis***	Indonesia	AF373923	AF373949
*Actias selene***	India	AF373928	AF373954
Actias luna	USA	AF015069	AF015048
Graellsia isabellae	Spain	AF015068	AF015047
*Argema mimosae***	Africa	AF373925	AF373951
Brahmaeidae			
Brahmaea certhia	China	AF234560	AF234583 (709 nt)

Acanthobrahmaea europaea	Italy	AF234558	AF234581 (709 nt)
Sphingidae, Macroglossinae			
Hyles lineata	USA	AF234567	AF234589
Xylophanes falco	USA	AF234580	AF234599 (709 nt)
Darapsa sp. (*myron* or *pholus*)	USA	AF234563	AF234585 (709 nt)
Sphecodina abbottii	USA	AF234575	AF234594 (709 nt)
Eumorpha pandorus	USA	AF234565	AF234588 (709 nt)
Aellopos tantalus	USA	AF234559	AF234582 (709 nt)
Hemaris thysbe	USA	AF234568	AF234590 (709 nt)
Sphingidae, Sphinginae			
Lapara coniferarum	USA	AF234569	AF234591 (709 nt)
Sphinx chersis	USA	AF234576	AF234596 (709 nt)
Ceratomia sp. (*amyntor* or *undulosa*) USA		AF234562	AF234584
Manduca sexta	USA	AF234571	AF234592 (709 nt)
Dolba hyloeus	USA	AF234579	AF234598
Sphingidae, Smerinthinae			
Paonias myops	USA	AF234574	AF234593 (709 nt)
Smerinthus cerisyi	USA	AF234576	AF234595 (709 nt)

* *EF-1α* sequences are each 1240 nucleotides in length. *DDC* sequences are each 1051 nucleotides in length, unless otherwise indicated within parentheses after the GenBank accession number.
** Taxa with sequence data new to this report.

Saturniidae and thereby permit a test of the monophyly of Saturniinae. The 48-taxon sample was designed to address relationships within Saturniinae, potentially with greater accuracy because most outgroup taxa are missing about a third of the *DDC* sequence (Tab. 2) and because homoplasy, largely due to synonymous substitutions, increased when sampling extended across families.

Data collection and assembly. – Specimens were alive until frozen at -85°C in 100% ethanol. Vouchers are stored in freezers at the Department of Entomology, University of Maryland College Park. Total nucleic acids were isolated and specific sequences were amplified by the polymerase chain reaction (for *EF-1α*) or by reverse transcription and then the polymerase chain reaction (for *DDC*). Sequences of primers and conditions for amplification, reamplification with nested primers, gel isolation, and automated sequencing have been described (*EF-1α*: Cho et al. 1995; *DDC*: Fang et al. 1997; Friedlander et al. 1998). For *EF-1α*, the 1240-basepair fragment between primers M3 and rcM4 was sequenced and analyzed. For *DDC*, the 1051-basepair fragment between primers 1.7dF and M7.5RC was sequenced and analyzed, unless otherwise indicated (Tab. 2).

Automated DNA sequencer chromatograms were edited and contiguous fragments were assembled using the GAP4 program within the Staden software package (Staden et al. 1999). Sequences were aligned using the Genetic Data Environment software package (version 2.2, Smith et al. 1994). There were no indels in any of the sequences. Amino acid data sets were conceptually translated from nucleotide sequences using MacClade (version 3.07, Maddison & Maddison 1992).

Data analysis. – Phylogenetic analyses were conducted on the 48- and 64-taxon data sets using *EF-1α* and *DDC* in combination, after demonstrating that the signals from the two genes were not in conflict (see below). Maximum parsimony (MP) analyses of total nucleotide and amino acid data sets were performed with PAUP*4.0b2 (Swofford 1998) using unordered character states and a heuristic search algorithm (TBR branch swapping, 100 sequence-addition replicates with random taxon addition). Bootstrap values (Felsenstein 1985) were also obtained using a heuristic search (typically, 1000 replications with TBR branch swapping and 10 random sequence-addition replicates per replication).

Maximum likelihood (ML) analyses of combined-gene nucleotide data sets were performed with PAUP*4.0b2, in each case under the simplest model providing fit statistically indistinguishable from the optimum, as judged by likelihood ratio tests (Huelsenbeck & Rannala 1997, see also Shultz & Regier 2000). A general time reversible model (general reference: Swofford et al. 1996; GTR: Rodriguez et al. 1990) was selected. Among-site rate variation was accommodated by fitting the frequency of character change to a gamma distribution, approximated by four discrete

categories. A likelihood ratio test showed that a separate category of invariant sites, also estimated by likelihood, significantly improved the model's fit ($P < 0.0005$); this is called the *GTR+G+I* model. A separate general time-reversible model in which characters were partitioned by codon position and by gene was also explored; this is called the *GTR-ssr* model. When the all-nucleotide data set was optimized on the MP tree, the GTR-ssr model yielded a ln-likelihood score approximately 1.5% lower than the GTR+G+I model (-18,342 versus -18,619). Efficient exploration of tree space in likelihood analyses entailed a sequence of heuristic searches and parameter (re-) optimizations (Shultz & Regier 2000; Regier et al. 2000). Parameters fitted to the ML topology were used to create corrected pairwise distance matrices from 1000 bootstrapped data sets under a minimum evolution model for subsequent analysis by neighbor joining.

Average pairwise sequence differences were calculated by gene and by codon position using PAUP*4.0b2. Base frequencies of terminal taxa were calculated by gene and by codon position, also using PAUP*4.0b2. The incongruence length difference test (Farris et al. 1995), implemented as the partition homogeneity test in PAUP*4.0b2, was used to test for conflicting signal between character partitions. Relative rates of nucleotide substitution by codon position and by gene were estimated from parsimony tree lengths when constrained to the same all-nucleotide-derived topology. The amount of synonymous nucleotide change was estimated in PAUP*4.0b2 as the difference in overall tree lengths derived from total amino acid change and (separately) from total nucleotide change when mapped onto the same MP topology (derived from analysis of total nucleotides).

Results and discussion

Suitability of EF-1α and DDC: Synonymous and non-synonymous changes, pairwise differences, base frequencies, and partition homogeneity tests. – Within Saturniidae, synonymous differences account for about 92% of total character variation, most of which resides at the third codon position and is approximately equally partitioned between *DDC* and *EF-1α*. By contrast, non-synonymous changes are more than threefold greater for *DDC* than *EF-1α* (see also Regier et al. 1998). Higher rates of non-synonymous change for *DDC* are also reflected in the maximum observed (uncorrected) pairwise differences across Saturniidae at the second codon position, which is 5% for *DDC* but only 1% for *EF-1α*. Maximum differences at the third codon position are 55% and 39% for *DDC* and *EF-1α*, respectively.

In extending the comparisons from Saturniidae to include the outgroups Brahmaeidae and Sphingidae, maximum pairwise differences at the second codon position approximately double (to 11% for *DDC*, to 2% for *EF-1α*), while differences at the third codon position also increase for *DDC* (to 64%) but remain unchanged for *EF-1α*. Our separate analysis of an all-taxon data set (Saturniidae + Brahmaeidae + Sphingidae; 64 taxa) and a 48-taxon Saturniidae-only data set was motivated by the possibility that the high pairwise differences across families, particularly in the third codon position, could mask multiple hits, undermining phylogenetic inference.

Both genes show homogeneous base frequencies across Saturniidae at all codon positions (P values from 0.58 to 1.00), further supporting their suitability for saturniid phylogenetics. With Brahmaeidae and Sphingidae included, only the third codon position for *EF-1α* becomes strongly non-homogeneous ($P < 0.001$). In support of combined analysis of total nucleotides from *DDC* and *EF-1α*, partition homogeneity tests reveal no conflict in the signals from *DDC* and *EF-1α*, either with ($P = 0.11$) or without ($P = 0.30$) inclusion of the brahmaeid and sphingid outgroups.

Monophyly, composition, and phylogenetic position of Saturniinae. – In the following discussion of our phylogenetic results, summarized in Fig. 3 and 4, we make a heuristic distinction between 'strongly supported' nodes, defined as those with bootstrap percentages (BP) ≥ 80%, and the other, more weakly supported nodes, i.e. those with BP < 80%. Low BP values can result either from low numbers of supporting characters or from a relative abundance of conflicting characters (or some combination).

Likelihood and parsimony analyses of the all-nucleotide data set for 64 taxa strongly support the separation of Saturniidae from the other two families sampled (BP = 100%; Fig. 3). Within Saturniidae, the two genera of Ceratocampinae are strongly grouped (BP ≥ 91%). However, there is no strong support for any grouping among the three subfamilies sampled. Similarly weak resolu-

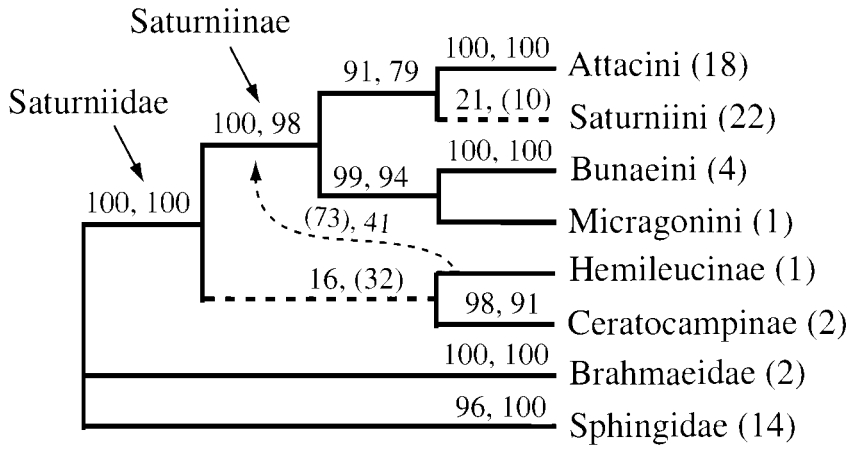

Figure 3. Summary of maximum likelihood topology based on analysis of total nucleotide sequences of *EF-1α* + *DDC* from 64 taxa (Saturniidae, Brahmaeidae, Sphingidae). Relationships among tribes, subfamilies, and families only are shown with the number of species sampled in each terminal group listed in parentheses after the name. Bootstrap percentages are listed above subtending branches (ML, MP strict consensus); bootstrap percentages in parentheses correspond to groups not present in the ML or MP (strict consensus) topology. The less likely, but more parsimonious, grouping of Hemileucinae as sister to Saturniinae, together with bootstrap percentages, is also shown. Solid lines identify groups that are supported by high (≥ 80%) BP values. Dashed lines identify groups whose relationships are considered provisional (BP < 80%). The lnL value for the fully resolved topology is -25162.569.

tion among the same three subfamilies was obtained in an exploratory sampling of 909 nucleotides of the *period* locus across 13 bombycoids that included seven saturniids (Regier et al. 1998).

Regarding the monophyly of Saturniinae vís a vís Micragonini, the present analysis (Fig. 3, 4) strongly supports the inclusion of Micragonini within Saturniinae (Oberprieler & Nässig 1994) over its segregation as a separate saturniid subfamily (Ludiinae: Lemaire & Minet 1999), thus supporting a common African origin for the Micragonini + Bunaeini clade (Tab. 1, Fig. 3); that is, our exemplar of Micragonini is strongly placed as sister group to the tribe Bunaeini, the latter assigned to Saturniinae by all previous authors, in our analyses of both nucleotides (BP ≥ 92%) and amino acids (BP = 94% under parsimony). With this inclusion, Saturniinae is very strongly separated (BP = 100%) from the other subfamilies sampled. While additional sequences from Micragonini are needed, as well as inclusion of Urotini and *Decachorda* (placed as Decachordini by Lemaire & Minet 1998), support for its inclusion in Saturniinae (Oberprieler & Nässig 1994, Oberprieler 1997) now seems strong.

Monophyly of tribes in Saturniinae and their interrelationships. – The 64-taxon and 48-taxon analyses give essentially the same relationships within Saturniinae. The two genera sampled from the African tribe Bunaeini are strongly grouped (BP = 100%) to the exclusion of all Saturniini. However, the monophyly of Bunaeini cannot be definitively assessed until sampling includes a greater diversity of bunaeine genera, and representatives of Urotini, speculated to lie phylogenetically within the former tribe (Oberprieler 1997).

Our analyses strongly corroborate a close relationship between Attacini and Saturniini, relative to the other tribes sampled (BP = 89%). This finding in turn argues strongly against one postulate of non-monophyly for Saturniini, namely, the suggestion (Oberprieler 1997) that the Madagascan genera *Antherina* and *Ceranchia* may be more closely related to the African tribe Bunaeini than to other Saturniini, which are mostly Oriental or Palearctic. In contrast, our data provide no strong evidence either way on the postulate of Saturniini paraphyly due to alliance of *Rhodinia* with Attacini (Peigler 1989), or on the general question of monophyly for this tribe. Thus, a monophyletic Saturniini is recovered by ML under both the (optimal) GTR+G+I and GTR-ssr models, but with low (17%) BP support. Under parsimony,

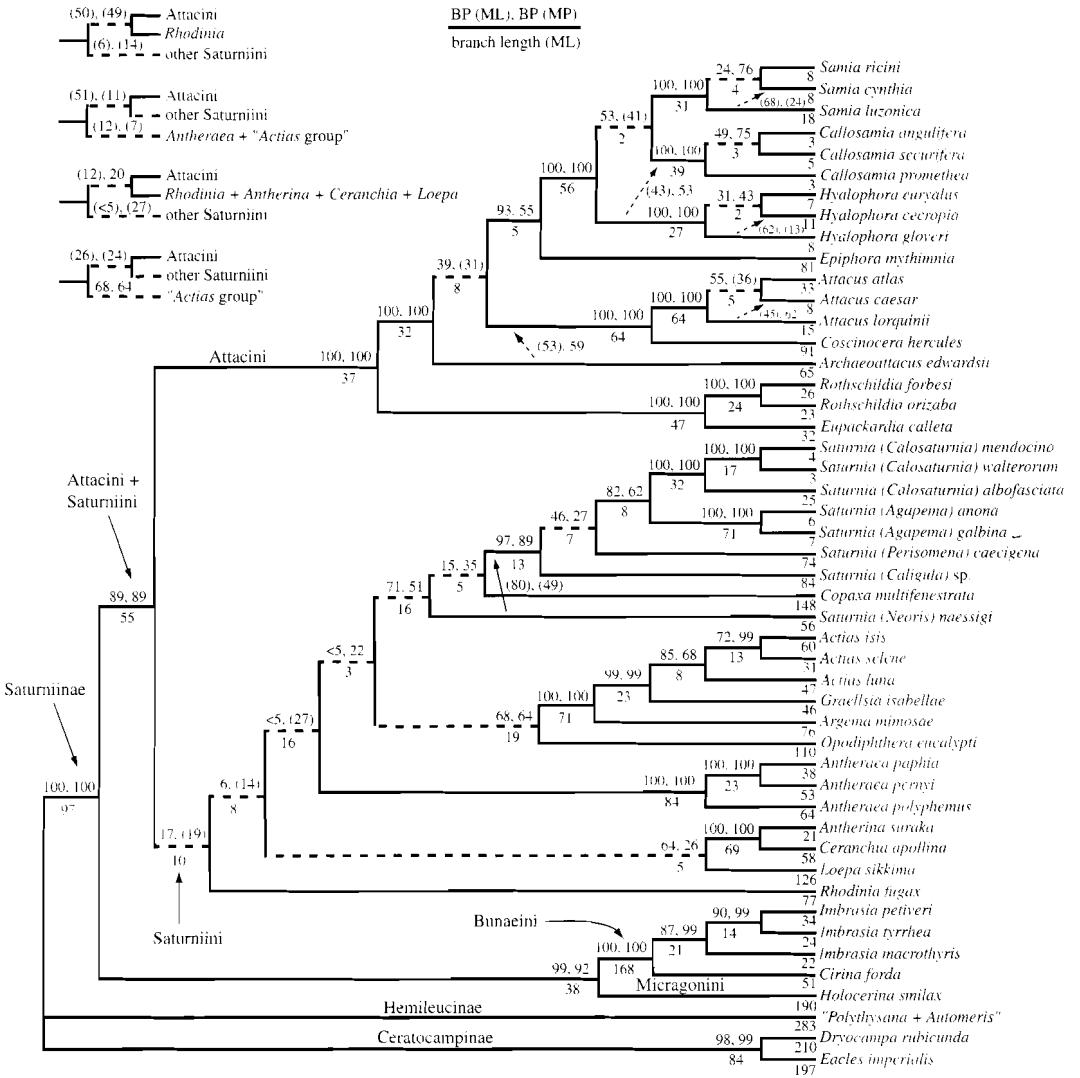

Figure 4. Maximum likelihood topology based on analysis of total nucleotide sequences of *EF-1α* + *DDC* from 48 taxa (Saturniidae only). Bootstrap percentages are listed above subtending branches (ML, MP strict consensus); bootstrap percentages in parentheses correspond to groups not present in the ML or MP (strict consensus) topology. ML-estimated numbers of nucleotide substitutions are shown below corresponding branches. The lnL value is -18599.015. Four alternative arrangements of taxa from Saturniini, together with bootstrap percentages, are summarized (upper left). In these alternative arrangements only, '*Actias* group' refers to *Actias* + *Argema* + *Opodiphthera*. Solid lines identify groups that are supported by high (≥ 80%) BP values. Dashed lines identify groups whose relationships are considered provisional (BP < 80%).

Saturniini are not monophyletic, but monophyly requires only one step beyond the minimum of 3259 steps. Four weakly-to-modestly supported alternatives to a monophyletic Saturniini, one of which groups *Rhodinia* with Attacini, are depicted in Figure 4 (upper left).

Lack of decisiveness on monophyly of Saturniini in our data reflects a general lack of strong resolution of deeper relationships among the genera in this tribe; support levels are much stronger in Attacini (Fig. 4). The reason for this difference is not obvious. The maximal pairwise divergences

within Saturniini are slightly higher than those in Attacini, but not dramatically so (e.g. approximately 0.26 versus 0.21, respectively, at the third codon position for EF-1α), and divergences across the strongly supported basal split in Attacini (e.g., 0.15-0.21 at the third codon position for EF-1α) are actually larger than those across any well-supported split within Saturniini. Thus, loss of signal due to greater divergence and saturation in Saturniini cannot easily explain the lack of resolution in that tribe. An earlier molecular study (Shimada et al.1995), using only 468 nucleotides of the arylphorin gene in a sparse taxon sample – four genera of Saturniini (2 species of *Antheraea*, 2 of *Actias*, 2 of *Saturnia*, and 1 of *Rhodinia*), one genus of Attacini (2 species of *Samia*), and outgroups from Bombycidae and Sphingidae – also failed to find strong evidence on monophyly of Saturniini.

An alternative possibility is the near-simultaneous divergence of basal lineages, implying that signal will be generally sparse. Arguing against this interpretation is the strong pairing (BP = 97%) of the two saturniine genera, *Antheraea* and *Antherina*, included in the aforementioned *period* locus study (Regier et al. 1998). In contrast, these genera are never decisively placed by our current data set, even when the taxon sample is reduced to match that of the *period* study (result not shown). That is, signal on deeper Saturniini divergences may actually be plentiful if the right gene is used. We are currently expanding the sample for *period*.

Generic relationships within Attacini. – Monophyly of Attacini is again strongly corroborated (BP = 100%), and generic relationships are unchanged from a previous study (Friedlander et al. 1998), with three qualifications. First, the placement of *Epiphora*, although unchanged across studies and analytical methods, receives considerably stronger support from our current ML analysis (BP = 93%) than under MP analysis in either study (BP = 66% and 55%). Second, *Archaeoattacus*, not strongly placed in either study, is now either sister group to *Attacus* + *Coscinocera* (weakly recovered by MP; Friedlander et al. 1998) or to Attacini - (*Rothschildia* + *Eupackardia*) (weakly recovered by ML, Fig. 4). Third, relationships across *Samia*, *Callosamia*, and *Hyalophora* are again unresolved, with similarly low levels of BP support for *Callosamia* + *Samia* and *Callosamia* + *Hyalphora*. Species relationships within these three genera are also ambiguous, although the genera themselves are well supported (BP = 100%).

Relationships within Saturniini. – Overall, intergeneric relationships within Saturniini, particularly at deeper levels, are less strongly supported than those in Attacini (Fig. 4). Several relatively recent divergences, however, are strongly resolved. Bootstrap support is 100% for the subtribe 'Actiens' of Bouvier (1936; see also Oberprieler & Nässig 1994), consisting of the green and yellow 'moon moths' with tailed hindwings in the genera *Argema*, *Actias*, and *Graellsia*. These three genera are also unusual in specializing on resinous hostplants (Peigler 1986). The Australian *Opodiphthera* likewise feeds on resinous plants, so its placement as the sister group to the 'moon moth' clade by our data, although not strongly supported, may be reasonable.

Within the 'moon moth' clade, our data strongly support a sister group relationship of the African *Argema* to *Actias* + *Graellsia*, which are Oriental and Holarctic in distribution. In turn, *Graellsia isabellae* is strongly supported as the sister species to a monophyletic *Actias*, the latter sampled from three species groups. Previously, Oberprieler and Nässig (1994) had synonymized the monotypic *Graellsia* with *Actias* because of evidence suggesting that *Actias* would thereby be rendered paraphyletic. For example, the green, short-tailed *Graellsia* resembles the pale green *A. luna* more than does the brown and yellow *A. isis*, which has long, straight tails on its hindwings. However, our results do not support the necessity for this synonymy, and we have therefore maintained the traditional usage of *Graellsia*.

The Madagascan endemics *Antherina* and *Ceranchia* are strongly supported as sister genera (BP = 100%). Their close relationship to *Loepa* is a novel, but only weakly supported, hypothesis.

Finally, with one exception, our analysis strongly supports (BP $\geq$ 89%) monophyly for the large and diverse genus *Saturnia* as redefined by Michener (1952; see Fig. 2), who synonymized eight smaller genera. The major uncertainty concerns the subgenus *Neoris*, on which the evidence is conflicting, as indicated by the 80% BP supporting its inclusion in *Saturnia* despite the fact that this placement does not occur in the optimal MP or ML trees. *Copaxa*, which splits *Neoris* from the other *Saturnia*, has the longest branch length in

the tribe and its placement could be spurious, although features of cocoon and larval morphology and of adult wing pattern suggest its close relationship to *Saturnia* (Packard 1914, Bouvier 1936, Wolfe 1993, D'Abrera 1998). Within *Saturnia*, the two multiply-sampled subgenera are both strongly recovered, and there is good support for the postulated New World clade consisting of *Calosaturnia* + *Agapema*, whose last common ancestor may have dispersed across Beringia from eastern Asia (Hogue et al. 1965). However, a close relationship between the Palearctic subgenera *Perisomena* and *Neoris*, advocated by Jordan (1911) and Peigler (1996), is not supported.

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