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 2,682 nucleotides from elongation factor 1-alpha and dops deoxytRNA kinase genes of six ingroup 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 + 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 are the genera Actias, Argema, and Graellsia, with their tallied 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.11-13. DNA has included 23 species, Argema four species, and Graellsia includes 16. 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 Reger et al.14 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 ingroup species and two outgroups. Two species were selected as outgroups: Eudia pavonia, and Saturnia pyri, simply to root the tree. The morphology plus behavior data matrix was assembled in NONA15 by implementing the heuristic commands (TBR, mult=1000, max=100, hold1000, hold=100). Bootstrap16, parsimony jackknife16, and Bremer support17 were calculated in NONA. All characters were non-additive and weighted equally.

The molecular parsimony analysis was conducted in PAUP18, 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 from different genera rarely give congruent topologies with strong support. We calculated Bremer support17, Bremer support, and Bremer support (Fig. 7). Bootstrap data matrices were combined and a heuristic search was conducted in PAUP* (TBR, max = 1000, 1000 reps, hold = 100).

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 groups, which differs from the molecular analysis (Fig. 8) which grouped Actias with Graellsia. Monophyly of Argema is retained in the morphological analysis, but close support was low (BP, JK < 50%). The molecular analysis resulted in a monophylectic clade, but support values were lower than values obtained for Graellsia + Actias + Argema (BP > 60%).

The total evidence analysis resulted in six most parsimonious trees (L = 636, CI = 0.74, RI = 0.62). All six trees were similar in topology, differing slightly in sister-group relationships of Actias dubernardi, A. rhodopneuma. Our study recovers a monophyletic Actias, A. sinensis basal to other species in the genus (BP = 76%). We examined the evolution of tail length in one of these six trees (Fig. 9).

Our results indicate that tail length evolved from none (Eudia + Saturnia) to short, and then long at least twice (once in the common ancestor of Argema and another time in Actias). Figure 9. Very long tails evolved independently in three different lineages (Actias, Graellsia, and Argema). This characteristic was noted previously for Actias and Argema,14 and we note here that Graellsia also crosses its tails. This character is a synapomorphy for all species of Actias included in this study.

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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REFERENCES