

Total Evidence Phylogenetic Analysis of the Moon Moths: Combining Morphology, Molecules, and Behavior (Lepidoptera: Saturniidae)

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ABSTRACT

A phylogenetic analysis of sixteen moon moth species was conducted using morphology, behavior, and molecules. Morphological and behavioral data comprised of 93 characters from the larva, pupa, and adult of all ingroup species and two outgroups. Molecular data included 2,662 nucleotides from elongation factor 1-alpha and dopa decarboxylase nuclear genes of six ingroups and two outgroups. Data types were analyzed separately, compared, and combined. The total evidence analysis resulted in six most parsimonious trees and the strict consensus reveals the following generic relationships: (outgroups (*Argema* (*Graellsia* + *Actias*))). Character evolution indicates that the posture at which hindwing tails are held evolved from an ancestral state in which tails were crossed before becoming parallel. The short hindwing tail evolved once and lengthened at least twice in different moon moth lineages.

INTRODUCTION

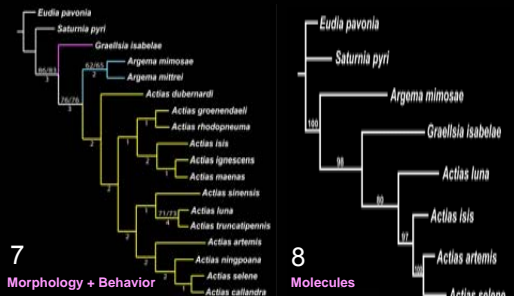
Moon moths in the genera *Actias*, *Argema*, and *Graellsia*, with their tailed hindwings and delicate green, yellow, and rose coloration are highly popular with lepidopterists and have been considered some of the most beautiful insects in the world⁽¹⁾. *Actias* includes 23 species, *Argema* four species, and *Graellsia* includes one⁽²⁾. The three genera are distributed mainly in tropical and sub-tropical Asia, with fewer representatives in temperate regions.

Many quasi-Hennigian hypotheses on moon moth relationships have been postulated, but a phylogenetic analysis including many species of moon moths has never been conducted. The only known phylogenetic analysis including the three genera is the molecular study by Regier *et al.*⁽³⁾ which included only five moon moth species. The purpose of this study is to use modern cladistic methodology to: (a) infer relationships of sixteen moon moth species using morphological, behavioral, and molecular data, (b) compare results from different data types, and (c) test the monophyly of the ingroup genera. A small sample of the adult and larva of several species are illustrated in color (Figs. 1–6).

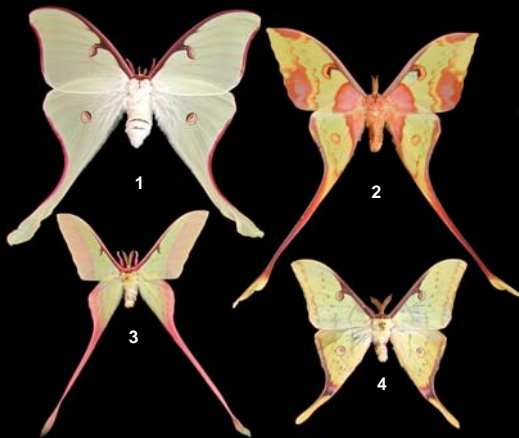
MATERIALS AND METHODS

Morphological data were recorded and coded from the larva, pupa, and adult of sixteen ingroups and two outgroups. Two species were selected as outgroups: *Eudia pavonia*, and *Saturmia pyri*, simply to root the tree. The morphology plus behavior data matrix was assembled in WinClada⁽⁴⁾ and analyzed in NONA⁽⁴⁾ by implementing the heuristic commands (TBR, mult*1000, max*, hold1000, hold/100). Bootstrap⁽⁵⁾, parsimony jackknife⁽⁶⁾, and Bremer support⁽⁷⁾ were calculated in NONA. All characters were non-additive and weighted equally.

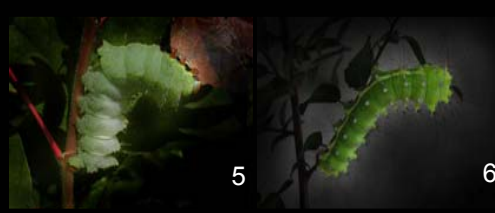
The molecular parsimony analysis was conducted in PAUP⁽⁸⁾, and a heuristic search was conducted by implementing TBR, 1000 maximum trees, 1000 replicates, 100 trees held, and only the best trees were kept. All characters were weighted equally. The combined total evidence analysis was conducted because molecular and morphological data independently gave different topologies with strong support (Figs. 7, 8). Data matrices were combined and a heuristic search was conducted in PAUP* (TBR, max = 1000, 1000 reps, hold = 100).



Figs. 7, 8. Morphological and molecular topologies. Fig. 7. The most parsimonious tree (L=225, CI=0.52, RI=0.61) obtained from the morphological analysis under unambiguous optimization. Bootstrap/jackknife (>50%) values are indicated above branches, Bremer values indicated below. Fig. 8. The most parsimonious tree (L=402, CI=0.88, RI=0.71) generated from the molecular analysis of six ingroup species and two outgroups. Bootstrap values (>50%) are indicated above each branch.



Figs. 1-4. Adults of *Actias*. 1. *Actias truncatipennis*, female, from Las Minas, Veracruz, Mexico. 2. *Actias maenas*, male, from Tapah, Perak, Malaysia. 3. *Actias dubernardi*, male, from Dahongshan, 1600 m, Shuizhou, Hubei Province, China. 4. *Actias sinensis*, male, from Jiantongshan, 1500 m, Hunan Province, China.



Figs. 5, 6. Larvae included in the morphological matrix. 5. *Actias sinensis*. 6. *Saturmia pyri*. The matrix included 19 larval characters.

RESULTS AND DISCUSSION

Morphology and molecules both strongly support the monophyly of all ingroup genera. However, morphology + behavior (Fig. 7) placed *Actias* and *Argema* as sister genera, which differs from the molecular analysis (Fig. 8) which grouped *Actias* with *Graellsia*.

Monophyly of *Actias* is retained in the morphological analysis, but clade support was low (BP, JK < 50%). The molecular analysis resulted in a monophyletic *Actias*, but support values were lower than values obtained for *Graellsia* + *Actias* + *Argema* (BP = 80%, 68%, respectively).

The total evidence analysis resulted in six most parsimonious trees (L = 836, CI = 0.74, RI = 0.62). All six trees were similar in topology, differing slightly in sister-group relationships of *Actias dubernardi*, *A. groenendaelli*, and *A. rhodopneuma*. The total evidence analysis recovers a monophyletic *Actias*, with *A. sinensis* basal to other species in the genus (BP = 76 %). We examined the evolution of tail length in one of the six trees (Fig. 9).

Our results indicate that tail length evolved from none (*Eudia* + *Saturmia*) to short, and then long at least twice (once in the common ancestor of *Argema* and another time in *Actias*, Fig. 9). Very long tails evolved independently in three different lineages (*Argema mitrei*, *Actias dubernardi*, *Actias isis* + *A. ignescens* + *A. maenas*).

The posture at which hindwing tails are held evolved from an ancestral state in which tails were crossed (*Argema* and *Graellsia*) before becoming held parallel (Fig. 9). This characteristic was noted previously for *Actias* and *Argema*⁽⁹⁾, and we note here that *Graellsia* also crosses its tails. This character is a synapomorphy for all species of *Actias* included in this study.

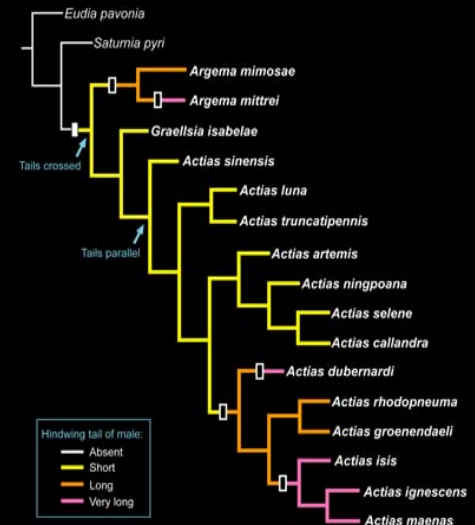


Fig. 9. One of six most parsimonious trees (L=836, CI=0.74, RI=0.62) from the total evidence dataset showing the evolution of the male hindwing tail under unambiguous optimization. State changes in tail length that are present in all six topologies are indicated on this tree. Tail length evolved once from no tail to short, and then lengthened multiple times. The posture at which hindwing tails are held evolved from an ancestral state in which tails were crossed before tails becoming held parallel. The white rectangle indicates a synapomorphy, unfilled rectangles indicate homoplasies.

CONCLUSIONS

Our study is the next step towards an understanding the relationships of moon moths. The analyses were conducted using all data available, but from the lack of full resolution and low support values for certain clades, it is evident that our knowledge is still incomplete. We would specifically suggest sequencing EF1- α and DDC for the ten ingroup taxa for which there was no molecular data. Additional molecular data will enrich the matrix presented in this current study, and may shed light on reasons why topologies of moon moths differ between morphology and molecular data.

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CONTRIBUTION BY AUTHOR

(JY): Coded morphological and behavioral characters. (RSP): Provided photographs, wrote several sections of the text for the accompanying manuscript. (AYK): Provided the molecular dataset, conducted phylogenetic analyses, and wrote part of the manuscript.

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