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A phylogeny of robber flies (Diptera: Asilidae) at the subfamilial level: molecular evidence

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Abstract

We present the first formal analysis of phylogenetic relationships among the Asilidae, based on four genes: 16S rDNA, 18S rDNA, 28S rDNA, and cytochrome oxidase II. Twenty-six ingroup taxa representing 11 of the 12 described subfamilies were selected to produce a phylogenetic estimate of asilid subfamilial relationships via optimization alignment, parsimony, and maximum likelihood techniques. Phylogenetic analyses support the monophyly of Asilidae with Leptogastrinae as the most basal robber fly lineage. Apocleinae + (Asilinae + Ommatiinae) is supported as monophyletic. The laphriinae-group (Laphriinae + Laphystiinae) and the dasypogoninae-group (Dasypogoninae + Stenopogoninae + Stichopogoninae + Trigonomiminae) are paraphyletic. These results suggest that current subfamilial classification only partially reflects robber fly phylogeny, indicating the need for further phylogenetic investigation of this group.

1. Introduction

Robber flies (Diptera: Asilidae) comprise one of the largest groups of extant flies (Hull, 1962). Asilids constitute more than 500 genera (Woodley, 1989) and more than 5500 species (Lehr, 1988) with a worldwide distribution except Antarctica. Species range in size from less than 1 cm to nearly 8 cm in length, and their prey consist of both small and large insects caught largely in flight. Asilid color patterns are simple: usually black, gray, or bronze, although some more colorful species appear to mimic bees and wasps.

The monophyly of Asilidae, including the problematic group Leptogastrinae (thread-waisted robber flies), is well-supported by synapomorphies including the fusion of the labella and prementum to form a heavily sclerotized, tube-like proboscis which contains the needle-like hypopharynx; the presence of a row or group of stout bristles along the lower edge of the face (mystax); and adult predatory behavior (Woodley, 1989; Yeates, 1994; Yeates and Wiegmann, 1999). Phylogenetic relationships of Asilidae with other families in Asiloidea have been recently investigated by molecular and morphological phylogenetic analyses (Wiegmann et al., 1993; Yeates, 2002). These analyses focused primarily on the monophyly of Asiloidea, a group including the families Asilidae, Apioceridae, Therevidae, Scenopinidae, Mydidae, Bombyliidae, and Apsilocephalidae (Yeates, 2002). Yeates (2002) used 101 discrete morphological characters to infer phylogenetic relationships among these families as well as other lower Brachycera (Fig. 1). His analysis supported a topology that placed Asilidae as sister to the clade ((Apioceridae + Mydidae) + (Scenopinidae + (Therevidae + Apsilocephalidae))).

Despite the considerable popularity of robber flies, and a rich history of extensive research on asilid morphology, taxonomy, and behavior (Bigot, 1857; Bromley, 1932; Enderlein, 1914; Hull, 1962; Karl, 1959; Loew, 1847; Leach, 1819; Macquart, 1838; Martin, 1968; Oldroyd, 1969; Osten Sacken and Baron von, 1884), a comprehensive phylogenetic hypothesis for the subfamilies has yet to emerge. Recent attempts to produce a phylogenetic hypothesis at this level have been based on intuition rather than a quantitative phylogenetic analysis (Lavigne et al., 1978; Papavero, 1973; Wood, 1981; Woodley, 1989) and have not received universal acceptance. Various authors have identified characters that

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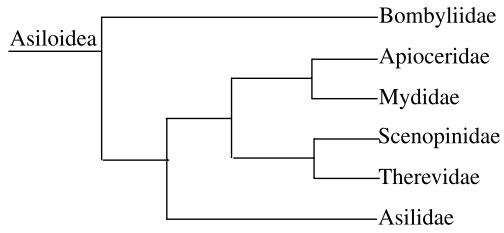

Fig. 1. Phylogeny of families comprising the Asiloidea based on Yeates (2002) with Apsilocephalidae collapsed within Therevidae.

may be useful in a phylogenetic analysis. These include features associated with palpal segments and female genitalia (Williston, 1908), marginal cells of the wings (Bromley, 1932; Engel, 1928), presence or absence of an apical spur on the foretibia (Loew, 1847; Hull, 1962), hair on the katatergite, and antennal segmentation and structure (Papavero, 1973). However, since none of these characters have ever been formally coded nor tested cladistically, their utility in deciphering asilid phylogeny has yet to be demonstrated. Moreover, the characters used to define subfamilies have never been assessed across the entire diversity of robber flies, and many groups do not fit well within these subfamilial groupings (Carrera, 1949; Hardy, 1948; Hermann, 1926; Williston, 1908).

Papavero (1973) presented an intuitive phylogeny for asilid subfamilies (Fig. 2), in which he organized the subfamilies into four major groups. The leptogastrinae-group, occupied solely by the subfamily Leptogastrinae, is characterized as having a long slender abdomen with the alula and pulvilli lacking. The asilinae-group consisting of the three *Asilus*-like subfamilies Asilinae, Ommatiinae, and Apocleinae, are characterized as having closed marginal wing cells and slender antennae. The laphriinae-group is comprised of the *Laphria*-like subfamilies Laphriinae and Laphystiinae, and is characterized as having closed marginal wing cells and clubbed antennae. The remainder of Asilidae is composed of morphologically diverse taxa placed in the dasypogoninae-group, including the subfamilies Dasypogoninae, Trigonomiminae, Stichopogoninae, and Stenopogoninae. This group is characterized by the presence of open marginal wing cells and an apical spur on the foretibia. The characters supporting Papavero's hypothesis are controversial, and there is a diversity of opinions among dipterists over the validity of these groups (Hardy, 1948; Hull, 1962; Martin, 1968; Oldroyd, 1969; Wood, 1981).

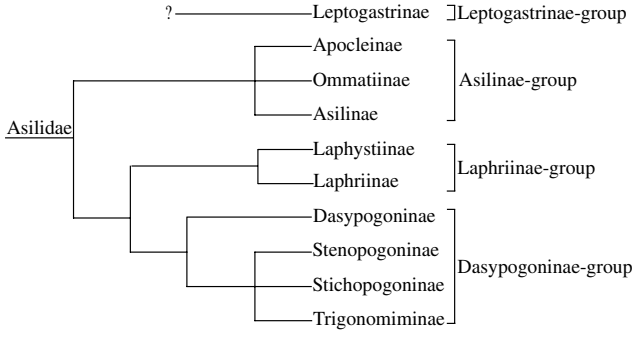

Fig. 2. Hypothetical phylogeny of the subfamilies of Asilidae as proposed by Papavero (1973). Stichopogoninae was raised to subfamily and the position of Leptogastrinae is unresolved.

One major concern is the phylogenetic placement of Leptogastrinae. Martin (1968) argued that they should be given full family status within Asiloidea based on 46 distinct morphological and behavioral features, and placed them as the most basal asilid lineage, sister group to the remaining Asilidae. Oldroyd (1969), however, rejected 43 of Martin's 46 "distinct traits" by showing that similar traits are scattered among members of the remaining subfamilies of Asilidae and are not unique to Leptogastrinae. Further, some morphologists have suggested that Leptogastrinae is nested within Asilidae as a highly derived group that has undergone a thinning of the abdomen, legs, and wings presumably to provide crypsis in grasses ("agrionism;" Janssens, 1954).

The placement of the subfamily Laphriinae, a group including a great diversity of bee mimics, has also been problematic. Hardy (1948) combined Dasypogoninae and Laphriinae as one subfamily, based on characters associated with antennae, palpi, and male and female external genitalia. Martin (1968) suggested that Dasypogoninae is sister group to Laphriinae based on the presence of two-segmented palpi and secondarily coalesced epandria. Wood (1981) hypothesized that Laphriinae + Laphystiinae may actually be nested within Dasypogoninae (and therefore should be demoted to tribal status), presumably based on Martin's (1968) speculation that Laphystiinae may be the "link" between Dasypogoninae and Laphriinae. Papavero (1973), however, placed Laphriinae as sister group to Laphystiinae, and this clade as sister to the dasypogoninae-group (Fig. 2) based on mesopleural bristle characters. Clearly a robust phylogeny of asilid subfamilies is needed to address these questions of relationship. We present the first comprehensive phylogenetic analysis of asilid phylogeny, with an emphasis on testing the validity of these subfamilial relationships.

2. Materials and methods

2.1. Taxon sampling

The number of subfamilies recognized within Asilidae varies with the classification scheme, but to date, 11 subfamilies have been proposed: Apocleinae, Asilinae, Dasypogoninae, Dioctriinae, Laphriinae, Laphystiinae, Leptogastrinae, Ommatiinae, Stenopogoninae,

Table 1
List of all taxa used in this analysis with GenBank Accession Nos.

Family	Subfamily	Tribe	Species	16S	18S	28S	COII
Outgroups							
Apioceridae			*Apiocera* sp.	AY325006	AY325037	AY325068	AY325099
Bombyliidae			*H. jaennickeana*	AY325005	AY325036	AY325067	AY325098
Mydidae			*M. clavatus*	AY324983	AY325014	AY325045	AY325076
Scenopinidae			*S. fenestralis*	AY325007	AY325038	AY325069	AY325100
Therevidae			*O. costalis*	AY325004	AY325035	AY325066	AY325097
Ingroups							
Asilidae	Apocleinae	Apocleini	*Efferia nemoralis*	AY325008	AY325039	AY325070	AY325101
Asilidae	Apocleinae	Apocleini	*Proctacanthus nearno*	AY325009	AY325040	AY325071	AY325102
Asilidae	Apocleinae	Apocleini	*Promachus bastardii*	AY324984	AY325015	AY325046	AY325077
Asilidae	Apocleinae	Apocleini	*Promachus* sp.	AY324985	AY325016	AY325047	AY325078
Asilidae	Asilinae	Asilini	*Machimus* sp.	AY325010	AY325041	AY325072	AY325103
Asilidae	Asilinae	Asilini	*Philonicus arizonensis*	AY324991	AY325022	AY325053	AY325084
Asilidae	Damalinae	Damalini	*Holcocephala abdominalis*	AY324999	AY325030	AY325061	AY325092
Asilidae	Dasypogoninae	Dasypogonini	*Saropogon fletcheri*	AY324995	AY325026	AY325057	AY325088
Asilidae	Dasypogoninae	Dasypogonini	*Senobasis corsair*	AY325002	AY325033	AY325064	AY325095
Asilidae	Dasypogoninae	Lastaurini	*Diogmites grossus*	AY324988	AY325019	AY325050	AY325081
Asilidae	Laphriinae	Andrenosomsini	*Andrenosoma fulvicauda*	AY324997	AY325028	AY325059	AY325090
Asilidae	Laphriinae	Atomosini	*Adelodus* sp.	AY324998	AY325029	AY325060	AY325091
Asilidae	Laphriinae	Dasylechiini	*Smeryngolaphria* sp.	AY324993	AY325024	AY325055	AY325086
Asilidae	Laphriinae	Laphriini	*Laphria* sp.	AY325011	AY325042	AY325073	AY325104
Asilidae	Laphriinae	Laphriini	*Maira* sp.	AY324986	AY325017	AY325048	AY325079
Asilidae	Laphystiinae	Laphystiini	*Laphystia* sp.	AY324996	AY325027	AY325058	AY325089
Asilidae	Leptogastrinae	Leptogastrini	*Leptogaster* sp.	AY325003	AY325034	AY325065	AY325096
Asilidae	Leptogastrinae	Leptogastrini	*Psilonyx annulatus*	AY325012	AY325043	AY325074	AY325105
Asilidae	Ommatiinae	Ommatius	*Ommatius* sp.	AY324987	AY325018	AY325049	AY325080
Asilidae	Stichopogoninae	Stichopogonini	*Stichopogon trifasciatus*	AY324989	AY325020	AY325051	AY325082
Asilidae	Stenopogoninae	Cyrtopogonini	*Holopogon currani*	AY324992	AY325023	AY325054	AY325085
Asilidae	Stenopogoninae	Stenopogonini	*Stenopogon martini*	AY325013	AY325044	AY325075	AY325106
Asilidae	Stenopogoninae	Stenopogonini	*Ospriocerus latipennis*	AY324990	AY325021	AY325052	AY325083
Asilidae	Stenopogoninae	Enigmomorphini	*Creolestes nigribarbis*	AY325000	AY325031	AY325062	AY325093
Asilidae	Stenopogoninae	Tillobromini	*Hypenetes critesi*	AY325001	AY325032	AY325063	AY325094
Asilidae	Stenopogoninae	Phellini	*Obelopherus landbecki*	AY324994	AY325025	AY325056	AY325087

Stichopogoninae, and Trigonomiminae (Damalinae) (Artigas and Papavero, 1988; Hull, 1962; Lehr, 2001; Papavero, 1973; Woodley, 1989). We obtained multiple exemplars representing 10 of the 11 subfamilies (all except Dioctriinae) and 18 tribes for a total of 26 ingroup taxa (Table 1). For this analysis the taxon representing Megapodinae (*Senobasis*) is collapsed within Dasypogoninae, as Megapodinae is not a widely accepted subfamily. While we recognize that our sampling does not broadly represent all asilid diversity, this choice of ingroup taxa adequately reflects subfamilial diversity and provides a good first estimate for these relationships. Outgroups were selected from five closely related families (Yeates, 2002) within the superfamily Asiloidea (Fig. 1): Mydidae (*Mydas clavatus*), Therevidae (*Ozodiceromyia costalis*), Bombyliidae (*Hemipenthes jaennickeana*), Apioceridae (*Apiocera* sp.), and Scenopinidae (*Scenopinus fenestralis*).

2.2. Laboratory methods

Genomic DNA was extracted from specimens preserved in 100% ethanol using the Qiagen Dneasy protocol for animal tissue (Valencia, CA). Muscle tissue was dissected from the leg and/or thorax region. Genomic DNA vouchers and specimen vouchers are deposited in the Insect Genomics Collection (IGC), M.L. Bean Museum, Brigham Young University.

Our molecular data set is comprised of four genes: 16S ribosomal (16S rDNA, 0.6 kb) and the protein coding gene cytochrome oxidase subunit II (COII, 0.6 kb) from the mitochondrion, and 18S ribosomal (18S rDNA, 2.0 kb) and 28S ribosomal (28S rDNA, 1.3 kb) from the nucleus. Primers for 16S rDNA are: 16Sa: 5′-CGC CTG TTT ATC AAA AAC AT-3′; 16Sb: 5′-CTC CGG TTT GAA CTC AGA TCA-3′. Primers for COII are: COII-2a: 5′-ATA GAK CWT CYC CHT TAA TAG AAC A-3′; COII-9b: 5′-GTA CTT GCT TTC AGT CAT CTW ATG-3′. Primers for 18S rDNA and 28S rDNA are given in Whiting (2001). The 18S rDNA, 28S rDNA, and COII were each amplified using a three-step PCR at 40 cycles with an annealing temperature of 50 °C for 18S rDNA and 28S rDNA and 49 °C for COII. The 16S rDNA gene was amplified using a touchdown method with the annealing temperature starting at 62 °C and decreasing to 42 °C over 40 cycles of a standard

three-step PCR. All PCR products were visualized via agarose gel electrophoresis to assure proper amplification and detect possible contamination using negative controls. Products were purified using Montage PCR Cleanup Kit (Millipore) and cycle-sequenced using BigDye Terminator chemistry (ABI). Sequences were generated using an ABI 3100 capillary sequencer at the DNA Sequencing Center, Brigham Young University. Complementary strands were sequenced with sufficient fragment overlap to reduce sequencing errors.

2.3. Data analysis

The alignment for COII was generated manually in Sequencher 4.1 (GeneCodes, 2002) and was based on conservation of codon reading frame, resulting in an alignment devoid of gaps. Sequences for the ribosomal genes were initially aligned manually in Sequencher 4.1 to identify conserved and variable regions. These regions were then subdivided into variable and conserved partitions in order to assist the search strategy in finding more optimal solutions during optimization alignment (Giribet, 2001). The partitions for each gene are as follow: 16S rDNA, 11 conserved regions and 6 variable regions; 18S rDNA, 14 conserved regions and 6 variable regions; 28S rDNA, 13 conserved regions and 5 variable regions. Two non-informative autapomorphic expansion regions in the D7A (42–333 bp) and D7B (4–49 bp) expansion regions of 28S rDNA were removed from the analysis for all taxa. All partitions were analyzed via Optimization Alignment (OA) in POY (Gladstein and Wheeler, 1999; Wheeler, 1996) to find a set of analytical parameters to be applied uniformly throughout the alignment and tree reconstruction process. OA was implemented using the following parameter set "-fitchtrees -noleading -norandomizeoutgroup -impliedalignment -sprmaxtrees 1 -tbrmaxtrees 1 -maxtrees 2 -holdmaxtrees 2 -slop 2 -checkslop 2 -buildspr -buildmaxtrees 1 -random 800 -treefuse -fuselimit 2 -fusemingroup 2 -fusemaxtrees 2 -numdriftchanges 5 -driftspr -numdriftspr 2 -drifttbr -numdrifttbr 2 -slop 2 -checkslop 2 -molecularmatrix 111.txt -seed -1." The COII data set was treated as pre-aligned data. Multiple parameter combinations for OA were employed using this search strategy in order to explore the sensitivity of the resulting topologies to variations in the cost ratios for gap insertion, transversion, and transition. The gap to nucleotide change ratio varied from 1 to 4 while the transversion to transition ratio varied from 1 to infinity (Table 2). The incongruence length difference (ILD) metric was used to select a parameter set that minimized incongruence among the data sets and was taken as the best-justified parameter values for phylogenetic inference under OA (Wheeler, 1995). Bootstrap values, Bremer support (BS), and partition Bremer support (PBS) values were calculated from the implied alignment of POY. Bremer support and partition Bremer support values were computed via a batch file crafted using TreeRot.v2a (Sorenson, 1999) implemented in PAUP* 4.0b.7 (Swofford, 2001) with 1000 random additions. Bootstraps were calculated via PAUP* 4.0b.7 (Swofford, 2001) with 1000 replicates of 50 random additions each. Likelihood analysis was

Table 2
Optimization alignment costs for partitioned and combined data across a range of cost parameter values, with incongruence length difference (ILD) scores

Gap:Tv:Ts	16S	Length of individual genes		con	Total length	ILD
		18S	28S			
1:1:0	902	555	882	1014	3524	0.0485
2:1:0	1046	749	1279	1014	4448	0.0809
3:1:0	1154	913	1646	1014	5172	0.0860
4:1:0	1239	1071	1942	1014	5921	0.1106
1:1:1	**1454**	**1010**	**1307**	**2316**	**6274**	**0.0298**
2:1:1	1622	1234	1719	2316	7236	0.0477
3:1:1	1718	1421	2083	2316	8003	0.0581
4:1:1	1811	1589	2421	2316	8862	0.0818
2:2:1	2381	1589	2222	3386	9934	0.0358
4:2:1	2704	2016	3034	3386	11,867	0.0613
6:2:1	2914	2369	3752	3386	13,386	0.0721
8:2:1	3075	2717	4453	3386	14,876	0.0837
3:3:1	3309	2147	3108	4405	13,513	0.0403
6:3:1	3771	2772	4356	4405	16,374	0.0653
9:3:1	4052	3299	5403	4405	18,646	0.0797
12:3:1	4347	3806	6456	4405	20,920	0.0911
4:4:1	4224	2696	4006	5430	17,097	0.0433
8:4:1	4826	3526	5638	5430	20,838	0.0680
12:4:1	5252	4230	7023	5430	23,916	0.0828
16:4:1	5627	4906	8445	5430	26,888	0.0922

The parameter combination treating the costs of gaps, transversions (Tv), and transitions (Ts) = 1 minimized the ILD value (in bold).

performed by using Modeltest (Posada and Crandall, 1998) to select a "justified" model of evolution. Likelihood analysis using the preferred model was executed using 15 random addition replicates with TBR branch swapping. In all analyses, trees were rooted to *Hemipenthes*, the taxon representing Bombyliidae, since this is considered to be the basal asiloid family in the Yeates (2002) analysis. The alignments are available at http://inbio.byu.edu/faculty/mfw2/whitinglab/.

3. Results

3.1. Phylogenetic analyses

Costs for OA analyses under the selected parameter values are summarized in Table 2. Weighting gaps, transitions, and transversions identically resulted in the minimal ILD value (0.0298) for all parameter combinations explored. This was the parameter combination used in all subsequent analyses. Analysis of the data using the parameters set to identity produced a single, fully resolved topology with a cost of 5834 steps. A parameter landscape summarizing the results from the sensitivity analyses for each node is shown in Fig. 3. The landscape depicts the parameters under which a particular relationship is monophyletic or not. For instance, Leptogastrinae is always monophyletic for all OA cost parameter values investigated, whereas the monophyly of all asilids, excluding leptogastrines, is supported under a more narrow range of parameter values (Fig. 3). Overall, these results suggest that the basal nodes within the phylogeny are more sensitive to fluctuations in parameter values than are the more apical portions of the topology.

The Bremer support and bootstrap values calculated from the implied alignment indicate that this topology is relatively well supported (Fig. 3). Of the 28 nodes, 24

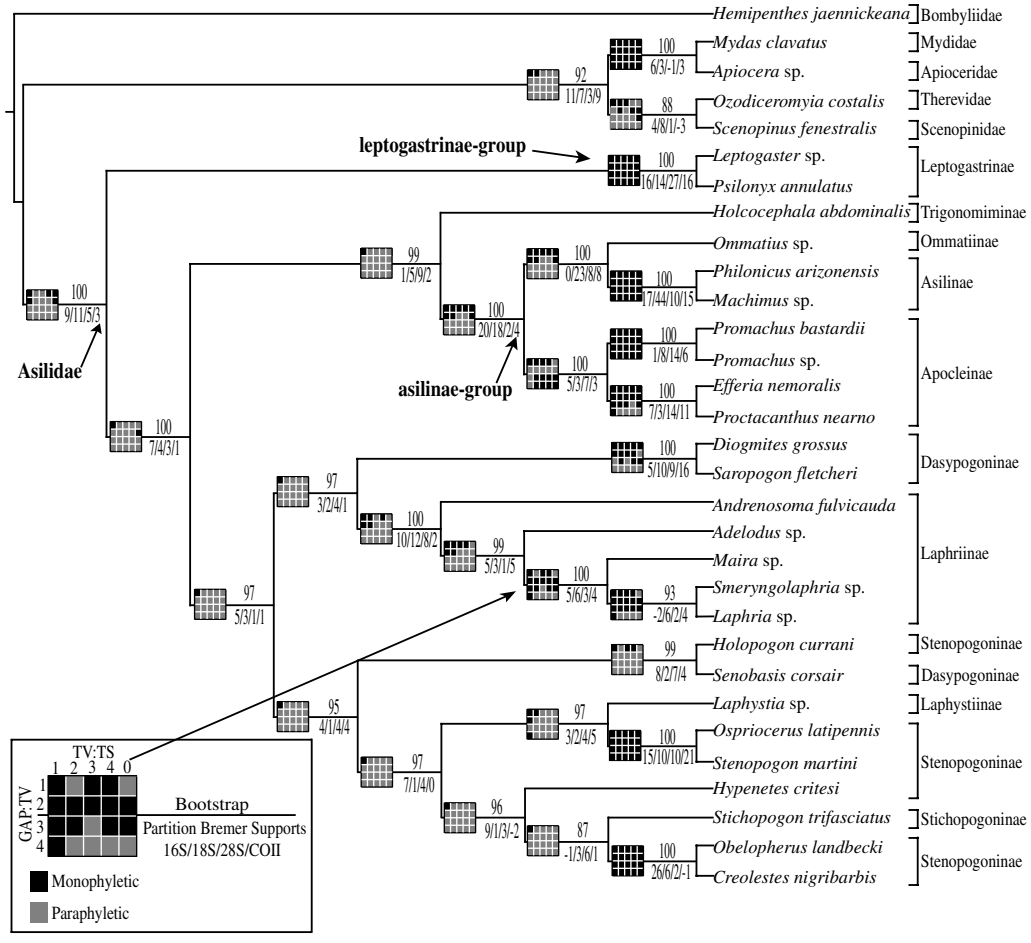

Fig. 3. Single optimization alignment topology for Asilidae and outgroups based on the combined analysis of 16S rDNA, 18S rDNA, 28S rDNA, and COII genes, summarizing results from the sensitivity analysis. This tree was reconstructed with gap, transversion (Tv), and transition (Ts) costs set to 1, and had a total cost of 5834. Non-parametric bootstrap values are given above the nodes, and partition Bremer support values are given below the nodes. The sensitivity landscape is depicted on each node with a dark box representing the parameter combination under which the node is supported as monophyletic and a gray box depicting the parameter combination under which the node is paraphyletic. Of the four higher-level groups defined by Papavero (1973), only the leptogastrinae-group and the asilinae-group are supported as monophyletic.

nodes had bootstrap values greater than 95 and all nodes had total Bremer support values of 5 or greater. Summing partition Bremer support values across the topology suggests that each gene provides roughly equivalent signal in constructing this topology (16S rDNA, 27.9%; 18S rDNA, 29.6%; 28S rDNA, 23%; and COII, 19.3%). Despite these high values, the sensitivity analysis suggests that the results may not be as well supported as the bootstrap and Bremer values indicate.

When the combined data set was executed through Modeltest, the General Time Reversible + Invariable site + Gamma distribution (GTR + I + G) model was selected as most appropriate for these data with the following parameter settings: Base frequencies: freqA = 0.3277; freqC = 0.1497; freqG = 0.1877; and freqT = 0.3349; Substitution model: Rate matrix *R*(a) [A–C] = 1.0000; *R*(b) [A–G] = 5.0149; *R*(c) [A–T] = 4.132; *R*(d) [C–G] = 0.3555; *R*(e) [C–T] = 10.2965; and *R*(f) [G–T] = 1.0000. Performing 15 random addition sequences with TBR branch swapping using the above parameters produced a single topology (score = 28883.17868; Fig. 4). This topology is similar to that of the OA tree, except that in the ML tree *Laphystia* is sister group to Laphriinae rather than sister to *Ospriocerus* + *Stenopogon*; Dasypogoninae is monophyletic in ML but paraphyletic in the OA topology, and Apocleinae is paraphyletic in ML but monophyletic in OA.

4. Discussion

4.1. Relationships among Asiloidea

Our data are congruent with the relationships among the families of Asiloidea as found by Yeates (2002, Fig. 1) and supports Asilidae as sister to the clade (Mydidae + Apioceridae) + (Therevidae + Scenopinidae). Asilidae is monophyletic (BS 28; Bootstrap 100), but the sensitivity analysis indicates that this monophyly occurs in a relatively narrow range of parameter values, due primarily to the nesting of *Mydas* + *Apiocera* within Asilidae for some parameter combinations.

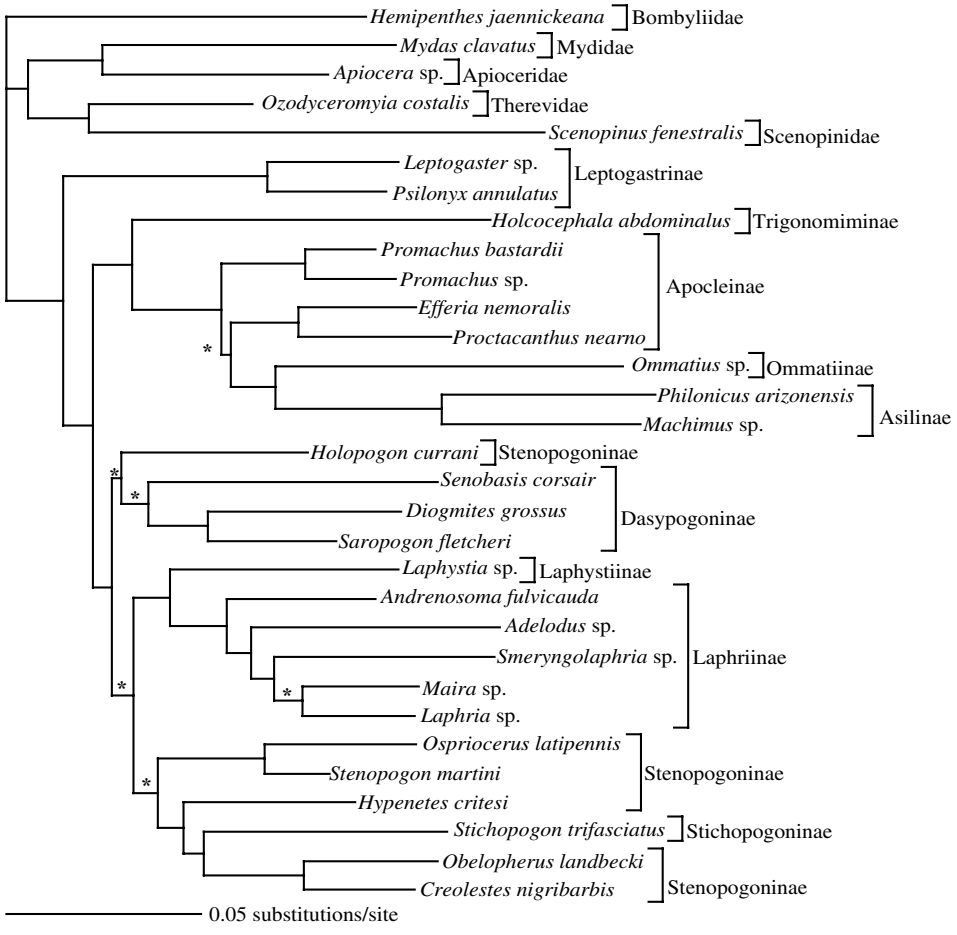

Fig. 4. Maximum likelihood topology for Asilidae and outgroups based on the combined analysis of 16S rDNA, 18S rDNA, 28S rDNA, and COII genes. This topology is based on the GTR + I + G model with a log likelihood score = 28883.17868. This topology is similar to the OA topology for the majority of nodes except as indicated by asterisks.

4.2. Phylogenetic status of leptogastrinae-group

Leptogastrinae is resolved as sister to the remaining Asilidae (BS 15; Bootstrap 100) under both OA and ML analyses. The sensitivity analysis shows that the basal placement of Leptogastrinae is relatively sensitive to parameter values. However, a portion of this sensitivity is due to outgroups nesting within the ingroups as described above. Overall, the basal placement of Leptogastrinae appears to be well supported via molecular data, with nearly 47% of the signal originating from 16S rDNA, whereas the other genes provide positive but lower support values. Note that the clade *Leptogaster* + *Psilonyx* is extremely well supported in this analysis, which further supports the monophyly of Leptogastrinae. Our result reinforces Martin's opinion (1968) that this lineage is distinct from that of other asilids and places it specifically as the most basal asilid group.

4.3. Monophyly of asilinae-group (Asilinae + Ommatiinae + Apocleinae)

The only higher level relationship suggested by Papavero (1973) supported in our analysis was the monophyly of the asilinae-group. This relationship is supported by high bootstrap (100) and Bremer support (44) values, is relatively robust to OA parameter values, and was retrieved in the ML analysis. All gene partitions support this relationship, with the majority of the signal originating from 18S rDNA and 16S rDNA. In the sensitivity analysis, the paraphyly of the asilinae-group only occurs when *Adelodus*, a member of the subfamily Laphriinae, is placed as sister group to the subfamily Asilinae. That molecular data support the monophyly of the asilinae-group suggests that the closed marginal wing cells and slender antennae used to characterize this group are valid synapomorphies. Within the asilinae-group our analysis supports two major lineages: Asilinae + Ommatiinae and Apocleinae. The former clade is well supported under OA and ML analyses, while the latter is only supported under OA analysis. An interesting result is the placement of the subfamily Trigonomiminae as the sister group to the asilinae-group, since morphology suggests a placement within the dasypogoninae-group (Papavero, 1973). However, our data indicate that the dasypogoninae-group is paraphyletic, and hence the morphological characters defining this group are not homologous.

4.4. Monophyly of laphriinae-group (Laphriinae + Laphystiinae)

Wood (1981) and Papavero (1973) suggested that Laphriinae + Laphystiinae is sister group to a presumably monophyletic dasypogoninae-group, defined as (*Dasypogoninae* + (*Trigonomiminae* + *Stenopogoninae* + *Stichopogoninae*)). In our analyses, the dasypogoninae-group is never monophyletic (described below). In OA analysis, Laphriinae is sister to *Diogmites* + *Sarapogon*, one lineage within Dasypogoninae, rather than sister group to Laphystiinae. This result, however, is not robust to changes in OA cost parameter values. The group Laphystiinae + Laphriinae is supported under ML and a subset of cost ratios under OA (Fig. 5). However, in ML Laphystiinae + Laphriinae is sister group to a paraphyletic assemblage of Stenopogoninae and Stichopogoninae, rather than to the dasypogoninae-group.

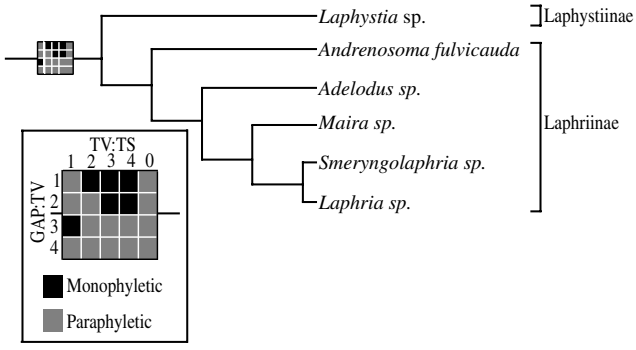

Fig. 5. Sensitivity analysis mapped on the topology for the hypothetical laphriinae-group as proposed by Papavero (1973). This relationship is recovered in the maximum likelihood tree (Fig. 4) and for a subset of cost parameter values in the optimization alignment tree, but is not recovered when gaps = transversions = transitions as in Fig. 3.

4.5. Paraphyly of dasypogoninae-group (Dasypogoninae + (Trigonomiminae + Stenopogoninae + Stichopogoninae))

The dasypogoninae-group is paraphyletic under OA and ML analyses, because of the placement of Laphystiinae and Laphriinae within this group. In all analyses, the subfamily Stenopogoninae is paraphyletic consisting of four clades in OA analysis and three clades under ML analysis. Stichopogoninae is placed as sister group to one of these lineages (*Obelopherus* + *Creolestes*) in both analyses, though with relatively low support values. Under OA analysis, Dasypogoninae is paraphyletic with *Senobasis* as sister to *Holopogon*, whereas this subfamily is monophyletic under ML analysis. The placement of *Senobasis* in a group distinct from other Dasypogoninae under OA, may lend credence to the hypothesis of Hull (1962), who placed this taxon in its own subfamily, Megapodinae. The single exemplar representing the family Trigonomiminae is placed as sister group to the asilinae-group in the ML analysis and under one cost parameter value for the OA analysis.

5. Conclusions

This analysis included 10 of the 11 recognized asilid subfamilies, with seven subfamilies represented by multiple exemplars. Of these seven subfamilies, only four were supported as monophyletic: Laphriinae, Asilinae, Apocleinae, and Leptogastrinae. Of the four higher-level groups defined by Papavero (1973), only the leptogastrinae-group and the asilinae-group are supported as monophyletic. Overall, our choice of molecular markers appears to provide good levels of support for most relationships and provide the first empirical test of robber fly phylogeny. These results suggest that current classification only partially reflects robber fly phylogeny, and that additional work from both a morphological and a well-sampled molecular perspective is needed to better establish these relationships. This molecular phylogeny of Asilidae has provided several resolved, well-supported nodes that can now be further tested via additional data. Our results suggest that many of the morphological characters used to define subfamilial groups (e.g., open marginal wing cells, foretibia apical spurs, two-segmented palpi, secondarily coalesced epandria, etc.) may be homoplastic. The evolution and diversification of robber flies is complex, and this work provides the first step towards deciphering their phylogeny.

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