TITLE:

Evolution of body size and wing shape trade-offs in arsenurine silkmoths

RUNNING HEAD:

EVOLUTIONARY TRADEOFFS IN SILKMOTHS

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ABSTRACT

One of the key objectives in biological research is understanding how evolutionary processes have produced Earth's biodiversity. These processes have led to a vast diversity of wing shapes in insects; an unanswered question especially pronounced in moths. As one of the major predators of nocturnal moths, bats are thought to have been involved in a long evolutionary arms race with their prey. In response, moths are thought to have evolved many counter strategies, such as diverse wing shapes and large body sizes. However, the tradeoffs between body size and wing shape are not well understood. Here we examined the evolution of wing shape in the wild silkmoth subfamily Arsenurinae (Saturniidae). By using phylogenomics and geometric morphometrics, we established the framework to evaluate potential evolutionary relationships between body size and wing shape. The phylogeny was inferred based on 781 loci from target capture data of 42 arsenurine species representing all 10 recognized genera.

We found there are evolutionary trade-offs between body size, wing shape, and the interaction of fore- and hindwing shape. Namely, body size decreases with increasing hindwing length, but increases as forewing shape becomes more complex. Additionally, hindwing shape has a significant effect on forewing shape complexity. The complex wing shapes that make Arsenurinae, and silkmoths as a whole, so charismatic are likely driven by the strong forces of natural selection and genomic constraints.

One other important outcome was discovering within our data one of the most vexing problems in phylogenetic inference – a region of a tree that possesses short branches and no “support” for relationships (i.e., a polytomy). These parts of the Tree of Life are often some of the most interesting from an evolutionary standpoint. To investigate this problem, we used reciprocal illumination to determine the most probable generic relationships within the

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Arsenurinae by inspecting differing phylogenetic inferences, alternative support values, quartets, and phylogenetic networks to reveal hidden phylogenetic signal.

KEYWORDS:

Lepidoptera, Saturniidae, Arsenurinae, phylogenomics, Anchored Hybrid Enrichment, geometric morphometrics, bat-moth interactions

The vast amount of Earth's diversity in faunal form and function lies in the arthropod Tree of Life, yet major questions persist: 1) How many arthropod species wait to be discovered and described? 2) What are the relationships across the arthropod Tree of Life, particularly towards the tips? and 3) What characters, traits, or interactions have allowed some lineages to become more diverse than others? The lineage that provides perhaps the most informative opportunities for answering these questions is the Insecta. Arguably the most successful lineage on the planet, insects have diversified to fill virtually all terrestrial and freshwater niches ((Ehrlich and Raven 1964), (Farrell et al. 1992), (Mitter et al. 1988), (Wiens et al. 2015), (Condamine 2016)), often evolving incredible traits to exploit them, such as wings and the ability to fly. These innovations provided both ecological opportunities and challenges to diversify in the face of new pressures, particularly predatory.

Between 50-70 million years ago, a major lineage of insect predator appeared - echolocating bats ((Jones et al. 2005), (Teeling et al. 2005), (Miller-Butterworth et al. 2007), (Shi and Rabosky 2015), (Lei and Dong 2016)). Nightly battles between moths and bats drove a predator-prey arms race ((Corcoran et al. 2009), (Conner and Corcoran 2012)) that produced remarkable anti-bat strategies such as ultrasonic-detecting ears ((Roeder and Treat 1957), (Scoble 1992)), ultrasound producing organs capable of jamming bat sonar ((Corcoran et al. 2011), (Barber and Kawahara 2013)) or warning of bad taste ((Dunning 1968), (Hristov

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and Conner 2005), (Barber et al. 2009), (Dowdy and Conner 2016)), and evasive flight strategies – such as aerial maneuvers or temporal partitioning of activity ((Lamarre et al. 2015), (Kawahara et al. 2018)). Amidst the emergence of these fierce predators, one of the most captivating lepidopteran radiations arose – the superfamily Bombycoidea ((Wahlberg et al. 2013), (Misof et al. 2014), (Kawahara and Barber 2015), (Kawahara et al. 2019)).

Some of the most spectacular anti-bat strategies can be found in the bombycoid sister lineages Saturniidae (wild silkmoths) and Sphingidae (hawkmoths) – two lineages with an incredible array of shapes and behavioral traits ((Barber and Kawahara 2013), (Breinholt and Kawahara 2013), (Kawahara and Breinholt 2014), (Barber et al. 2015), (Rubin et al. 2018)). The divergent life-history strategies of these two lineages has likely played a major role in driving their diversity (Hamilton et al. 2019). For example, the majority of hawkmoths are “income breeders” ((Janzen 1984), (Tammeru and Haukioja 1996)); adults live for a relatively long time period (weeks to months) during which they feed on nectar while traveling long distances looking for mates, mate multiple times, retain the eggs internally for long periods of time to allow egg maturation, and searching for their appropriate larval host plants (frequently highly specific and toxic) upon which to lay their eggs. Their incredibly fast and maneuverable flight, including the ability to hover and fly backwards, as well as their sleek appearances, has earned them a popular caricature as the “fighter jets” of the moth world ((Roeder 1974), (Rydell and Lancaster 2000)). In addition, many sphingid lineages possess ears or ultrasound producing organs that have independently evolved multiple times to detect and respond to their echolocating predators ((Barber and Kawahara 2013), (Kawahara and Barber 2015)), whereas some sphingids possess neither. This apparent vulnerability begs the question: How do lineages that cannot hear bat echolocation survive the nightly gauntlet?

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Initial answers can be found in the Saturniidae, generally the most charismatic lineage of moth due to their large body sizes, striking colors and patterns, and elaborate wing shapes. As “capital breeders”, they possess a very different life-history strategy from their sister lineage ((Janzen 1984), (Tammaru and Haukioja 1996)). Adults typically only live one or two weeks after emergence, and importantly, they do not feed as adults as they lack functional mouthparts (see Janzen 1984). Most of the saturniid life span encompasses the larval period, during which they do not sequester toxins – a markedly different strategy from their sister lineage. It is during this critical stage that they gather as many resources as possible to fuel the adult stage, where they usually mate once with the first available mate and the female almost immediately lays her eggs on what are generally non-toxic (but nutritionally poor) plants (e.g., most saturniids feed on trees, whereas most sphingids feed on flowering plants) ((Scoble 1992), (Morton 2009)). As adults, saturniids possess neither ears nor ultrasound-producing organs, yet they are far from defenseless. As with the sphingids, saturniids are often quite large moths, and this sheer body size itself could forestall bat attack ((Roeder 1974), (Jacobs and Bastian 2016); also see Discussion). Saturniid bodies are also covered in dense scales, creating a “furry” appearance, that absorb bat biosonar and may effectively reduce the moth’s detectability distance by bats (Neil et al. 2020). Once detected, some saturniids thwart their echolocating bat prey by luring attacks to non-essential wing areas. A number of species possess long hindwing tails with twisted and cupped ends that rotate behind them in flight, likely creating a sensory illusion that fools the bat into attacking these appendages ((Barber et al. 2015), (Rubin et al. 2018)). The efficacy of this trait is scaled with hindwing tail length, as increasingly long tails increasingly draw bat attack towards these non-essential appendages, away from the vital body core, allowing the moth to escape more often (Rubin et al. 2018). While long tails provide the greatest benefit for predator escape, simply having an elongated hindwing also provides protection; experimentally elongating the

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hindwing of a saturniid moth elevates its escape success ~25% (Rubin et al. 2018). Interestingly, studies on multiple Lepidoptera groups have shown that severe damage or entire removal of the hindwings does not greatly inhibit flight, while similar forewing damage or removal renders the animal flightless ((Jantzen and Eisner 2008), (Le Roy et al. 2019), (Stylman et al. 2019)). Within the Saturniidae, several subfamilies and multiple genera include tailed and non-tailed species, providing an excellent opportunity to understand the evolution of lineages with anti-bat wing traits and those without.

One of these subfamilies, the Arsenurinae, are large, cryptic moths that mostly inhabit low to mid-elevation tropical forests throughout the Neotropics (Lemaire and Minet 1998). Importantly, across the 10 genera and 89 species (Kitching et al. 2018), they possess a tremendous amount of divergence in wing shape ((Zhong et al. 2016), (Barber et al. 2015), (Rubin et al. 2018)). Phylogenetic relationships among the genera of Arsenurinae have been hypothesized three times ((Michener 1952), (Peigler 1993), (De Camargo et al. 2009)), with major differences in the generic relationships postulated each time (Supp. Fig. 1). While the study of Michener (1952) was pre-cladistic, it contrasted derived and ancestral character states as indicators of phylogenetic affinities. Peigler (1993) and DeCamargo et al. (2009) undertook cladistic studies that used morphological characters to infer the monophyly of the Arsenurinae and its constituent genera. Unfortunately, the only consistent phylogenetic relationships on which they all agreed were that *Dysdaemonia* and *Titaea* were sister genera, and that *Almeidaia* was sister to all other Arsenurinae (Supp. Fig. 1). As a result, we have lacked a robust phylogeny and understanding of relationships that could be used to test hypotheses regarding the group's evolution. Recently, Rubin and Hamilton et al. (2018) densely sampled the Saturniinae, using phylogenomics, to provide the clearest picture so far of the relationships within this subfamily; however, that study lacked sampling outside of the Saturniinae. Hamilton et al. (2019) then examined relationships within the superfamily

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Bombycoidea and identified diversification rate shifts that may be attributed to the predatory pressure of bats, but Arsenurinae sampling was severely limited with only two lineages included.

To build the evolutionary framework from which we can trace the path of potential anti-bat traits, we inferred the first molecular phylogeny of the subfamily Arsenurinae. We subsequently investigated the evolution of wing shape and body size by using geometric morphometrics from natural history collection specimens and tested whether there have been evolutionary trade-offs between body size, fore- and hindwing shape, and the presence of hindwing tails as a hypothesis-generating approach for future trait-based work.

MATERIAL AND METHODS

Taxon Sampling

We sampled 36 out of 89 described species from the ten arsenurine genera (see Kitching et al. 2018): *Almeidaia* Travassos, 1937; *Arsenura* Duncan, 1841; *Caio* Travassos & Noronha, 1968; *Copiopteryx* Duncan, 1841; *Dysdaemonia* Hübner, [1819]; *Grammopelta* Rothschild, 1907; *Loxolomia* Maassen, 1869; *Paradaemonia* Bouvier, 1925; *Rhescyntis* Hübner, [1819]; and *Titaea* Hübner, [1823]. To examine the placement of Arsenurinae wing shape morphospace within the context of the Saturniidae, we digitally imaged and quantified the shape complexity (as principal components) of 968 male specimens comprising 174 species across the eight subfamilies (see Supp. Tables 2 & 3). Ingroup sampling of images (i.e., the Arsenurinae) comprised 545 specimens corresponding to 50 species of the 89 total ((Kitching et al. 2018); see Supp. Tables 4 & 5). Outgroup lineages were chosen because they possess high variation in wing shape morphology, allowing the capture of the morphological diversity outside of the Arsenurinae.

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175 *Molecular Data*

176 We obtained specimens for phylogenetic analyses from the molecular collections of
177 the McGuire Center for Lepidoptera and Biodiversity (MGCL) at the Florida Museum of
178 Natural History (FLMNH), the University of Maryland (UMD), and the Muséum national
179 d'Histoire naturelle in Paris (MNHN). Twenty-six pinned or papered museum specimens,
180 some as old as 36 years, were used for sequencing, to compare recovery of DNA with 16
181 traditionally-stored molecular collections specimens in $\geq 95\%$ ethanol and -80°C (see
182 (McGaughan 2020); Supp. Table 1, Supp. Texts 1 & 2).

183 Genomic DNA from molecular collection specimens was extracted from the thorax or
184 leg(s) using OmniPrep Genomic DNA Extraction Kits (G-Biosciences, St. Louis, MO, USA)
185 or DNeasy Blood and Tissue Kits (Qiagen, Valencia, CA, USA). We used the protocol for
186 extracting DNA from historical museum specimens that was developed and outlined in
187 Hamilton et al. (2019). DNA concentration was evaluated through agarose gel electrophoresis
188 and fluorometry using a Qubit 2.0 (Invitrogen, Thermo Fisher Scientific). The LEP1
189 Anchored Hybrid Enrichment (AHE) targeted sequencing kit (Breinholt et al. 2018), an
190 Agilent Custom SureSelect Target Enrichment kit, was used to target 855 loci. Library
191 preparation and Illumina HiSeq 2500 sequencing (PE100) was carried out at RAPiD
192 Genomics, Gainesville, FL, USA. Tissues from the molecular samples, as well as all extracts,
193 are preserved at -80°C in the MGCL; wing vouchers are stored following the methods of Cho
194 et al. (2016) and stored at the MGCL.

195 A previously developed bioinformatics pipeline was used to prepare sequences for
196 phylogenetic inference (Breinholt et al. 2018). The pipeline uses a probe-baited iterative
197 assembly that extends beyond the probe region, checks for quality and cross contamination
198 due to barcode leakage, removes paralogs, and returns a set of aligned orthologs for each

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locus and taxon of interest. To accomplish these tasks, the pipeline uses the *Bombyx mori* genome (Xia et al. 2004) and the LEP1 AHE reference library. Loci for phylogenetic analysis were selected by applying a cutoff of $\geq 50\%$ sampled taxa recovery (i.e., for a locus to be included in the analysis, the locus had to be recovered in at least 50% of the sampled taxa). The pipeline evaluates density and entropy at each site of a nucleotide sequence alignment. As established by Hamilton et al. (2019), we elected to trim with entropy and density cutoffs only in “flanking” regions, allowing the “probe” region to be converted into amino acid sequences. For a site (outside of the probe region) to remain, that site must pass a 60% density and 1.5 entropy cutoff, rejecting sites that fail these requirements. A higher first value (60) increases the coverage cutoff (e.g., a site is kept if 60% of all taxa are represented at that site). A higher second value (1.5) increases the entropy cutoff (i.e., entropy values represent the amount of saturation at a site); sites with values higher than 1.5 possess higher saturation and are thus deleted. AliView v1.18 (Larsson 2014) was used to translate to amino acids, check for frame shifts, recognize and remove stop codons (if present), and edit sequencing errors or lone/dubious indels. Because flanking sequences are generally non-coding and sites have been deemed homologous (following assembly and alignment), these flanking sequences were separated from the exons, then combined and treated together as an independent partition. Following the filtering steps in the bioinformatics pipeline (i.e., site orthology, and density and saturation evaluation), the flanking partition is viewed as a SNP supermatrix, where each site is homologous, but uninformative sites, saturated sites, or sites with large amounts missing data have been removed. All pipeline analyses, including phylogenomic analyses (below) were conducted on the University of Florida High-Performance Computing Center HiPerGator2 (<http://www.hpc.ufl.edu/>).

Phylogenetics

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Phylogenomic inferences were conducted under likelihood optimality criteria in IQ-TREE multicore version 1.6.9 (Nguyen et al. 2015). To examine phylogenetic signal, we evaluated nucleotide and amino acid datasets. Four supermatrix datasets were built for phylogeny inference: 1) AA = an amino acid supermatrix (777 loci) composed of translated probe region loci; 2) Pr = probe region-only, as nucleotides (778 loci; each locus modeled by sites); 3) Pr+Fl = a probe + flanking supermatrix (782 loci; each locus modeled by sites); and 4) Fl = flanking supermatrix only (modeled by sites). Because concatenation has been shown to fail when there are high levels of incomplete lineage sorting (see Mendes and Hahn 2017), we assessed the impact of potential gene-tree discordance ((Maddison 1997), (Slowinski and Page 1999), (Edwards 2009)) by inferring a phylogeny for each individual locus from the Pr+Fl supermatrix using IQ-TREE, with subsequent species tree estimation performed in ASTRAL-III version 5.6.3 ((Mirarab et al. 2014), (Mirarab and Warnow 2015), (Zhang et al. 2017)) (see Supplemental Trees). For both nucleotide and amino acid datasets, the ‘-m TEST’ command was used in IQ-TREE to perform a search for the most appropriate model of amino acid or nucleotide substitution. Traditional branch support values were computed via 1000 random addition sequence (RAS) replicates, and 1000 replicates each for both ultrafast bootstraps (UFBS) (‘-bb’ command), as well as SH-aLRT tests (‘-alrt’ command). Nodes were classified as “robust” if they were recovered with support values of UFBS ≥ 95 and SH-aLRT ≥ 80 ((Minh et al. 2013), (Guindon et al. 2010)). ASTRAL support values (ASV) – local posterior probabilities (Sayyari and Mirarab 2016), were used to evaluate node support on the species tree. ASTRAL support values were determined to be “robust” if nodes were recovered with local posterior probabilities ≥ 0.95 .

Because phylogenomic datasets can produce incongruent topologies and artificially inflate traditional nodal support values (e.g., due to the increase in total number of sites, the sampling variance for a particular branch is low - see Reddy et al. 2017), we evaluated

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additional modes of topological support. To identify potential rogue taxa or unstable regions in the tree, we ran RogueNaRok ((Aberer and Stamatakis 2011), (Aberer et al. 2012)), <http://rnr.h-its.org/>, on the 1000 ultrafast bootstrap trees and the consensus tree from the Pr+Fl supermatrix. Potential rogue taxa were removed, and phylogenetic inference was rerun ('AA minus *Grammopelta*', 'Pr+Fl minus *Grammopelta*'). Disagreements among loci were estimated using concordance factors (gCF – gene concordance factor of (Minh et al. 2018)) calculated in IQ-TREE 1.7-beta12, on both the Pr+Fl supermatrix constrained tree #1 (see below and Supplemental Trees) and the ASTRAL tree. Internode Certainty scores (IC) were calculated using QuartetScores (Zhou et al. 2020) to infer a measure of support for the reference topology compared with the frequency of the most prevalent alternative topology. Quartets were then investigated to inspect relationships at nodes with low support and evaluate whether one topology was occurring more often than others. Quartet testing was carried out using Likelihood Mapping (Strimmer and vonHaeseler 1997) in IQ-TREE (Nguyen et al. 2015). In this hypothesis-testing framework, we computed the likelihood of possible relationships that can be constructed from possible quartets of the taxa. For example, we looked at the subfamily level by testing whether Arsenurinae was more closely related to the Agliinae, Salassinae, or Saturniinae. We also investigated the genus level by grouping lineages, according to the phylogeny, and evaluating (from a quartet standpoint) whether lineages were found more closely related to another. Quartet likelihood mapping is also a way to inspect the information (i.e. phylogenetic) content of a dataset. To investigate potential gene tree/species tree incongruence, we computed a phylogenetic network in SplitsTree4 version 4.14.4 (Huson and Bryant 2006). Because the gene trees do not include identical sets of taxa, the 'SuperNetwork' method was implemented, using uncorrected p-distance and Jukes-Cantor models, in which the edge weights equaled "TreeSizeWeightedMean". We attempted to investigate networks using PhyloNet 3 ((Than et

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al. 2008), (Wen et al. 2018)). Due to the computational resources this approach requires, we reduced the taxon set to a single representative for each genus and attempted to evaluate the “best” total log probability score from the inferred networks, after testing whether the number of reticulations had been from 0 to 14 (the total number of tips in this reduced taxon set). Finally, using the process of reciprocal illumination, we coalesced these outcomes and reran phylogenetic inference with two unrooted constrained topologies: (#1) a simplified tree with the major generic relationships reduced to a polytomy ((((*Copiopteryx*, *Rhescyntis*), (*Titaea*, *Dysdaemonia*), *Grammopelta*, *Paradaemonia*, *Caio*, *Loxolomia*), *Arsenura*), *Almeidaia*, (*Salassa*, *Agliia*)); and (#2) a simplified tree based on the topology of the ‘Pr+Fl minus *Grammopelta*’ to see where *Grammopelta* would be placed (see Supplemental Trees). To test whether one topology was better than another, we ran Shimodaira-Hasegawa tests in the R package ‘phangorn’ (Schliep 2010), under 10,000 bootstrap replicates. All trees were rooted with six outgroup species from the subfamilies Saturniinae, Salassinae, and Agliinae (Supp. Table 1). All consensus alignment FASTA files, loci information, partition files, tree files, and other essential data files used for phylogenetic inference are available as supplementary materials on the Dryad Data Repository (doi:??). All data matrices and resulting trees are available in TreeBASE (??).

Digitization

The majority of specimens digitized were from the MGCL collection. Additional images of *Almeidaia aidae*, *Caio richardsoni*, and *Arsenura drucei* were obtained from the Barcode of Life Datasystems (BOLD); www.boldsystems.org (Ratnasingham and Hebert 2007). Further images of *Almeidaia aidae* were obtained from specimens at the MNHN and from Eurides Furtado (Furtado 2004) (see the non-LEP/non-MGCL codes in Supp. Tables 2-5). Specimens from these alternative sources were used because these species were

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unavailable in the MGCL (e.g., *Almeidaia* specimens are exceedingly rare in collections). Images of the right wing (or left wing, if right side was damaged or broken) were taken with a Canon EOS Rebel T3i digital camera with EF 35mm F2.0 lens. Pinned specimens were mounted onto a piece of clay and carefully placed on an opaque acrylic sheet (355 x 355 x 3 mm) in a way that the wings were in a horizontal plane. If needed, the wings of pinned specimens were slightly moistened with 90% EtoH in order to provide transparency for visualizing the area of overlap between the forewing (FW) and hindwing (HW). A metric ruler was included with each image to provide scale. If the fore- and hindwing were not in one plane, two images were taken.

Measurements used for comparative analyses included FW length (FW_L), HW length (HW_L), and type of HW tail shape as a categorical variable. FW length was chosen because it is widely used as a proxy for body size (Miller 1977). Images were processed in ImageJ (Abràmoff et al. 2004) to obtain measurements of the wings. FW and HW length were measured using the included ruler for scale. Forewing length was measured as the straight-line distance between a point placed at the middle of the base of the wing junction to the thorax, and one placed at the apex of the forewing (Supp. Fig. 2a). Hindwing length was measured from a point placed at the middle of the wing junction to the thorax, to the point of the deepest curve (convex) along the tornal edge of the hindwing (i.e., most prominent extension or curvature on the tornal margin) or the tornal end of the hindwing tail (Supp. Fig. 2b). Photoshop CS6 Version 13.0 was used to obtain the wing shape outlines used in the Elliptical Fourier analyses. To do this, FW and HW were highlighted and cut out separately with the quick selection tool (standard set up: size 22 pixels, hardness 100%, and spacing 25%). The wing of choice was selected and colored pure black, with the background converted to pure white. Images were converted to black and white with the greyscale function. If images of the left wings were used, they were flipped horizontally to have the

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same orientation as the other images. Specimens imaged at the MGCL had a green label with “MGCL #####” added with the specimen. All images are available in the supplemental information within the Dryad Digital Repository (doi:??).

Elliptical Fourier Descriptor analysis

Analyzing shape variation among objects can reveal insights into their function and the underlying mechanisms leading to their variation. Geometric morphometrics (GM) is a powerful tool to study phenotypic variation and covariation, as size and shape are treated as separate variables (Zelditch et al. 2012). Elliptical Fourier Descriptor (EFD) methods were developed for fitting curves to complex closed contours (Kuhl and Giardina 1982) and can be used to analyze the outlines of objects and numerically describe shapes that have few or no identifiable homologous (i.e., explicit) landmarks ((Iwata et al. 1998), (Chitwood 2014), (Bonhomme et al. 2013), (Bonhomme et al. 2014)), while eliminating size as a variable in shape. Instead of using traditional landmark data to quantify Lepidoptera wing shape (e.g., (Nath and Devi 2009), (Chazot et al. 2016), (Zhong et al. 2016)), we investigated the usefulness of EFD using the R program ‘Momocs’ (Bonhomme et al. 2014). EFD has been used frequently in plants to study the evolution of leaf shape ((Chitwood and Naylor 2012), (Chitwood et al. 2012a), (Chitwood et al. 2012b), (Chitwood et al. 2013), (Chitwood et al. 2014)), but rarely in animals ((Rohlf and Archie 1984), (Felice and O’Connor 2014), (Zhan and Wang 2012), (Sharma et al. 2017)). Recently however, it has been shown to be a powerful approach to study shape evolution in Lepidoptera ((Rubin et al. 2018), (Hegedus et al. 2018)). We chose male specimens because they fly long distances looking for mates, while females generally sit in the vegetation very near where they emerge from the pupa (e.g., Arsenurinae pupate in the soil), waiting for males to find them, which thus potentially exposes males to higher bat predation pressure (see (Rutowski 1982), (Acharya 1995)).

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To quantify wing shape and morphospace, the first harmonic (i.e., defines the best-fitting ellipse) was used to normalize the harmonic coefficients and render them invariant to size and rotation. This approach allows quantifiable analysis of shape when there are few or no identifiable homologous landmarks ((Iwata and Ukai 2002), (Bonhomme et al. 2014), (Chitwood 2014), (Chitwood and Sinha 2016)). EFD uses the first ellipse to normalize for rotation, translation, size and orientation, then uses harmonic coefficients for subsequent statistical analysis, Principle Components Analysis (PCA), and visualization. Outlines were defined as the closed polygon formed by the (x; y) coordinates of bounding pixels. A smoothing iteration was visually determined by eye and set to 500 for both HW and FW, using the function ‘`coo_smooth`’. The harmonics were then estimated using the function ‘`calibrate_harmonicpower_efourier`’ to evaluate the number needed to effectively describe the shapes in this analysis, without overparameterization, and where 99% of the power is captured. For forewings (FW) the number of harmonics were set at 8, while the hindwings (HW) were set at 9. Prior to EFD, all images were stacked and centered on top of each other (‘`stack`’ and ‘`coo_center`’), scaled to the same size (‘`coo_scale`’), and then put into the same direction (‘`coo_slidedirection`’) to avoid poor alignment and rotation of the shapes. The harmonic coefficients were then used for subsequent statistical analysis (EFD), PCA to reduce the dimensionality of the `efourier` coefficients, and visualization of the morphospace. HW morphospace was then plotted and visualized for all specimens designated by subfamily, tribe, genus, or species, and HW shape category (i.e., “none” = no tail, “lobe” = bulge of HW material (both small and long), “small” tail, “medium” tail, “long” tail, and “extra long” tail), as well as for the Arsenurinae (ingroup) using only the genus, species, and HW shape category descriptor. FW morphospace was plotted and visualized using the same categories, in particular the HW shape category, in order to visualize potential relationships between the two variables.

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375 *Trait Evolution*

376 Phylogenetic relationships present problems for statistical inference because lineages
377 are evolutionarily related and therefore the data are not independent. Our major questions
378 asked whether there were evolutionary trade-offs between body size, wing shape, and the
379 presence of hindwing tails. To evaluate the appropriateness of testing for statistical
380 significance, we investigated the phylogenetic signal (Pagel's λ) in our measured traits (i.e.,
381 HW shape, FW shape, body size (FW length = proxy for body size in moths), and HW
382 length). After accounting for phylogeny, we tested for the effects of one trait on another by
383 performing Phylogenetic Generalized Least Squares (PGLS) regression, under the 'mvOU'
384 (multivariate Ornstein-Uhlenbeck) model of evolution (i.e., the mvOU model provided a
385 better fit to the data than the BM (Brownian Motion) or EB (Early Burst) models) using the R
386 package 'Rphylopars' (Goolsby 2017a); this program can estimate evolutionary covariance
387 while accounting for within-species variation and missing data (see (Bruggeman et al. 2009),
388 (Goolsby 2015), (Goolsby 2017b), (Goolsby 2017a)). In order to probe our dataset for
389 possible evolutionary tradeoffs, we asked these questions: 1. Is HW length a predictor of
390 FW_L [body size]?; 2. Is FW_L [body size] a predictor of HW length?; 3. Is HW length a
391 predictor of FW shape complexity?; and 4. Is FW shape complexity a predictor of HW
392 length? (see Table 1). Additionally, phylogenetic ANOVA were conducted using the R
393 package 'phytools' (Revell 2011) to test whether the type of hindwing (as multistate
394 categorical variables) had an effect on another trait (HW shape, FW shape, or body size). We
395 coded the HW categories into discrete states (based on the results of Rubin et al. 2018): none
396 (i.e., no HW tail) = 0, a small lobe = 1, a long lobe = 2, a small tail = 3, medium tail = 4, or
397 extra long tail = 5 (Fig. 2). To do this, we reduced our dataset to only our ingroup, the
398 Arsenurinae, by removing the outgroup tips from our preferred tree, the constrained tree (#1).

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The tree was then converted into a relative-rate scaled ultrametric tree using the ‘chronopl’ command in the R package ‘ape’ (Paradis and Schliep 2019). This approach produces a tree whose branches are scaled to evolutionary rates, rather than a dated tree, and provides a means to understand evolutionary changes over relative “time” in the group being investigated. Our quantitative dataset was then matched to the ingroup species and tips in the tree. Trait distributions were also visualized using the contMap function in ‘phytools’ (Revell 2011).

RESULTS

Phylogenetics

All initial datasets contained 42 taxa (36 ingroup Arsenurinae and 6 outgroup Saturniidae lineages). We evaluated how museum specimens performed in sequencing (i.e., papered or pinned), and did not observe any qualitatively significant differences in recovery of genetic data (Supp. Table 1, Supp. Text 2). Importantly, our datasets do not possess GC-bias (Bossert et al. 2017), with 41.7% GC content. The sequence information found within the ‘Pr’ dataset comprised 185,007 bp, of which 55,209 were informative sites, and a -1479454.703 log-likelihood score for the consensus tree. The sequence information found within the ‘AA’ dataset comprised 60,425 sites, of which 4,709 were informative sites, and a -294666.796 log-likelihood score for the consensus tree. The sequence information found within the ‘Pr+Fl’ dataset comprised 267,958bp, of which were 93,428 informative sites, and a -2273571.993 log-likelihood score for the consensus tree. The sequence information found within the ‘Fl’ dataset comprised 80,276 bp, of which 35,647 were informative sites, and a -817959.106 log-likelihood score for the consensus tree. See Supplemental Trees for these material.

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In the initial phylogenetic inferences, we only see two recurring outcomes: 1) All genera are monophyletic entities and well-supported; and 2) *Almeidaia* – the only representative of tribe Almeidaiini – is always sister to the rest of the Arsenurinae, a clade forming the Arsenurini tribe. Generic relationships within this latter tribe are rather unresolved, except for the sister group relationship between *Dysdaemonia* and *Titaeta*. We often found *Copiopteryx* and *Rhescyntis* as sister lineages, with *Paradaemonia* as the sister lineage to those two; the ‘AA’ and ‘Pr’ inferences identify the same three lineages as a monophyletic group, but with differing lineages as the sister to the other two (see Supplemental Trees). From the ‘AA’ and ‘Fl’ datasets, we recovered a “well-supported” placement of *Loxolomia* as the sister to the rest of the Arsenurini, whereas in the ‘Pr’, ‘Pr+Fl’, and ASTRAL datasets *Arsenura* is found in that position. The lineages whose topological locations change depending on the dataset are *Caio*, *Grammopelta*, and *Loxolomia*. In the ASTRAL tree, these three genera were never found as sister to a single other genus, but always form independent lineages branching from the tree stem (see Supplemental Trees). In the ‘AA’ tree, *Caio* is sister to *Arsenura*, and *Grammopelta* is sister to *Copiopteryx*, *Paradaemonia* and *Rhescyntis*. In the ‘Pr’ tree, *Caio* is sister to *Titaeta* and *Dysdaemonia*, and *Grammopelta* and *Loxolomia* are sister lineages. In the ‘Fl’ tree, *Caio* is sister to *Paradaemonia*, and *Grammopelta* is sister to *Copiopteryx* and *Rhescyntis* (see Supplemental Trees). One additional common outcome is that across all trees there are very short branches and low support values associated with the early branching of the Arsenurini tribe. In particular, the ASTRAL tree, with its branch lengths in coalescent units, starkly highlights the lack of signal at this depth (see Supplemental Trees).

One of the most vexing problems in phylogenetic inference occurs when a region of a tree possesses short branches and no “support” for relationships (i.e., a polytomy). In our initial inferences, we consistently see this “unfortunate” pattern where a large polytomy (if

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nodes with low support are collapsed) occurs along the backbone of the Arsenurini (see Fig. 1 and Supp. Trees). Consequently, we aimed to determine whether hidden phylogenetic signal could be found in the data. First, we searched for “rogue” taxa that were potentially negatively influencing topological differences and support. Only one taxon was identified as a potential “rogue taxon”, *Grammopelta lineata* – a monotypic genus. However, *Grammopelta* was not seen as an exceptionally divergent or problematic taxon. The leaf stability index (lsDif) is a measure of the stability of a node in a set of trees, based on quartet frequencies (Thorley and Wilkinson 1999), with values ranging between 0 (unstable) and 1 (stable), but the *Grammopelta* leaf stability index, lsDif = 0.722367, was only slightly lower than those of other well supported taxa - *Dysdaemonia* and *Titaea*, both with an lsDif = 0.775075. An alternative statistic, the taxonomic instability index, measures the stability of a node in a set of trees based on unweighted patristic distances (Maddison and Maddison 2011), where the higher the annotation value, the more unstable the taxon, but the *Grammopelta* taxonomic instability index (87511.46) was again only slightly higher than other well supported taxa (e.g., *Dysdaemonia* sister to *Titaea* = 76089.91). Nevertheless, we decided to remove the taxon and rerun the phylogeny inference to investigate how topologies or support changed.

After removing *Grammopelta*, the sequence information found within the ‘AA minus *Grammopelta*’ dataset comprised 60,425 sites, of which 4,670 were informative sites, and a -291394.450 log-likelihood score for the consensus tree. The sequence information found within the ‘Pr+Fl minus *Grammopelta*’ dataset comprised 267,958 bp, of which 92,647 were informative sites, and a -2214908.564 log-likelihood score for the consensus tree. When we compare the topological outcomes, we see that removing *Grammopelta* generally increases “traditional” support values, placing *Paradaemonia* and *Caio* as sister lineages, and provides good support for a clade comprising these two lineages as sister to *Copiopteryx* + *Rhescyntis*.

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Titaea and *Dysdaemonia* are sister lineages, with *Loxolomia* the sister to those two, with good support for these three lineages as sister to the previously mentioned four, and *Arsenura* and *Almeidaia* sister to the rest of the Arsenurinae. When included, *Grammopelta* is sister to *Caio*, though not well supported, and this clade is sister to ((*Rhescyntis* and *Copiopteryx*), (*Paradaemonia*, (*Titaea* and *Dysdaemonia*))), with *Loxolomia* sister to this group, and *Arsenura* and *Almeidaia* sister to the rest of the Arsenurinae (see Supplemental Trees).

We also investigated additional modes of topological support. One alternative measure of node support is to look at concordance factors, in particular, gCF values - the percentage of “decisive” gene trees containing a particular branch of interest. Using the ‘Pr+Fl’ and ASTRAL phylogenies (see Supp. Trees and Fig. 1 for these values on the preferred tree), we see that the underlying data contain much discordance – only 17 nodes within the Arsenurinae possess a gCF ≥ 50 and most of those correspond to nodes that define genera as well as intrageneric nodes (see Supplemental Trees), outcomes we already felt comfortable with. For example, the individual genera within the Arsenurinae are robustly supported as monophyletic; all are ≥ 50 gCF. We also see that seemingly undisputed nodes (e.g., the placement of *Almeidaia* as sister to the *Arsenura*, which is then sister to the rest of the Arsenurinae) have gCF values of slightly below 50 (40.6 and 49.8 respectively) – values still relatively high when compared with other nodes. Unfortunately, again we see that there is no resolution within the medium depth nodes, simply a star phylogeny (if “poorly” supported nodes are collapsed), and it is unknown whether this represents a hard or soft polytomy.

Another alternative measure of node support utilizes Internode Certainty (IC) scores, quartet-based measures for estimating internode certainty (Zhou et al. 2020). Importantly, IC scores were designed to quantify the level of incongruence in phylogenetic data sets, whereas traditional support values (i.e., bootstrap and posterior probability) aim to predict the

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correctness (congruence) of the tree topology. One important assumption underlying the QP-IC score is that the four subsets of taxa around a given internal branch are correctly resolved; because of this, we tested how IC scores (specifically, the QP-IC score - quadripartition internode certainty) would change depending on alternative placements of ambiguous taxa (e.g., *Grammopelta*, *Loxolomia*). According to Zhou et al. (2020), scores will fall between 1 and -1, summarizing the diversity and strength of conflicting signals into a single number. When scores approach 1 or -1, this suggests that a given internal branch is either supported (1) or contested (-1), whereas scores close to 0 indicate high levels of incongruence and a lack of phylogenetic signal. When we look at these scores on the 'Pr+Fl' phylogeny, we see that only 10 nodes have a QP-IC score ≥ 0.50 , again with most of these representing genera or intrageneric relationships, whereas the other nodes possess values near 0 (Supp. Text 3). Following our taxon placement tests, we found alternative placements of *Grammopelta* and other taxa did not fundamentally change the pattern or strength of QP-IC score, and so only these scores are reported. For genera, we see there is good support for the reference topology (i.e., all genera are monophyletic) versus the frequency of the most prevalent alternative topology. We also find support for *Almeidaia* as sister to the rest of the Arsenurinae, but very little support for any between-genera relationships, outside of *Titaea* and *Dysdaemonia*. Unfortunately, this measure of support again confirms the high levels of incongruence amongst the individual gene trees.

Likelihood mapping was used to examine the clustering of user-defined subgroups, in a hypothesis-testing framework, as well as to visualize the phylogenetic content of the AHE dataset (Strimmer and vonHaeseler 1997). By looking at the distribution of quartets, we see there is significant signal to expect a well-resolved tree, with signal split evenly among the quartets (Supp. Fig. 3). To reconstruct a better understanding of the Arsenurinae backbone, we computed the likelihood of relationships that could be constructed from possible quartets

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of the taxa. First, we looked at the subfamily level by testing whether Arsenurinae is more closely related to Agliinae, Salassinae, or Saturniinae (Supp. Fig. 4). We found that 100% of the quartets possess an (Arsenurinae + Agliinae) ~ (Salassinae + Saturniinae) relationship. Second, at the generic level we find that a relationship of (a *Loxolomia*/*Grammopelta* clade + a *Titaea*/*Dysdaemonia* clade) ~ (a *Copiopteryx*/*Rhescyntis* + a *Paradaemonia*/*Caio* clade) is found in 69.6% of investigated quartets (with the other subfamily representatives – i.e., outgroups, *Almeidaia*, and *Arsenura* ignored) and had the best likelihood score (-2358325.992) with the shortest total tree length (2.200) (Supp. Fig. 5). Additional combinations of hypotheses were tested, though only one had an equal score and tree length – a relationship of (*Almeidaia* + a *Loxolomia*/*Caio*/*Grammopelta* clade) ~ (a *Copiopteryx*/*Rhescyntis* + a *Paradaemonia*/*Titaea*/*Dysdaemonia* clade) is found in 51.6% of quartets, whereas (*Almeidaia* + a *Copiopteryx*/*Rhescyntis* clade) ~ (a *Loxolomia*/*Caio*/*Grammopelta* clade + a *Paradaemonia*/*Titaea*/*Dysdaemonia* clade) represents 37.9% of quartets (the other subfamily representatives and *Arsenura* were ignored) (Supp. Fig. 6).

To evaluate gene tree/species tree incongruence in an alternative framework, we computed phylogenetic networks. Networks can help visualize reticulate relationships among taxa (i.e., due to hybridization, horizontal gene transfer, or recombination). This approach allows for comparison of the underlying phylogenetic signal because phylogenetic trees exclude all incompatible edges, whereas phylogenetic networks include them; the exclusion of an edge from a network is a powerful statement about relationships (Schliep et al. 2017). The inferred SplitsTree4 network indicates low amounts of reticulation and a large star relationship, with no intergeneric structure, except that *Loxolomia* and *Grammopelta* are closely related (Supp. Fig. 7). Unfortunately, no significant information could be gathered from the PhyloNet analyses because each time a new reticulation event was added, the

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likelihood score would become better, with no apparent stabilization or asymptotic behavior. Though we attempted to look at up to 14 reticulation events (i.e., equal to the total number of tips in the reduced tree), the computational resources needed (both computing power and time), resulted in this not being feasible to reasonably arrive at an answer for all 14; we tested 1 to 9 events.

Overall, to infer the most probable relationships within the Arsenurinae, we inspected the differing phylogenetic inferences, alternative support values (e.g., concordance factors, IC scores), quartets (e.g., Likelihood Mapping), and phylogenetic networks. We then inferred two unrooted constrained topologies. The constrained inference (#1) produced a consensus tree with a -2273610.971 log-likelihood score (Fig. 1). The constrained inference (#2) produced a consensus tree with a -2273675.522 log-likelihood score. We then tested whether the constrained topologies (#1 and #2) were better than the ‘Pr+Fl’ topology or each other, by using Shimodaira-Hasegawa tests under 10,000 bootstrap replicates. We find the constrained tree (#1) is not significantly better than the ‘Pr+Fl’ tree, but the constrained tree (#1) is significantly better than the constrained tree (#2). We could not statistically compare these trees with the ‘Pr+Fl minus *Grammopelta*’ tree because of the missing taxon (see Supplemental Trees).

Collectively, these findings indicate *Almeidaia* is the sister lineage to all other Arsenurinae, with *Arsenura* likely the next branching lineage after *Almeidaia*. The results also indicate that within the Arsenurini *Copiopteryx* and *Rhescyntis* are sister lineages, and that *Titaeta* and *Dysdaemonia* are also sister lineages. The problematic lineages (i.e., those that topologically unstable) are *Caio*, *Grammopelta*, and *Loxolomia*. When *Grammopelta* is removed from the inference (‘Pr+Fl minus *Grammopelta*’), *Caio* is sister to *Paradaemonia*. When *Grammopelta* is allowed to place itself in the phylogeny (constrained #2), it is inferred to be the sister lineage of a (*Copiopteryx* + *Rhescyntis*) + (*Caio* + *Paradaemonia*) clade,

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albeit weakly supported. In the ‘Pr+Fl’ and constrained (#1) trees, *Grammopelta* and *Loxolomia* are sister lineages, and *Caio* is sister to *Paradaemonia* (see Supplemental Trees). A synthesis of these findings provides our best estimate of the Arsenurinae phylogeny, the constrained tree (#1) (Fig. 1), and guides the trait evolution analyses (below).

Elliptical Fourier Descriptor analysis

To examine the evolution of wing shape across the Arsenurinae, we conducted geometric morphometric analysis of 968 male specimens, mostly from natural history collections, comprising 174 species across the eight Saturniidae subfamilies (see Supp. Tables 2 & 3). Ingroup morphological sampling comprised 545 specimens corresponding to 50 species of the currently recognized 89 Arsenurinae species (see (Kitching et al. 2018); Supp. Tables 4 & 5). Across the Saturniidae, HW morphospace was visualized for all specimens at the subfamily, tribe, genus, and species level, as well as by HW shape category descriptor. Within the Arsenurinae (i.e., ingroup), morphospace was visualized at the genus and species level, as well as by HW shape category descriptor (Figs. 2 & 3). FW morphospace was plotted and visualized for the same categories, with particular focus on the HW shape category, to visualize potential trait relationships (Figs. 4 & 5). Background shapes in the morphospace plots are hypothetical shape space based on the specimens being analyzed.

When HW morphospace is plotted, we observe a crescent shape of morphospace that is filled by all sampled genera across the family (Fig. 2). If we classify specimens by subfamily, we see that the Arsenurinae and Saturniinae generally occupy the same morphological space, independently, as found by Rubin and Hamilton et al. (2018), suggesting there are likely evolutionary forces (e.g. bat predation and genomic constraints) driving lineages into this similar shape space (Fig. 2a). We see there is overlap in occupied

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shape space between HW shape categories ‘none’ and ‘lobe’, ‘lobe’ and ‘small’, ‘small’ and ‘medium’, but ‘long’ and ‘extra long’ are distinct (Fig. 2b). When we examine just the Arsenurinae (ingroup), again we see the same areas/paths of morphospace being filled, but this time it is more clear that there are three distinct shape spaces – with no overlap between *Copiopteryx* (1), *Dysdaemonia*, *Paradaemonia*, and *Titaea* (2), and *Almeidaia*, *Arsenura*, *Caio*, *Grammopelta*, *Loxolomia*, and *Rhescyntis* (3) (Fig. 3a). When we look to see if there is overlap in occupied shape space among the HW shape categories in the Arsenurinae, we see overlap between ‘none’ and ‘lobe’, ‘lobe’ and ‘small’, ‘small’ and ‘medium’, but ‘long’ and ‘extra long’ are distinct (Fig. 3b). Additional Arsenurinae (within genera) plots can be found in the Supplemental Information.

When we examine FW shape, we again see that the Saturniinae and Arsenurinae overlap in morphospace, though this overlap also occurs more broadly across the family (Fig. 4a). When we just examine the Arsenurinae, we see similar overlap and variation, but certain genera are distinct (*Almeidaia*, *Dysdaemonia*, *Loxolomia*, *Rhescyntis*, and *Titaea*) from others (*Arsenura*, *Caio*, *Grammopelta*, and *Paradaemonia*) (Fig. 5a). If we look at whether there is a potential link between FW shape and HW shape, patterns are hard to perceive due to a large amount of variation and overlap (Fig. 4b). When evaluating only the Arsenurinae, an observable pattern becomes clearer between FW shape and HW shape (Fig. 5b); those with ‘long’ or ‘extra long’ hindwings are similar, as well as ‘small’ and ‘medium’, suggesting there may be physiological/developmental “rules” to wing shape. And lastly, from a taxonomic standpoint an interesting outcome was revealed – EFD wing shape morphometrics can be used for species identification/delimitation, at least when investigating male specimens (e.g., within each genus, there are significant differences between certain species’ wing shape morphospace, in particular *Caio* and *Paradaemonia*; see the additional Arsenurinae genera plots found in the Supplemental Information).

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624

625 *Trait Evolution*

626 The main aim of this research was to determine whether there are evolutionary
627 relationships between body size (measured as FW length - a proxy for body size), wing shape
628 (quantified as principal components), and the presence of tails (Table 1; see Fig. 6) in the
629 Arsenurinae, in the hope of testing for these patterns across the Saturniidae in the future. The
630 first four principal components were used in the trait evolution analyses, with the first two the
631 most informative (i.e., PC1 and PC2 explained $\geq 75\%$ of the variation in both FW and HW
632 shape). Before investigating the evolution of these traits across the Arsenurinae, we evaluated
633 whether there was significant phylogenetic signal in our data (Pagel's $\lambda = 0.974$). A
634 likelihood ratio test suggested there was significant signal in the phylogenetic residuals ($p =$
635 2.186×10^{-22}) (i.e., close relatives are more similar than random pairs of species; Revell 2010).

636 To answer our questions relating to wing shape and body size tradeoffs, we used
637 phylogenetic generalized least squares regressions (PGLS) and phylogenetic ANOVAs. We
638 found that HW length (HW_L) is a predictor of body size (FW_L) (i.e., $FW_L \sim HW_L$; $p =$
639 0.001). When we ask if body size determines the length of HW a moth lineage will have, we
640 find that body size does not predict the length of the hindwing (i.e. $HW_L \sim FW_L$; $p =$
641 0.1131). When we ask whether the length of the HW determines forewing shape, we find that
642 HW length (HW_L) is a predictor of FW shape complexity (PC1 of FW shape) (i.e., $PC1 \sim$
643 HW_L ; $p = 2.2 \times 10^{-16}$). And lastly, we do not find that FW shape complexity predicts HW
644 length (i.e., $HW_L \sim FW_PC1$; $p = 0.8423$). See Table 1 for questions tested and the
645 outcomes. See Figure 6 for shape changes across the phylogeny. To investigate the effects of
646 HW shape category (i.e., whether or not there is a HW tail and if so what kind) on body size
647 and FW shape we employed phylogenetic ANOVAs. We find there was no significant effect
648 of HW shape category on body size ($p = 0.502$), but there was a significant effect of HW

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shape category on FW shape complexity (for both FW_PC1 and FW_PC2; $p = 0.001$). See Supp. Figures 8 and 9 for the changes in principal component morphospace across the HW and FW shapes.

DISCUSSION

For biologists to understand the evolution of traits, measured variables must be investigated within a solid phylogenetic framework. Phylogenomics has provided systematists with the capability to gather vast amounts of genomic sequence data in the hope of establishing those robust foundations from which evolutionary hypotheses can be tested. This molecular framework can then be integrated with other data (e.g., ecological, behavioral, morphology) to better understand how divergent forms and functions have evolved. To produce the first molecular phylogeny of the subfamily Arsenurinae, we employed Anchored Hybrid Enrichment (AHE) targeted-sequencing phylogenomics ((Lemmon et al. 2012), (Breinholt et al. 2018)). Within this phylogenetic context, we investigated the evolution of wing shape across the subfamily by integrating geometric morphometrics from natural history collection specimens to ask whether there were evolutionary trade-offs between body size, wing shape, and the presence of hindwing tails.

Phylogeny

One (hopeful) outcome of phylogenomics is that a tree will be inferred with well-resolved nodes so that robust evolutionary inferences can be made. But even in the “age of phylogenomics” there are areas of the Tree of Life where it seems that no matter the amount of data thrown at a problem, robust resolution remains elusive. While “unfortunate”, these parts of the Tree of Life are also often some of the most interesting from an evolutionary

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standpoint. The Neotropical subfamily Arsenurinae appears to be one of these cases. Following initial phylogenetic inference, a large polytomy remained unresolved in the backbone of the Arsenurinae phylogeny, although all genera are monophyletic and well-supported (see Fig. 1 and Supp. Trees). One important aim was then to determine if there was hidden phylogenetic signal not being detected in traditional phylogenetic inference, and if so, where and how could it be used to piece together the evolutionary history of the group into a meaningful story.

To investigate whether hidden “support” could be found within this polytomy (i.e., a putative rapid diversification), we inspected tree topology (both supermatrix and species tree), rogue taxon analysis, alternative nodal support values (e.g., concordance factors, IC scores), quartets (e.g., Likelihood Mapping), network analyses, and constrained topology tests to arrive at a “most probable” relationship within the Arsenurinae (Fig. 1). Collectively, these analyses strongly indicate that *Almeidaia* is the sister lineage to all other Arsenurinae (the Arsenurini tribe), and that *Arsenura* is likely the next branching lineage. The results also indicate that *Copiopteryx* and *Rhescyntis* are sister lineages, as well as *Dysdaemonia* and *Titaea*. The phylogenetic network analysis indicates that *Grammopelta* and *Loxolomia* are closely related sister lineages, a finding also seen in Peigler (1993). When we compare our results to those hypothesized past relationships (Supp. Fig. 1; (Michener 1952), (Peigler 1993), (De Camargo et al. 2009)), we find little consistency (Fig. 1), except for the placement of *Almeidaia* and the sister relationship between *Dysdaemonia* and *Titaea*.

The ASTRAL tree is particularly informative because its topological structure and branch lengths (represented in coalescent units) reveal a likely diversification event in this group’s evolutionary history – an event that led to the evolution of the major genera. Though the conflicting signal that we discovered produces a polytomy, this could represent an important evolutionary event, depending on whether it is a “hard” or “soft” polytomy. Hard

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polytomies represent a hypothesis that a common ancestral lineage speciated into multiple lineages at the “exact” same time, although most inferred polytomies are thought to be soft – meaning that they would be resolved if data of higher quality were available. While our data are comprehensive, they are not representative of the entire genome. But interestingly, an independent phylogenomic dataset (co-author RR, in prep) using ultraconserved elements (UCE) also faces the exact same problem, supporting the hypothesis of a hard polytomy.

There are multiple additional reasons for why we find this polytomy, for example the phylogenetically-informative sites may be scattered in the “noisy” genes, such that individual gene trees do not possess much useful information. This would help answer why gCF values are low; our ability to resolve single locus gene trees is limited due to their short length (i.e., lack of molecular evolutionary signal). For example, the gCF value can be as low as 0% if no single gene tree contains a branch present in the reference tree. This is an important point because relationships with short branch lengths are hard to resolve, particularly with short loci – a common feature of target capture phylogenomic data, indicating that many of our loci contain genuinely conflicting signal. Lastly, all but two genera in the subfamily have fewer than 10 described species (*Grammopelta* = 1 species; *Almeidaia* = 2; *Loxolomia* = 3; *Rhescyntis* and *Titaia* = 5; *Dysdaemonia* = 6; *Caio* and *Copiopteryx* = 7), whereas *Paradaemonia* and *Arsenura* have 20 and 34 species, respectively. It is possible that many lineages have gone extinct, but at this time it is unknown whether this disparity was due to an elevated extinction rate in some lineages, a mass extinction event, a generic-level burst of diversification within this relictual lineage, or simply that a lack of taxonomic effort has not yet discovered the full extant diversity in this subfamily. Based on our previous knowledge of the bat-moth arms race, we propose a burst of diversification occurred in the Arsenurinae (as evidenced by the phylogeny), but at this time it is unknown what exactly drove the burst. A dated phylogeny would help us answer this question, but due to the paucity of informative

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Saturniidae and Bombycoidea fossils (Breinholt and Kawahara 2013) this was not undertaken. Our findings suggest this region of the Tree of Life may truly be unresolvable. But at this time, we cannot pinpoint the mechanism that allowed for this rapid split of lineages, perhaps more taxon sampling combined with biogeographical and ecological data will better answer this in the future.

Morphology & Trait Evolution

The Arsenurinae is not a “species rich” lineage of Saturniidae, but their diverse wing shape morphospace and discovery to be a relictual lineage (Hamilton et al. 2019) can shed significant light on the evolution of the Saturniidae. The subfamily possesses a number of wing shapes that are shared with other subfamilies (see Rubin et al. 2018). For example, Arsenurinae and Saturniinae occupy the same morphological space, suggesting there are likely multiple evolutionary forces (e.g. bat predation and genomic constraints) driving these lineages into this convergent shape space (Figs. 2a & 4a). Future work will have to parse whether these forces are more heavily weighted towards genetic constraints, or whether natural selection (perhaps by bat predation) has played the larger role. We are only now beginning to answer some of the more fascinating questions in the evolution of the Saturniidae – e.g., whether tails evolved using the same path, over and over, and whether long hindwing tails gradually evolved in a progressive manner from no tails. Similar to the findings of Rubin and Hamilton et al. (2018), our results indicate that hindwing tails evolved rapidly and not transitionally.

The most ecologically elucidating aspect of this investigation was determining there were evolutionary trade-offs between body size, forewing shape, hindwing length, and the presence of tails (Table 1; see Fig. 6): 1) body size and the length of the HW covary inversely to one another, such that as HW length increases, body size decreases – interestingly, this

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trait (i.e., a very small body size and extra-long tails) has evolved independently in the long-tailed African saturniid genus *Eudaemonia* (Rubin et al. 2018); 2) the complexity of FW shape also covaries in opposition to the length of the HW, such that as HW length increases, FW shape becomes less complex; and 3) the type of HW shape category that a lineage possesses has an effect on FW shape, such that as lineages move through the shape states from no tail to long tail, the FW becomes less complex. These results also make intuitive sense – for example, the largest body sizes in the Arsenurinae are found in the genus *Rhescyntis*, a lineage without hindwing tails (Fig. 6) but instead possess large lobed hindwings (Fig. 1), a trait experimentally shown to reduce capture success by bats, as well as having evolved independently in the Saturniidae tribe Attacini (Rubin et al. 2018). And lastly, there are a number of saturniid lineages that are small and do not have hindwing tails. While we see that FW shape complexity does not predict HW length, those Arsenurinae possessing a long hindwing tail exhibit less complex FW shape – a finding likely important on a macroevolutionary scale, given the possible flight constraints imposed by carrying long hindwing tails (reviewed in Le Roy et al. (2019)).

There are many reasons why these tradeoffs might occur across evolutionary time. Perhaps there may be an energetic cost to building a long hindwing tail during pupal development, which might limit the resources available for other structures (such as large bodies or convoluted forewing shapes) (Shingleton et al. 2007). Alternatively, it could be that because large body size is also a corollary of being a capital breeder (i.e., needing to store resources as a larvae for future flight as adults because the lineage does not nectar feed as adults), Saturniidae lineages cannot possess a large body size if they also have long tails – potentially explaining why we do not see more species with tails across the family. Detailed kinematic analysis of diverse silkmoths in flight could provide the explanatory power, as it is possible that increasing the complexity of the hindwings imposes limitations on body size

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and FW shape – structures more critical to moth flight than hindwings (Jantzen and Eisner 2008). For example, based on our observations (RR pers. obs.), long-tailed saturniids (e.g., *Copiopteryx* and *Eudaemonia*) have a different flight behavior than non-tailed species – i.e., long-tailed species tend to flutter highly erratically yet surprisingly maneuverable. In contrast, genera like *Arsenura* and *Titaea* exhibit a more powerful flight, potentially due to the larger wing surface area. Future research should also look into whether less complex forewing shape creates more uniform power during flight. Additionally, butterfly fore- and hindwings appear to be subject to differing selective pressures (Owens et al. 2020) and have been linked with microhabitat (Chazot et al. 2016), which will be an interesting question to consider in future research, as members of the Arsenurinae, and Saturniidae at large, have a wide geographical spread across a variety of environments. It has also recently been seen that, in butterflies, the hindwing is important in gliding performance (Stylman et al. 2019). And lastly, research on the effects of wing damage to specific regions of the fore- and hindwings indicates that altering the shape of the leading edge of the forewing has the greatest impact on flight parameters (Le Roy et al. 2019). We suggest that more work is needed in the Saturniidae family in general to understand the kinematic effects of complex FW and HW shape.

Arsenurine moths, like other saturniids, do not possess many of the anti-bat traits that can be found in their sister lineage, the sphingids, such as ears or ultrasound producing organs. One intriguing, but as yet untested, hypothesis is that body size and FW shape are anti-bat strategies. Insectivorous bats are gape-limited predators (Santana 2016). This functionally-restricted morphology may restrict bats to attacking only “appropriately sized” prey. Thus, large-bodied moths may effectively reduce or eliminate predation events from small bat species. In fact, some of the largest moths are thought to avoid predation simply because their size prevents bats from capturing them (Roeder and Treat 1970). Small myotid

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bats, when offered dead, tethered moths of various sizes, have been shown to attack small moths approximately 50% more frequently than large ones (Barclay and Brigham 1994). In addition, while experimental evidence indicates that hindwing tails are a highly-effective anti-bat evolutionary strategy that has evolved multiple times within the Saturniidae, it is not yet clear whether complex forewing shape can function to thwart bat attack ((Barber et al. 2015), (Rubin et al. 2018)). Our present morphometric and phylogenetic investigation into the Arsenurinae suggests that these hindwing appendages come with a body size and forewing shape trade-off. These results provide another layer of insight into the evolutionary forces structuring wing shape in the Arsenurinae and likely the Saturniidae.

FUNDING

This work was supported by the National Science Foundation (NSF grant number 1557007 to AYK, NSF IOS-1121739, 1121807, 1920895, and 1920936 to AYK and JRB, NSF DBI 1349345 and 1601369 to AYK, and PRFB 1612862 to CAH); National Environmental Research Council (NERC grant number NE/P003915/1 to IJK); French National Research Agency (ANR grant SPHINX 16-CE02-0011-01 to RR).

ACKNOWLEDGEMENTS

We thank Vincent Bonhomme for his help with Momocs. We are indebted to Charles Mitter and Kim Mitter for the use of their specimens in the UMD collection, as well as Jon Heppner for the use of specimens in his collections (FLMNH) – this work would absolutely not have been done without their help. We thank those who helped collect specimens (too many to list here). Kelly Dexter assisted with DNA extractions and additional lab work; Ryan

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824 St. Laurent provided input with Arsenurinae systematics. Sammantha Epstein and the
825 FLMNH volunteer team in the Kawahara Lab helped voucher specimens. UF undergraduates
826 Adena Mahadai, Shaelyn McGiveron, Dominique Philoctete, and Neeka Sewnath helped with
827 specimen digitization, as did UF researcher Geena Hill and visiting researchers James Adams
828 and John Snyder. We acknowledge the UF HPC for providing computational support and
829 assistance. Hamilton thanks AYK and Charles Cobb for their guidance as sponsoring
830 scientists during the PRFB.

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833 DATA AVAILABILITY

834 Data available from the Dryad Digital Repository: [http://dx.doi.org/10.5061/dryad.\[NNNN\]](http://dx.doi.org/10.5061/dryad.[NNNN])

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836

837 LITERATURE CITED

838 Aberer A.J., Krompass D., Stamatakis A. 2012. Pruning rogue taxa improves phylogenetic
839 accuracy: an efficient algorithm and webservice. *Syst Biol.* 62:162–166.

840 Aberer A.J., Stamatakis A. 2011. A simple and accurate method for rogue taxon
841 identification. *IEEE.* 118–122.

842 Abràmoff M.D., Magalhães P.J., Ram S.J. 2004. Image processing with ImageJ.
843 *Biophotonics Int.* 1–7.

844 Acharya L. 1995. Sex-biased predation on moths by insectivorous bats. *Anim. Behav.*
845 49:1461–1468.

EVOLUTIONARY TRADEOFFS IN SILKMOTHS

- 846 Barber J.R., Chadwell B.A., Garrett N., Schmidt-French B., Conner W.E. 2009. Naïve bats
847 discriminate arctiid moth warning sounds but generalize their aposematic meaning. JEB.
848 212:2141–2148.
- 849 Barber J.R., Kawahara A.Y. 2013. Hawkmoths produce anti-bat ultrasound. Biology Lett.
850 9:20130161–20130161.
- 851 Barber J.R., Leavell B.C., Keener A.L., Breinholt J.W., Chadwell B.A., McClure C.J.W., Hill
852 G.M., Kawahara A.Y. 2015. Moth tails divert bat attack: evolution of acoustic deflection.
853 PNAS. 112:2812–2816.
- 854 Barclay R., Brigham R.M. 1994. Constraints on optimal foraging: a field test of prey
855 discrimination by echolocating insectivorous bats. Anim. Behav. 48:1013–1021.
- 856 Bonhomme V., Picq S., Gaucherel C., Claude J. 2014. Momocs: outline analysis using R. J.
857 Stat. Soft. 56:1–24.
- 858 Bonhomme V., Prasad S., Gaucherel C. 2013. Intraspecific variability of pollen morphology
859 as revealed by elliptic Fourier analysis. Plant Syst. Evol. 299:811–816.
- 860 Bossert S., Murray E.A., Blaimer B.B., Danforth B.N. 2017. The impact of GC bias on
861 phylogenetic accuracy using targeted enrichment phylogenomic data. Mol Phylogenet
862 Evol. 111:149–157.
- 863 Breinholt J.W., Earl C., Lemmon A.R., Lemmon E.M., Xiao L., Kawahara A.Y. 2018.
864 Resolving relationships among the megadiverse butterflies and moths with a novel
865 pipeline for anchored phylogenomics. Syst Biol. 67:78–93.

Hamilton, Winiger, Rubin, Breinholt, Rougerie, Kitching, Barber, Kawahara

- 866 Breinholt J.W., Kawahara A.Y. 2013. Phylotranscriptomics: saturated third codon positions
867 radically influence the estimation of trees based on next-gen data. *Genome Biol Evol.*
868 5:2082–2092.
- 869 Bruggeman J., Heringa J., Brandt B.W. 2009. PhyloPars: estimation of missing parameter
870 values using phylogeny. *Nucleic Acids Res.* 37:179–184.
- 871 Chazot N., Panara S., Zilbermann N., Blandin P., Le Poul Y., Cornette R., Elias M., Debat V.
872 2016. Morpho morphometrics: Shared ancestry and selection drive the evolution of wing
873 size and shape in *Morpho* butterflies. *Evolution.* 70:181–194.
- 874 Chitwood D.H. 2014. Imitation, Genetic Lineages, and Time Influenced the Morphological
875 Evolution of the Violin. *PLoS ONE.* 9:e109229.
- 876 Chitwood D.H., Headland L.R., Kumar R., Peng J., Maloof J.N., Sinha N.R. 2012a. The
877 developmental trajectory of leaflet morphology in wild tomato species. *Plant Physiol.*
878 158:1230–1240.
- 879 Chitwood D.H., Headland L.R., Ranjan A., Martinez C.C., Braybrook S.A., Koenig D.P.,
880 Kuhlemeier C., Smith R.S., Sinha N.R. 2012b. Leaf asymmetry as a developmental
881 constraint imposed by auxin-dependent phyllotactic patterning. *The Plant Cell.* 24:2318–
882 2327.
- 883 Chitwood D.H., Kumar R., Headland L.R., Ranjan A., Covington M.F., Ichihashi Y., Fulop
884 D., Jiménez-Gómez J.M., Peng J., Maloof J.N., Sinha N.R. 2013. A quantitative genetic
885 basis for leaf morphology in a set of precisely defined tomato introgression lines. *The*
886 *Plant Cell.* 25:2465–2481.

EVOLUTIONARY TRADEOFFS IN SILKMOTHS

- 887 Chitwood D.H., Naylor D.T. 2012. Conflict between intrinsic leaf asymmetry and phyllotaxis
- 888 in the resupinate leaves of *Alstroemeria psittacina*. Front. Plant Sci. 3:1–11.
- 889 Chitwood D.H., Ranjan A., Martinez C.C., Headland L.R., Thiem T., Kumar R., Covington
- 890 M.F., Hatcher T., Naylor D.T., Zimmerman S., Downs N., Raymundo N., Buckler E.S.,
- 891 Maloof J.N., Aradhya M., Prins B., Li L., Myles S., Sinha N.R. 2014. A modern
- 892 ampelography: a genetic basis for leaf shape and venation patterning in grape. Plant
- 893 Physiol. 164:259–272.
- 894 Chitwood D.H., Sinha N.R. 2016. Evolutionary and Environmental Forces Sculpting Leaf
- 895 Development. Cur. Bio. 26:R297–R306.
- 896 Cho S., Epstein S.W., Mitter K., Hamilton C.A., Plotkin D., Mitter C., Kawahara A.Y. 2016.
- 897 Preserving and vouchering butterflies and moths for large-scale museum-based molecular
- 898 research. PeerJ. 4:e2160–11.
- 899 Condamine F. 2016. Global patterns of insect diversification: Towards a reconciliation of
- 900 fossil and molecular evidence? Sci. Rep. 6:310.
- 901 Conner W.E., Corcoran A.J. 2012. Sound Strategies: The 65-Million-Year-Old Battle
- 902 Between Bats and Insects. Annu. Rev. Entomol. 57:21–39.
- 903 Corcoran A.J., Barber J.R., Conner W.E. 2009. Tiger moth jams bat sonar. Science. 325:325–
- 904 327.
- 905 Corcoran A.J., Barber J.R., Hristov N.I., Conner W.E. 2011. How do tiger moths jam bat
- 906 sonar? J. Exp. Biol. 214:2416–2425.

Hamilton, Winiger, Rubin, Breinholt, Rougerie, Kitching, Barber, Kawahara

- 907 De Camargo A.J.A., Mielke O.H., Casagrande M.M. 2009. Cladistic analysis of the
- 908 subfamily Arsenurinae (Lepidoptera, Saturniidae) based on adult morphology.
- 909 Zootaxa.:1–34.
- 910 Dowdy N.J., Conner W.E. 2016. Acoustic Aposematism and Evasive Action in Select
- 911 Chemically Defended Arctiine (Lepidoptera: Erebiidae) Species: Nonchalant or Not?
- 912 PLoS ONE. 11:e0152981–20.
- 913 Dunning D.C. 1968. Warning sounds of moths. Zeitschrift für Tierpsychologie. 25:129–138.
- 914 Edwards S.V. 2009. Is a new and general theory of molecular systematics emerging?
- 915 Evolution. 63:1–19.
- 916 Ehrlich P.R., Raven P.H. 1964. Butterflies and plants: a study in coevolution. Evolution.
- 917 18:586.
- 918 Farrell B.D., Mitter C., Futuyma D.J. 1992. Diversification at the insect-plant interface.
- 919 BioScience. 42:34–42.
- 920 Felice R.N., O’Connor P.M. 2014. Ecology and caudal skeletal morphology in birds: the
- 921 convergent evolution of pygostyle shape in underwater foraging taxa. PLoS ONE.
- 922 9:e89737.
- 923 Furtado E. 2004. Almeidaia aidae Mielke & Casagrande: seus estágios imaturos e notas
- 924 taxonômicas (Lepidoptera, Saturniidae, Arsenurinae, Almeidaiini). Revista Brasileira de
- 925 Zoologia. 21:663–669.
- 926 Goolsby E. 2017a. Rphylopars: fast multivariate phylogenetic comparative methods for
- 927 missing data and within-species variation. Methods Ecol. Evo. 8:22–27.

EVOLUTIONARY TRADEOFFS IN SILKMOTHS

- 928 Goolsby E.W. 2015. Phylogenetic Comparative Methods for Evaluating the Evolutionary
929 History of Function-Valued Traits. *Syst Biol.* 64:568–578.
- 930 Goolsby E.W. 2017b. Rapid maximum likelihood ancestral state reconstruction of continuous
931 characters: A rerooting-free algorithm. *Ecol Evol.* 7:2791–2797.
- 932 Guindon S., Dufayard J.-F., Lefort V., Anisimova M., Hordijk W., Gascuel O. 2010. New
933 algorithms and methods to estimate Maximum-Likelihood phylogenies: assessing the
934 performance of PhyML 3.0. *Syst Biol.* 59:307–321.
- 935 Hamilton C.A., Laurent R.A.S., Dexter K., Kitching I.J., Breinholt J.W., Zwick A.,
936 Timmermans M.J.T.N., Barber J.R., Kawahara A.Y. 2019. Phylogenomics resolves
937 major relationships and reveals significant diversification rate shifts in the evolution of
938 silk moths and relatives. *BMC Evol. Biol.* 19:1–13.
- 939 Hegedus M., DeVries P., Penz C.M. 2018. The Influence of Mimicry on Wing Shape
940 Evolution in the Butterfly *Papilio dardanus* (Lepidoptera: Papilionidae). *Ann. Entomol.*
941 *Soc. Am.* 112:33–43.
- 942 Hristov N.I., Conner W.E. 2005. Sound strategy: acoustic aposematism in the bat–tiger moth
943 arms race. *Naturwissenschaften.* 92:164–169.
- 944 Huson D.H., Bryant D. 2006. Application of phylogenetic networks in evolutionary studies.
945 *Mol Biol Evol.* 23:254–267.
- 946 Iwata H., Niikura S., Matsuura S., Takano Y., Ukai Y. 1998. Evaluation of variation of root
947 shape of Japanese radish (*Raphanus sativus* L.) based on image analysis using elliptic
948 Fourier descriptors. *Euphytica.* 102:143–149.

Hamilton, Winiger, Rubin, Breinholt, Rougerie, Kitching, Barber, Kawahara

- 949 Iwata H., Ukai Y. 2002. SHAPE: A computer program package for quantitative evaluation of
950 biological shapes based on elliptic Fourier descriptors. *J Hered.* 93:384–385.
- 951 Jacobs D.S., Bastian A. 2016. Predator-prey interactions: co-evolution between bats and their
952 prey. Springer International Publishing.
- 953 Jantzen B., Eisner T. 2008. Hindwings are unnecessary for flight but essential for execution
954 of normal evasive flight in Lepidoptera. *PNAS.* 105:16636–16640.
- 955 Janzen D. 1984. Two ways to be a tropical big moth: Santa Rosa saturniids and sphingids.
956 Oxford Surveys in Evolutionary Biology. 1:140.
- 957 Jones K.E., Bininda-Emonds O.R.P., Gittleman J.L. 2005. Bats, clocks, and rocks:
958 diversification patterns in Chiroptera. *Evolution.* 59:2243–2255.
- 959 Kawahara A.Y., Barber J.R. 2015. Tempo and mode of antibat ultrasound production and
960 sonar jamming in the diverse hawkmoth radiation. *PNAS.* 112:6407–6412.
- 961 Kawahara A.Y., Breinholt J.W. 2014. Phylogenomics provides strong evidence for
962 relationships of butterflies and moths. *P Roy Soc B-Biol Sci.* 281:1–8.
- 963 Kawahara A.Y., Plotkin D., Espeland M., Meusemann K., Toussaint E.F.A., Donath A.,
964 Gimnich F., Frandsen P.B., Zwick A., Reis dos M., Barber J.R., Peters R.S., Liu S., Zhou
965 X., Mayer C., Podsiadlowski L., Storer C., Yack J.E., Misof B., Breinholt J.W. 2019.
966 Phylogenomics reveals the evolutionary timing and pattern of butterflies and moths.
967 *PNAS.* 116:22657–22663.
- 968 Kawahara A.Y., Plotkin D., Hamilton C.A., Gough H., Laurent R.S., Owens H.L., Homziak
969 N.T., Barber J.R. 2018. Diel behavior in moths and butterflies: a synthesis of data
970 illuminates the evolution of temporal activity. *Org. Divers. Evol.* 18:13–27.

EVOLUTIONARY TRADEOFFS IN SILKMOTHS

- 971 Kitching I., Rougerie R., Zwick A., St Laurent R., Ballesteros Mejia L., Kawahara A.,
972 Kitching I., St Laurent R., Ballesteros Mejia L., Kawahara A., Kitching I., Hamilton C.,
973 St Laurent R., Naumann S., Ballesteros Mejia L., Kawahara A. 2018. A global checklist
974 of the Bombycoidea (Insecta: Lepidoptera). *BDJ*. 6:e22236–13.
- 975 Kuhl F.P., Giardina C. 1982. Elliptic Fourier features of a closed contour. *Computer Graphics*
976 and Image Processing. 18:236–258.
- 977 Lamarre G.P.A., Mendoza I., Rougerie R., Decaëns T., Hérault B., Bénéfuz F. 2015. Stay out
978 (almost) all night: contrasting responses in flight activity among tropical moth
979 assemblages. *Neotrop Entomol*. 44:109–115.
- 980 Larsson A. 2014. AliView: a fast and lightweight alignment viewer and editor for large
981 datasets. *Bioinformatics*. 30:3276–3278.
- 982 Le Roy C., Cornette R., Llaurens V., Debat V. 2019. Effects of natural wing damage on flight
983 performance in *Morpho* butterflies: what can it tell us about wing shape evolution? *JEB*.
984 222:1–10.
- 985 Lei M., Dong D. 2016. Phylogenomic analyses of bat subordinal relationships based on
986 transcriptome data. *Sci. Rep*. 6:27726.
- 987 Lemmon A.R., Emme S.A., Lemmon E.M. 2012. Anchored Hybrid Enrichment for
988 massively high-throughput phylogenomics. *Syst Biol*. 61:727–744.
- 989 Maddison W. 1997. Gene trees in species trees. *Syst Biol*. 46:523–536.
- 990 Maddison W.P., Maddison D.R. 2011. Mesquite: a modular system for evolutionary analysis.

Hamilton, Winiger, Rubin, Breinholt, Rougerie, Kitching, Barber, Kawahara

- 991 McGaughran A. 2020. Effects of sample age on data quality from targeted sequencing of
992 museum specimens: what are we capturing in time? BMC Genomics. 21:1–10.
- 993 Mendes F.K., Hahn M.W. 2017. Why concatenation fails near the anomaly zone. Syst Biol.
994 67:158–169.
- 995 Michener C.D. 1952. The Saturniidae (Lepidoptera) of the Western Hemisphere -
996 Morphology, Phylogeny, and Classification. Bull. Am. Mus. Nat. Hist. 98:341–501.
- 997 Miller W.E. 1977. Wing measure as a size index in Lepidoptera: the family Olethreutidae.
998 Ann. Entomol. Soc. Am. 70:253–256.
- 999 Miller-Butterworth C.M., Murphy W.J., O'Brien S.J., Jacobs D.S., Springer M.S., Teeling
1000 E.C. 2007. A family matter: conclusive resolution of the taxonomic position of the long-
1001 fingered bats, *Miniopterus*. Mol Biol Evol. 24:1553–1561.
- 1002 Minh B.Q., Hahn M., Lanfear R. 2018. New methods to calculate concordance factors for
1003 phylogenomic datasets. bioRxiv.:1–7.
- 1004 Minh B.Q., Nguyen M.A.T., Haeseler von A. 2013. Ultrafast approximation for phylogenetic
1005 bootstrap. Mol Biol Evol. 30:1188–1195.
- 1006 Mirarab S., Reaz R., Bayzid M.S., Zimmermann T., Swenson M.S., Warnow T. 2014.
1007 ASTRAL: genome-scale coalescent-based species tree estimation. Bioinformatics.
1008 30:i541–i548.
- 1009 Mirarab S., Warnow T. 2015. ASTRAL-II: coalescent-based species tree estimation with
1010 many hundreds of taxa and thousands of genes. Bioinformatics. 31:i44–i52.

EVOLUTIONARY TRADEOFFS IN SILKMOTHS

- 1011 Misof B., Liu S., Meusemann K., Peters R.S., Donath A. 2014. Phylogenomics resolves the
1012 timing and pattern of insect evolution. *Science*. 346:763–767.
- 1013 Mitter C., Farrell B., Wiegmann B. 1988. The phylogenetic study of adaptive zones: has
1014 phytophagy promoted insect diversification? *Am. Nat.* 132:107–128.
- 1015 Morton E.S. 2009. The Function of Multiple Mating by Female *Promethea* Moths,
1016 *Callosamia promethea* (Drury) (Lepidoptera: Saturniidae). *Am. Midl. Nat.* 162:7–18.
- 1017 Nath R., Devi D. 2009. Venation pattern and shape variation in wing of *Antheraea*
1018 *assamensis* (Lepidoptera: Saturniidae) of Assam, India. *Int. J. Trop. Insect Sci.* 29:70–10.
- 1019 Neil T.R., Shen Z., Robert D., Drinkwater B.W., Holderied M.W. 2020. Thoracic scales of
1020 moths as a stealth coating against bat biosonar. *J. R. Soc. Interface*. 17:1–10.
- 1021 Nguyen L.-T., Schmidt H.A., Haeseler von A., Minh B.Q. 2015. IQ-TREE: a fast and
1022 effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol*
1023 *Evol.* 32:268–274.
- 1024 Owens H.L., Lewis D.S., Condamine F.L., Kawahara A.Y., Guralnick R.P. 2020.
1025 Comparative phylogenetics of *Papilio* butterfly wing shape and size demonstrates
1026 independent hindwing and forewing evolution. *Syst Biol.* in press.
- 1027 Paradis E., Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and
1028 evolutionary analyses in R. *Bioinformatics*. 35:526–528.
- 1029 Peigler R.S. 1993. Cladistic analysis of the genera of the subfamily Arsenurinae. *J. Lepid.*
1030 *Soc.* 47:211–228.

Hamilton, Winiger, Rubin, Breinholt, Rougerie, Kitching, Barber, Kawahara

- 1031 Ratnasingham S., Hebert P.D.N. 2007. BOLD: The Barcode of Life Data System
- 1032 (www.barcodinglife.org). Mol. Ecol. Notes. 7:355–364.
- 1033 Reddy S., Kimball R.T., Pandey A., Hosner P.A., Braun M.J., Hackett S.J., Han K.-L.,
- 1034 Harshman J., Huddleston C.J., Kingston S., Marks B.D., Miglia K.J., Moore W.S.,
- 1035 Sheldon F.H., Witt C.C., Yuri T., Braun E.L. 2017. Why Do Phylogenomic Data Sets
- 1036 Yield Conflicting Trees? Data Type Influences the Avian Tree of Life more than Taxon
- 1037 Sampling. Syst Biol. 66:857–879.
- 1038 Revell L.J. 2010. Phylogenetic signal and linear regression on species data. Methods Ecol.
- 1039 Evo. 1:319–329.
- 1040 Revell L.J. 2011. phytools: an R package for phylogenetic comparative biology (and other
- 1041 things). Methods Ecol. Evo. 3:217–223.
- 1042 Roeder K.D. 1974. Acoustic sensory responses and possible bat-evasion tactics of certain
- 1043 moths. Proceedings of the Canadian society of zoologists annual meeting.:71–78.
- 1044 Roeder K.D., Treat A.E. 1957. Ultrasonic Reception by the Tympanic Organ of Noctuid
- 1045 Moths. J. Exp. Zool. 134:127–157.
- 1046 Roeder K.D., Treat A.E. 1970. An acoustic sense in some hawkmoths (Choerocampinae). J.
- 1047 Insect Physiol. 16:1069–1086.
- 1048 Rohlf F.J., Archie J.W. 1984. A comparison of Fourier methods for the description of wing
- 1049 shape in mosquitoes (Diptera: Culicidae). Syst Biol. 33:302–317.
- 1050 Rubin J.J., Hamilton C.A., McClure C.J.W., Chadwell B.A., Kawahara A.Y., Barber J.R.
- 1051 2018. The evolution of anti-bat sensory illusions in moths. Sci. Adv. 4:1–9.

EVOLUTIONARY TRADEOFFS IN SILKMOTHS

- 1052 Rutowski R. 1982. Mate choice and lepidopteran mating behavior. *Fla. Entomol.* 65:72–82.
- 1053 Rydell J., Lancaster W.C. 2000. Flight and thermoregulation in moths were shaped by
1054 predation from bats. *Oikos.* 88:13–18.
- 1055 Santana S.E. 2016. Quantifying the effect of gape and morphology on bite force:
1056 biomechanical modelling and in vivo measurements in bats. *Funct Ecol.* 30:557–565.
- 1057 Sayyari E., Mirarab S. 2016. Anchoring quartet-based phylogenetic distances and
1058 applications to species tree reconstruction. *BMC Genomics.* 17:101–113.
- 1059 Schliep K., Potts A.J., Morrison D.A., Grimm G.W. 2017. Intertwining phylogenetic trees
1060 and networks. *Methods Ecol. Evo.* 62:162–9.
- 1061 Schliep K.P. 2010. phangorn: phylogenetic analysis in R. *Bioinformatics.* 27:592–593.
- 1062 Scoble M.J. 1992. *The Lepidoptera. Form, function and diversity.* Oxford University Press.
- 1063 Sharma P.P., Santiago M.A., Kriebel R., Lipps S.M., Buenavente P.A.C., Diesmos A.C.,
1064 Janda M., Boyer S.L., Clouse R.M., Wheeler W.C. 2017. A multilocus phylogeny of
1065 Podoctidae (Arachnida, Opiliones, Laniatores) and parametric shape analysis reveal the
1066 disutility of subfamilial nomenclature in armored harvestman systematics. *Mol*
1067 *Phylogenet Evol.* 106:164–173.
- 1068 Shi J.J., Rabosky D.L. 2015. Speciation dynamics during the global radiation of extant bats.
1069 *Evolution.* 69:1528–1545.
- 1070 Shingleton A.W., Frankino W.A., Flatt T., Nijhout H.F., Emlen D.J. 2007. Size and shape:
1071 the developmental regulation of static allometry in insects. *BioEssays.* 29:536–548.

Hamilton, Winiger, Rubin, Breinholt, Rougerie, Kitching, Barber, Kawahara

- 1072 Slowinski J., Page R. 1999. How should species phylogenies be inferred from sequence data?
1073 Syst Biol. 48:814–825.
- 1074 Strimmer K., vonHaeseler A. 1997. Likelihood-mapping: A simple method to visualize
1075 phylogenetic content of a sequence alignment. PNAS. 94:6815–6819.
- 1076 Stylman M., Penz C.M., DeVries P. 2019. Large Hind Wings Enhance Gliding Performance
1077 in Ground Effect in a Neotropical Butterfly (Lepidoptera: Nymphalidae). Ann. Entomol.
1078 Soc. Am. 113:15–22.
- 1079 Tammaru T., Haukioja E. 1996. Capital breeders and income breeders among Lepidoptera:
1080 consequences to population dynamics. Oikos. 77:561–564.
- 1081 Teeling E.C., Springer M.S., Madsen O., Bates P., O'Brien S.J., Murphy W.J. 2005. A
1082 molecular phylogeny for bats illuminates biogeography and the fossil record. Science.
1083 307:580–584.
- 1084 Than C., Ruths D., Nakhleh L. 2008. PhyloNet: a software package for analyzing and
1085 reconstructing reticulate evolutionary relationships. BMC Bioinformatics. 9:1–16.
- 1086 Thorley J.L., Wilkinson M. 1999. Testing the phylogenetic stability of early tetrapods. J.
1087 Theor. Biol. 200:343–344.
- 1088 Wahlberg N., Wheat C.W., Peña C. 2013. Timing and patterns in the taxonomic
1089 diversification of Lepidoptera (butterflies and moths). PLoS ONE. 8:e80875.
- 1090 Wen D., Yu Y., Zhu J., Nakhleh L. 2018. Inferring Phylogenetic Networks Using PhyloNet.
1091 Syst Biol. 67:735–740.

EVOLUTIONARY TRADEOFFS IN SILKMOTHS

- 1092 Wiens J.J., Lapoint R.T., Whiteman N.K. 2015. Herbivory increases diversification across
- 1093 insect clades. *Nat Comms.* 6:8370.
- 1094 Xia Q., Zhou Z., Lu C., Cheng D., Dai F., Bin Li, Zhao P., Zha X., Cheng T., Chai C., Pan
- 1095 G., Xu J., Liu C., Lin Y., Qian J., Hou Y., Wu Z., Li G., Pan M., Li C., Shen Y., Lan X.,
- 1096 Yuan L., Li T., Xu H., Yang G., Wan Y., Zhu Y., Yu M., Shen W., Wu D., Xiang Z., Yu
- 1097 J., Wang J., Li R., Shi J., Li H., Li G., Su J., Wang X., Li G., Zhang Z., Wu Q., Li J.,
- 1098 Zhang Q., Wei N., Xu J., Sun H., Le Dong, Liu D., Zhao S., Zhao X., Meng Q., Lan F.,
- 1099 Huang X., Li Y., Fang L., Li C., Li D., Sun Y., Zhang Z., Yang Z., Huang Y., Xi Y., Qi
- 1100 Q., He D., Huang H., Zhang X., Wang Z., Li W., Cao Y., Yu Y., Yu H., Li J., Ye J.,
- 1101 Chen H., Zhou Y., Bin Liu, Wang J., Ye J., Ji H., Li S., Ni P., Zhang J., Zhang Y., Zheng
- 1102 H., Mao B., Wang W., Ye C., Li S., Wang J., Wong G.K.-S., Yang H. 2004. A draft
- 1103 sequence for the genome of the domesticated silkworm (*Bombyx mori*). *Science.*
- 1104 306:1937–1940.
- 1105 Zelditch M.L., Swiderski D.L., Sheets H.D. 2012. Geometric morphometrics for biologists: a
- 1106 primer. Academic Press.
- 1107 Zhan Q.B., Wang X.L. 2012. Elliptic Fourier analysis of the wing outline shape of five
- 1108 species of antlion (Neuroptera: Myrmeleontidae: Myrmeleontini). *Zool. Stud.* 51:399–
- 1109 405.
- 1110 Zhong M., Hill G.M., Gomez J.P., Plotkin D. 2016. Quantifying wing shape and size of
- 1111 saturniid moths with geometric morphometrics. *J. Lepid. Soc.* 70:1–9.
- 1112 Zhou X., Lutteropp S., Czech L., Stamatakis A., Looz M.V., Rokas A. 2020. Quartet-Based
- 1113 Computations of Internode Certainty Provide Robust Measures of Phylogenetic
- 1114 Incongruence. *Syst Biol.* 69:308–324.

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FIGURES

Figure 1 – The preferred Arsenurinae phylogeny, constrained inference (#1), based on 782 AHE loci. Grey shading on branches indicate the problematic region along the backbone of the phylogeny. Nodes with support values with SH-aLRT & bootstrap (BS) values = 100% are indicated with a black circle. Support values below these are stated. Nodes with concordance factors ≥ 50 are indicated with a white box. Hindwing tail designations: extra-long tail (red), medium tail (green), small tail (blue), long lobe (orange), small lobe (yellow), no tail (white). Generalized wing shapes for genera or species groups within a genus are placed at the tips, in black. Photographs represent a generalized species from the genera sampled in the phylogeny.

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Figure 2 – Geometric morphometric analysis of hindwing (HW) shape reveals changes in morphospace across the Saturniidae subfamilies. Principal components (PC1~PC2) are plotted to visualize hindwing morphospace by A) subfamily, showing Saturniinae and Arsenurinae have convergently evolved into the same morphospace; and B) Hindwing (HW) shape morphospace plotting the hindwing tail categories “none”, “lobe”, “small”, “medium”, “long”, and “extra long”. Dots represent individual specimens in the analysis. Hypothetical shape approximations are plotted in the background to aid in visualizing shape change.

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1137

1138 **Figure 3** – Geometric morphometric analysis of hindwing (HW) shape reveals changes in
 1139 morphospace across the Arsenurinae genera. Principal components (PC1~PC2) are plotted to
 1140 visualize hindwing morphospace by A) Genus; and B) Hindwing (HW) shape morphospace
 1141 plotting the hindwing tail categories “none”, “lobe”, “small”, “medium”, “long”, and “extra
 1142 long”. Dots represent individual specimens in the analysis. Hypothetical shape
 1143 approximations are plotted in the background to aid in visualizing shape change.

1144

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1145

1146 Figure 4 – Geometric morphometric analysis of forewing (FW) shape reveals changes in
 1147 morphospace across the Saturniidae subfamilies. Principal components (PC1~PC2) are
 1148 plotted to visualize forewing morphospace by A) Subfamily, showing Saturniinae and
 1149 Arsenurinae have convergently evolved into the same morphospace; and B) Forewing (FW)
 1150 shape morphospace plotting the hindwing tail categories “none”, “lobe”, “small”, “medium”,
 1151 “long”, and “extra long” overlaid on FW shape. Dots represent individual specimens in the
 1152 analysis. Hypothetical shape approximations are plotted in the background to aid in
 1153 visualizing shape change.

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1154

1155

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1156 Figure 5 – Geometric morphometric analysis of forewing (FW) shape reveals changes in
 1157 morphospace across the Arsenurinae genera. Principal components (PC1~PC2) are plotted to
 1158 visualize forewing morphospace by A) genus; and B) Forewing (FW) shape morphospace
 1159 plotting the hindwing tail categories “none”, “lobe”, “small”, “medium”, “long”, and “extra
 1160 long” overlaid on FW shape. Dots represent individual specimens in the analysis.
 1161 Hypothetical shape approximations are plotted in the background to aid in visualizing shape
 1162 change.

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Figure 6 – Trait evolution across the Arsenurinae phylogeny to visually inspect whether there are evolutionary relationships between body size (Left; measured as FW length - a proxy for body size), HW length (middle Left), HW shape complexity (middle Right; as principal components); and FW shape complexity (Right; as principal components).

TABLE

Table 1 – Trait tests between body size (measured as FW length - a proxy for body size), and FW shape and HW length (as principal components). Information included are the specific tests and their significance.

Questions	PGLS regression	significance?	p-value
Is HW length a predictor of FW_L [body size]?	FW_L ~ HW_L	YES	0.001115
Is FW_L [body size] a predictor of HW length?	HW_L ~ FW_L	NO	0.1131
Is HW length a predictor of FW shape complexity?	FW_PC1 ~ HW_L	YES	2.20E-16
Is FW shape complexity a predictor of HW length?	HW_L ~ FW_PC1	NO	0.8423

APPENDICES

SUPPLEMENTAL FIGURES

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1179 Supplemental Figure 1 – The past hypothesized Arsenurinae relationships of Michener
 1180 (1952) (left), Peigler (1993) (middle), and De Camargo et al. (2009) (right), reproduced for
 1181 this publication.
 1182
 1183 Supplemental Figure 2 – Illustrations for how forewing (FW) length and hindwing (HW)
 1184 length measurements were made.
 1185
 1186 Supplemental Figure 3 – Quartet likelihood mapping to inspect the phylogenetic content of
 1187 the dataset. By looking at the distribution of quartets, we see that there is significant signal to
 1188 expect a well-resolved tree, with signal split evenly among the quartets.
 1189
 1190 Supplemental Figure 4 – Quartet likelihood mapping of possible relationships at the
 1191 subfamily level. We tested whether Arsenurinae was more closely related to the Agliinae,
 1192 Salassinae, or Saturniinae. We found that 100% of the quartets possess an (Arsenurinae +
 1193 Agliinae) ~ (Salassinae + Saturniinae) relationship.
 1194
 1195 Supplemental Figure 5 – Quartet likelihood mapping of possible relationships at the genus
 1196 level. We tested relationships by grouping lineages, according to the phylogeny, and
 1197 evaluating (from a quartet standpoint) whether lineages were found more closely related to
 1198 another. We find that a relationship of (a *Loxolomia*/*Grammopelta* clade + a
 1199 *Titaea*/*Dysdaemonia* clade) ~ (a *Copiopteryx*/*Rhescyntis* + a *Paradaemonia*/*Caio* clade) is
 1200 found in 69.6% of investigated quartets (with the other subfamily representatives, *Almeidaia*,
 1201 and *Arsenura* ignored).
 1202

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Supplemental Figure 6 – Quartet likelihood mapping of possible relationships at the genus level. We tested relationships by grouping lineages, according to the phylogeny, and evaluating (from a quartet standpoint) whether lineages were found more closely related to another. We find that a relationship of (*Almeidaia* + a *Loxolomia*/*Caio*/*Grammopelta* clade) ~ (a *Copiopteryx*/*Rhescyntis* + a *Paradaemonia*/*Titaea*/*Dysdaemonia* clade) is found in 51.6% of quartets, whereas (*Almeidaia* + a *Copiopteryx*/*Rhescyntis* clade) ~ (a *Loxolomia*/*Caio*/*Grammopelta* clade + a *Paradaemonia*/*Titaea*/*Dysdaemonia* clade) represents 37.9% of quartets (the other subfamily representatives and *Arsenura* were ignored). This relationships was the only other of those tested that had an equal score and tree length to Supplemental Figure 4.

Supplemental Figure 7 – To investigate potential gene tree/species tree incongruence, we computed a phylogenetic network. The inferred SplitsTree4 network indicates low amounts of reticulation and a large star relationship, with no intergeneric structure, except that *Loxolomia* and *Grammopelta* are closely related

Supplemental Figure 8 – Plot of changes in hindwing (HW) PC morphospace.

Supplemental Figure 9 – Plot of changes in forewing (FW) PC morphospace.

Directory “Arsenurinae_genera_morphospace_plots” – HW and FW plots for all Arsenurinae genera and species investigated herein.

SUPPLEMENTAL TABLES

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1227 Supplemental Table 1 – Tab-delimited file of information for specimens used in phylogenetic
1228 inference. Included are the specimen codes, taxonomy, their condition (i.e., molecular,
1229 papered, or pinned specimen), and year they were collected.

1230

1231 Supplemental Table 2 – Tab-delimited file of hindwing (HW) images used to quantify the
1232 shape complexity (as principal components) of 968 male specimens comprising 174 species
1233 across the eight Saturniidae subfamilies.

1234

1235 Supplemental Table 3 – Tab-delimited file of forewing (FW) images used to quantify the
1236 shape complexity (as principal components) of 968 male specimens comprising 174 species
1237 across the eight Saturniidae subfamilies.

1238

1239 Supplemental Table 4 – Tab-delimited file of ingroup (i.e., the Arsenurinae) hindwing (HW)
1240 images used to quantify the shape complexity (as principal components) of 545 male
1241 specimens comprising 50 of the 89 currently recognized species.

1242

1243 Supplemental Table 5 – Tab-delimited file of ingroup (i.e., the Arsenurinae) forewing (FW)
1244 images used to quantify the shape complexity (as principal components) of 545 male
1245 specimens comprising 50 of the 89 currently recognized species.

1246

1247 **SUPPLEMENTAL TEXT**

1248 Supplemental Text 1 – Tab-delimited file of locus recovery for each AHE locus used in
1249 phylogenetic inference. A cutoff of $\geq 50\%$ was applied to the sampled taxa (i.e., for a locus to
1250 be included in the analysis, the locus had to be recovered in at least 50% of the sampled
1251 taxa).

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1252

1253 Supplemental Text 2 – Tab-delimited file of taxon recovery for each AHE taxon used in the
1254 phylogenetic inference.

1255

1256 Supplemental Text 3 –The file is a text file with a newick tree embedded within. QP-IC
1257 scores (quadripartition internode certainty) can be found at nodes.

1258

1259 SUPPLEMENTAL TREES

1260 Arsenurinae.AA.noGrammopelta.pdf & .tre – Newick tree file and PDF from the amino acid
1261 (AA) phylogeny without *Grammopelta*.

1262

1263 Arsenurinae.AA.pdf & .tre – Newick tree file and PDF from the amino acid (AA) phylogeny.

1264

1265 Arsenurinae.PROBE.pdf & .tre – Newick tree file and PDF from the nucleotide ‘Pr’
1266 phylogeny.

1267

1268 Arsenurinae.concat.constraint1.pdf & .tre – Newick tree file and PDF from the constrained
1269 inference (#1) nucleotide dataset ‘Pr+Fl’ constrained with ‘Constraint_tree_01’.

1270

1271 Arsenurinae.concat.constraint2.pdf & .tre – Newick tree file and PDF from the constrained
1272 inference (#2) nucleotide dataset ‘Pr+Fl’ constrained with ‘Constraint_tree_02’.

1273

1274 Arsenurinae.concat.noGrammopelta.pdf & .tre – Newick tree file and PDF from the
1275 nucleotide ‘Pr+Fl’ phylogeny without *Grammopelta*.

1276

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- 1277 Arsenurinae.concat.pdf & .tre – Newick tree file and PDF from the nucleotide ‘Pr+Fl’
 1278 phylogeny.
 1279
 1280 Arsenurinae.flanking.pdf & .tre – Newick tree file and PDF from the nucleotide ‘Fl’
 1281 phylogeny.
 1282
 1283 Constraint_tree_01.pdf & .tre – Newick tree file and PDF of the ‘constrained inference (#1)’
 1284 phylogeny.
 1285
 1286 Constraint_tree_02.pdf & .tre – Newick tree file and PDF of the ‘constrained inference (#2)’
 1287 phylogeny.
 1288
 1289 concordance.factor.ASTRAL.tree.pdf & .tre – Newick tree file and PDF from the ASTRAL
 1290 inference, with concordance factors placed at nodes.
 1291
 1292 concordance.factor.concat.tree.pdf & .tre – Newick tree file and PDF from the constrained
 1293 inference (#1) nucleotide dataset, with concordance factors placed at nodes.
 1294