



A molecular phylogenetic analysis of genus *Anevrina* (Diptera: Phoridae), with the description of a new species and updated world key

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Abstract

Here we report on the first molecular phylogenetic study of the phorid genus *Anevrina* using a combination of nuclear (28S) and mitochondrial (12S, ND1 and CO1) genes for a total of 2220bp. Both maximum parsimony and Bayesian analyses recovered *Anevrina* as a monophyletic lineage within a broad sampling of phorid taxa that included 13 genera from 4 subfamilies. The higher-level relationships of phorid taxa based on the molecular tree were (Sciadocerinae + ((Hypocerinae + Phorinae) + Metopininae)). Relationships of species within *Anevrina* were also fully resolved with strong branch support in the form of posterior probabilities, bootstrap values, and decay indices. Two major clades were identified within *Anevrina*: ((*A. luggeri* + *A. macateei*) + (*A. curvinervis* + *A. unispinosa*)), which was joined as a sister group to ((*A. variabilis* + *A. thoracica*) + (*A. olympiae* + *A. urbana*)). A new and first Neotropical species, *A. neotropica*, from Costa Rica is described, illustrated, and included in an updated world key. *Anevrina setigera* (Loew, 1874) is synonymized with *A. urbana* (Meigen, 1830), new synonymy.

Key words: Diptera, Phoridae, *Anevrina*, new species, phylogeny

The genus *Anevrina* Lioy is a small group of large, mostly dark colored, phorid flies that are distributed throughout the northern hemisphere. Several species have been reported to visit the corpses of small mammals (Wood 1906; Lundbeck 1922; Disney et al. 1981) and/or have been collected from small mammal burrows, including gophers, *Thomomys talpoides*, (Hackman, 1963, 1967; Borgmeier 1963; Brown 1992), moles, *Talpa europea*, (Malloch 1908; Falcoz 1912; Lundbeck 1922), groundhogs, *Marmota monax*, (Borgmeier 1963; Brown 1992), and ground squirrels, *Spermophilus pygmaeus* (M. Mostovski, personal communication). In addition, Lundbeck (1922) observed *A. unispinosa* (Zetterstedt) mating while on the corpse of a sparrow (Passeridae), indicating that some species may be opportunistic on non-mammalian vertebrates. Although the life history of some *Anevrina* species is poorly known, the larvae of all species are probably scavengers or necrophagous on small vertebrates (Brown 1992, 1995; Disney 1994).

The most recent taxonomic revision of *Anevrina* is that of Brown (1995), who recognized 11 extant species and one fossil species, the extinct *A. oligocaenica* (Brues 1939) from Baltic amber. Brown (1995) also hypothesized species-level relationships using morphological characters, especially chaetotaxy of the legs and male genitalia, and provided a key to species. Since Brown's (1995) revision, four additional species have been described: *A. capillata* (Michailovskaya 1999), *A. wyatti* (Disney 2006), *A. microcilia* (Liu & Fang 2006) and *A. glabrata* (Liu & Zhu 2006). In this paper, a new species from Costa Rica, the first known from the Neotropical Region, is described and illustrated and an updated checklist and world key is provided. Furthermore, we report on a preliminary molecular phylogenetic study of 21 phorid taxa, representing 13 genera in 4 subfamilies, including 8 species of *Anevrina*.

Methods and material

Specimens. A list of analyzed taxa with collection details is presented in Table 1. The monophyly and relationships of 8 species of *Anevrina* were investigated within a broad sampling of phorid taxa that included 13 genera and 4 subfamilies (i.e., Sciadocerinae, Hypocerinae and Phorinae sensu Brown 1992 and Metopininae). A *Callomyia* species (Diptera: Platypezidae) was utilized as the outgroup. Voucher specimens are stored at the Natural History Museum of Los Angeles County (LACM). Surplus genomic extracts are stored at -70°C in the Department of Biology at California State University, Bakersfield (CSUB).

TABLE 1. List of taxa analyzed with collection data.

Taxa	Subfamily	Locality
<i>Anevrina curvinervis</i> (Becker)	Phorinae	Guelph, ON, CANADA
<i>Anevrina thoracica</i> (Meigen)	Phorinae	Guelph, ON, CANADA
<i>Anevrina olympiae</i> (Aldrich in Brues)	Phorinae	Guelph, ON, CANADA
<i>Anevrina urbana</i> (Meigen)	Phorinae	Guelph, ON, CANADA
<i>Anevrina luggeri</i> (Aldrich)	Phorinae	Guelph, ON, CANADA
<i>Anevrina macateei</i> (Malloch)	Phorinae	Guelph, ON, CANADA
<i>Anevrina variabilis</i> (Brues)	Phorinae	Monrovia, CA, USA
<i>Anevrina unispinosa</i> (Zetterstedt)	Phorinae	Šur, SLOVAKIA
<i>Borophaga verticalis</i> Borgmeier	Hypocerinae	Edmonton, AB, CANADA
<i>Borophaga subsultans</i> (L.)	Hypocerinae	Guelph, ON, CANADA
<i>Chaetogodavaria sinica</i> Liu	Hypocerinae	Doi Inthanon, THAILAND
<i>Stichillus</i> sp.	Hypocerinae	Cumbre Alto Beni, BOLIVIA
<i>Triphleba</i> sp.167	unknown	Zurquí de Moravia, COSTA RICA
<i>Apodicrania molinai</i> Borgmeier	Metopininae	Corcovado NP, COSTA RICA
Undescribed Genus #2(LACM)	Metopininae	Cocha Cashu, PERU
<i>Melaloncha horologia</i> Brown	Metopininae	50 km N Caranavi, BOLIVIA
<i>Phalacrotophora halictorum</i> (Melander & Brues)	Metopininae	Monrovia, CA, USA
<i>Apocephalus spinosus</i> Brown	Metopininae	Rios Paraisos, COSTA RICA
<i>Gymnophora spiracularis</i> Borgmeier	Metopininae	El Rodeo, COSTA RICA
<i>Megaselia impariseta</i> Bridarolli	Metopininae	Hamilton, NEW ZEALAND
Outgroups		
<i>Sciadocera rufomaculata</i> White	Sciadocerinae	AUSTRALIA
	Family	
<i>Callomyia</i> sp.	Platypezidae	Edmonton, AB, CANADA

DNA extraction, PCR amplification, and sequencing. Genomic DNA was extracted using the DNAeasy Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Four different DNA fragments comprising portions of the nuclear 28S rRNA (615bp) and mitochondrial 12S rRNA (384bp), cytochrome oxidase I (COI; 796bp) and NADH1 dehydrogenase (ND1; 425bp) genes were amplified and sequenced using the oligonucleotide primers listed in Table 2. All PCR amplifications were performed in 50µl volume using annealing temperatures ranging from 40 to 52°C. Amplified products were purified on a QiaQuick PCR column (Qiagen, Valencia, CA). DNA sequencing was performed using either the ABI d-Rhodamine Dye Terminator or Big Dye ver. 3 Cycle Sequencing Ready Reaction Kits (Perkin-Elmer, Foster City, CA) in 5µl volume. Purified sequencing reactions were submitted to the University of Florida's DNA Sequencing Core Facility for sequencing of both strands on an ABI 377 DNA sequencer. Sequence

electropherograms were read and edited using ABI's Sequence Navigator software. Multiple sequence alignment was initially carried out using CLUSTAL X (Thompson et al. 1997) using default parameters. The resulting alignment was further optimized by excluding unalignable hypervariable regions that were present in the rRNA partitions. All sequences have been deposited in GenBank under the accession numbers listed in Table 2. The aligned dataset (in Nexus format) is available at <http://www.phorid.net/phoridae.html>.

TABLE 2. Oligonucleotide primers utilized in this study with GenBank accession numbers for sequences.

Gene & Primer Code*	Sequence (5'-3')	Reference	GenBank Accession Numbers
Mitochondrial			
12S-F (SR-J-14199)	TACTATGTTACGACTTAT	Kambhampati & Smith (1995)	GU559891 – GU559912
12S-R (SR-N-14594)	AAACTAGGATTAGATACCC		
COI-F (C1-J-2183)	CAACAYTTATTTTGATTYYTTYGG	Simon <i>et al.</i> (1994)	GU559934 – GU559955
COI-R (TL2-N-3014)	TCCATTGCACTAATCTGCCATATTA		
ND1-F (N1-J-11861)	ATCATAACGAAAYCGAGGTAA	Smith & Brown (2008)	GU559956 – GU559977
ND1-R (N1-N-12530)	CAACCTTTTWTGTGATGC		
Nuclear			
28S (28E)	CGTAACTTCGGGATAAGGATTGGCT CTG	Moulton & Wiegmann (2007)	GU559913 – GU559933
28S (rc28p)	TGGTATGCGTAGAAGTGTTTGGC		

*mitochondrial primer nomenclature based on Simon *et al.* (1994); 3' nucleotide position based on sequence of *Drosophila yakuba* (Clary and Wolstenholme, 1985)

Phylogenetic Analysis. Summary statistics for the DNA sequence data were calculated using both MEGA version 4 (Tamura et al. 2007) and PAUP* (Swofford 2003) (Table 3). Phylogenetic relationships were estimated using maximum parsimony (MP) in PAUP* and Bayesian methods using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Parsimony analysis was carried out on each individual data partition and on the combined dataset using the multiple equally parsimonious heuristic search option with tree bisection-reconnection and 500 random addition sequence replicates (Table 3). Support for specific nodes on the MP trees was estimated by bootstrap analysis (Felsenstein 1985) (1000 replications with 10 random addition sequence replicates). Bremer support indices (decay indices) (Bremer 1994) were also calculated using AUTODECAY, ver. 4.0 (Eriksson 1998) and visualized using TREEVIEW (Page 1996).

The evolutionary model utilized for Bayesian analysis was selected by MODELTEST 3.7 (Posada & Crandall 1998, 2001), using the Akaike information criterion (AIC) (Posada & Buckley 2004), and corresponded to the GTR + I + G model. We analyzed our dataset under the recommended model using a Markov chain Monte Carlo (MCMC) search strategy in MrBayes. We conducted two independent runs on the data and for each run the MCMC process was set so that four chains (three heated and one cold) ran simultaneously for a total of 500,000 generations (sampling every 200 generations). A majority rule consensus was created from the remaining trees (n=3752).

Results and discussion

A total of 2,220 aligned bases (including gaps) of DNA sequence were obtained for a total of 22 taxa from portions of the nuclear 28S rRNA (615 bp) and mitochondrial 12S rRNA (384 bp), COI (796 bp) and ND1 (425 bp) genes. Of the 2,220 characters, 737 (33%) were variable, and 511 (23%) were parsimony informative. A summary of character statistics and results of unweighted parsimony analyses is presented in Table 3.

TABLE 3. Summary of tree and character statistics for the individual and combined molecular datasets.

Data Partition	Bases + Gaps	PICs	TL	EPTs	CI	RI	Monophyly of <i>Anevrina</i> Supported?
Cytochrome Oxidase 1 (CO1)	796	283	1358	2	0.38	0.61	Yes
NADH 1 Dehydrogenase (ND1)	425	130	621	1	0.43	0.57	Yes
12S rDNA (12S)	384	80	311	132	0.60	0.53	No/unresolved
28S rDNA (28S)	615	18	89	2877	0.89	0.10	No/unresolved
All Genes	2220	511	2430	1	0.36	0.36	Yes

PICs: number of parsimony informative characters; **TL:** tree length; **EPTs:** number of equally parsimonious trees; **CI:** consistency index; **RI:** retention index

Phylogenetic relationships were inferred using both parsimony and Bayesian methods. The topologies of the trees resulting from the two different analyses were largely congruent and differed only in the placement of some taxa within the Metopininae. The higher-level relationships among phorid subfamilies and species-level relationships within *Anevrina* were identical for both types of analyses.

Our discussion of phylogenetic relationships is based on the single most parsimonious tree (TL = 2430) for the combined dataset (Fig. 1). Parsimony analyses based on the single gene datasets (see Table 3) generally resulted in trees with less resolution and support due to fewer included characters. The higher-level relationships of phorid taxa based on the combined dataset were (Sciadocerinae + ((Hypocerinae + Phorinae) + Metopininae)) (Fig. 1), differing from the hypothesis of relationships based on morphology by Brown (1992), which were (Sciadocerinae + (Hypocerinae + (Phorinae + Metopininae))). Although not a focus of the present study, our preliminary molecular data suggests that *Triphleba* is not the sister-group to Metopininae as proposed by Brown (1992). This result, however, must remain subject to further verification as it is based on only a single specimen of *Triphleba* and could be an artifact of insufficient taxon sampling. Indeed, many more taxa and sequences are being analyzed by us in an effort to elucidate the higher-level relationships of phorid subfamilies and genera, including the phylogenetic placement of *Triphleba*.

The genus *Anevrina* belongs in the subfamily Phorinae, hypothesized to be the second most primitive lineage in the Phoridae (Brown 1992). In a previous study, Cook et al. (2004) sequenced portions of the mitochondrial 12S and 16S genes from a single specimen of *A. thoracica* and found that this species was placed within a morphologically broadly defined Phorinae and was the sister taxon to *Conicera* + *Spiniphora*. A limitation of the aforementioned study was the lack of primitive-grade taxa representation from the subfamilies Sciadocerinae and Hypocerinae (e.g., *Borophaga*, *Chaetogodavaria*, *Stichillus*) (sensu Brown 1992), which could have profound effects on character polarization and ultimate placement of *Anevrina* within the tree. The sister group to *Anevrina* is not known, but is currently being investigated by us (unpublished) in our ongoing studies of basal phorid relationships. By including exemplars of both basal (e.g., Sciadocerinae and Hypocerinae) and higher (Metopininae) phoridae in our molecular dataset, however, we can effectively assess the monophyly of representative *Anevrina* species.

The genus *Anevrina* is characterized by a setulose wing vein Rs and a convoluted process of the subepandrial plate (Brown 1992). Analysis of molecular characters recovered *Anevrina* as a monophyletic lineage within a broad sampling of phorid taxa (Fig. 1). The most recent taxonomic revision of *Anevrina* is that of Brown (1995), who hypothesized species-level relationships using morphological characters, but also mentioned that hind tibial setation (representing 3 of 6 morphological characters utilized by Brown (1995)) is a fairly labile trait that may or may not reflect evolutionary relatedness. However, Brown (1995) did identify two unequivocal groupings: (1) (*A. curvinervis* + *A. macateei*) (character 6) and (2) (*A. thoracica* + *A. urbana* + *A. variabilis* + (*A. setigera* + *A. sphaeropyge*)) (characters 1, 4–5). Brown (pers. obs.) later noted that *A. luggeri* possessed peglike setae (character 6) and thus should be grouped with *A. curvinervis* + *A. macateei*.

Although the present study only included about half of the known species of *Anevrina*, two major clades were identified: ((*A. variabilis* + *A. thoracica*) + (*A. olympiae* + *A. setigera*)) (Group I; Fig. 1), which was joined as a sister group to ((*A. luggeri* + *A. macateei*) + (*A. curvinervis* + *A. unispinosa*)) (Group II; Fig. 1). In

comparison to Brown's (1995) morphological tree, our molecular tree (Fig. 1) has both similarities and differences, which will not be fully resolved until we are able to obtain representatives of all known *Anevrina* species for molecular analysis. Despite the limitation of inadequate taxon sampling, the molecular tree can provide some preliminary insights into the species-level relationships within *Anevrina*. First, Group I (Fig. 1) is mostly comprised of species that possess a narrow, elongate surstylus (character 5 of Brown 1995) and a dorsal seta on the hind tibia (character 4 of Brown 1995). The lone exception is *A. olympiae*, which lack these two characters. Second, Group II (Fig. 1) is mostly comprised of species that possess peglike setae on the inner face of the hind femur (present in male specimens only) (see Figs. 12–15). The lone exception is *A. unispinosa*, which lack these setae (Fig. 5; but see below and Fig. 18). Within Group II (Fig. 1), the *A. luggeri* + *A. macateei* grouping is characterized by a linear arrangement of < 20 peglike setae (Figs. 12–13). Lastly, although all known species of *Anevrina* are restricted to the northern hemisphere, the molecular tree indicates no obvious phylogeographic structure in species' distribution, possibly indicating that Old World species have been introduced into the New World, and vice versa.

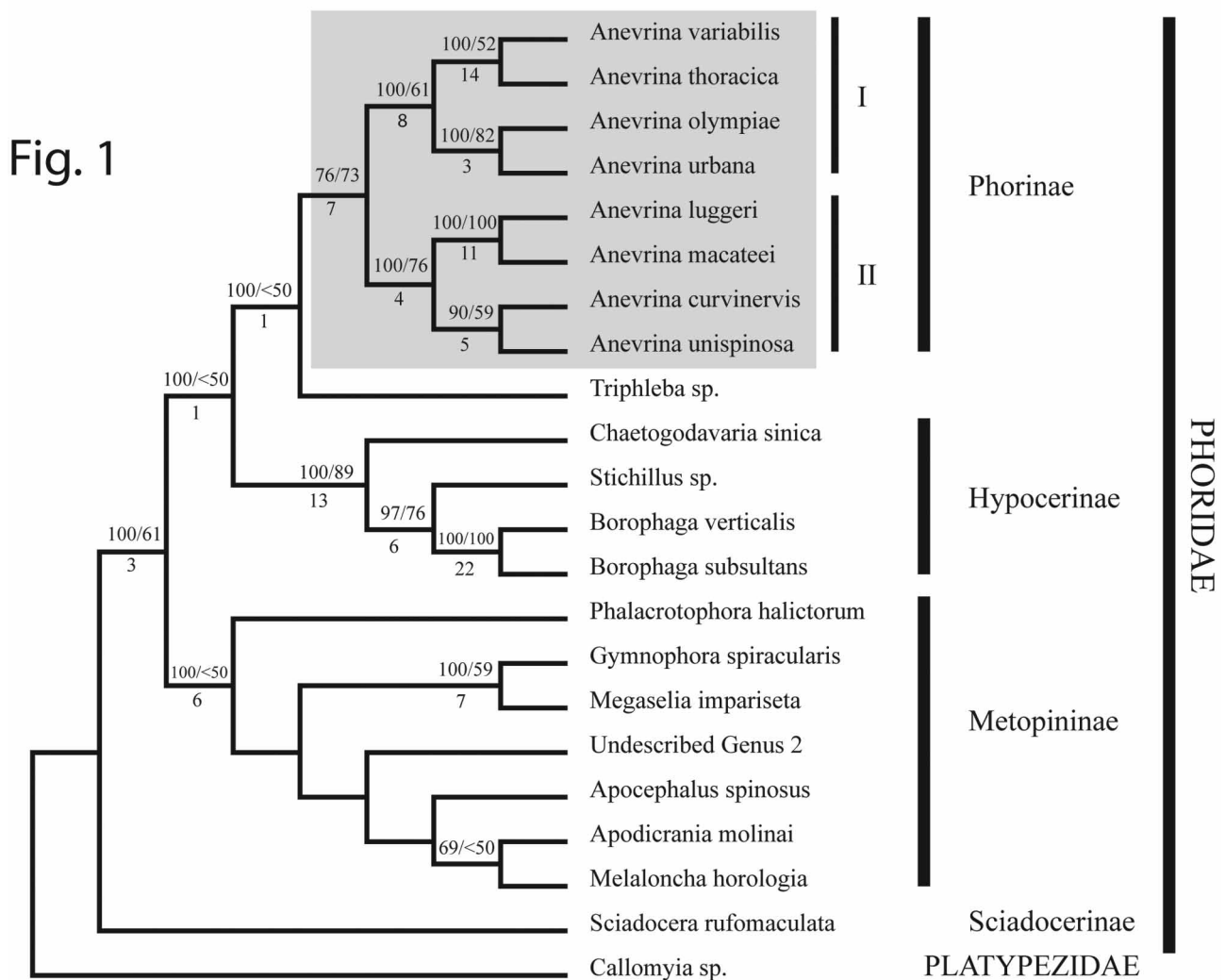
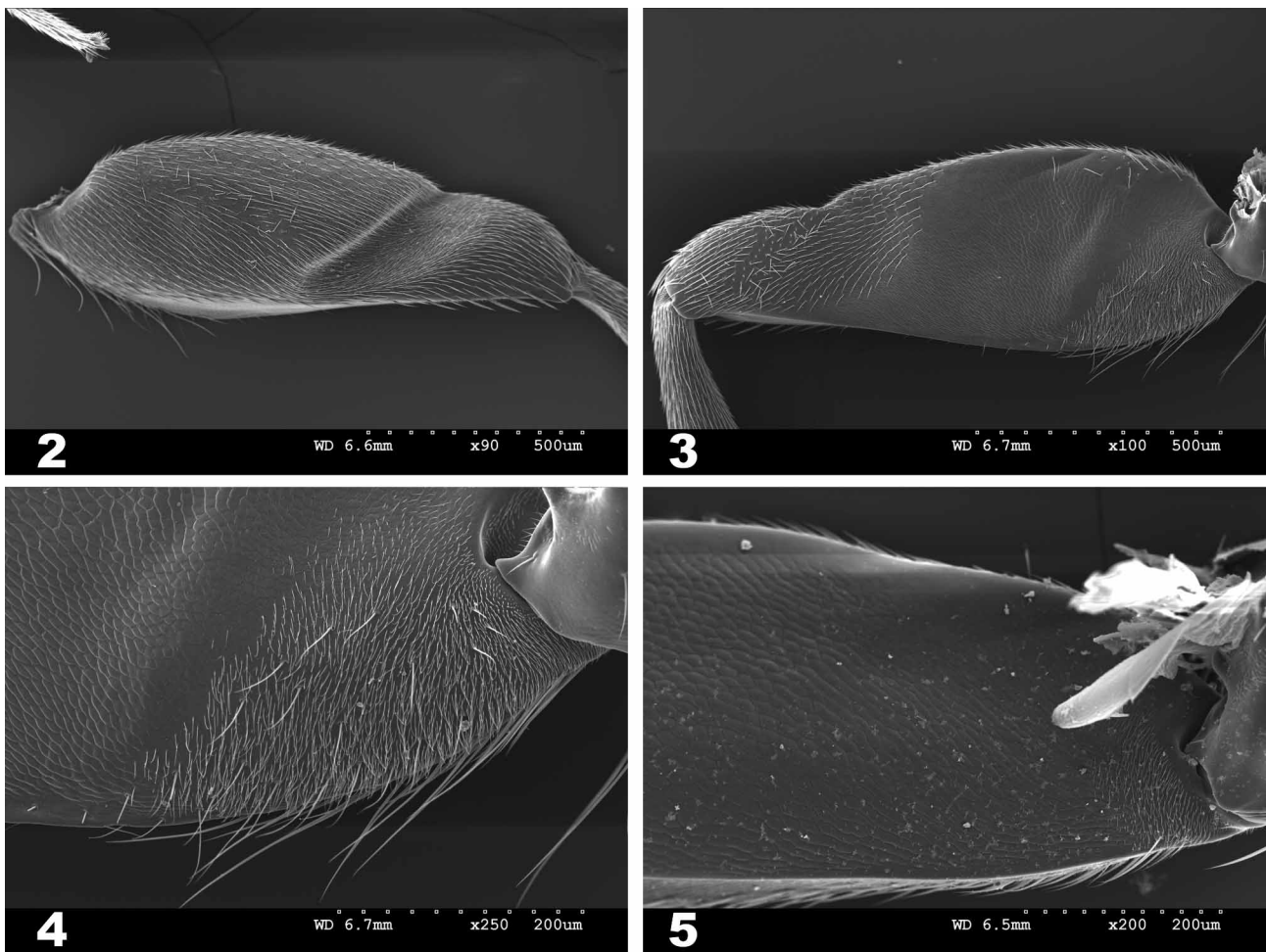


FIGURE 1. Single most parsimonious tree based on simultaneous analysis of portions of four genes (2220 bp including gaps) for 21 phorid taxa including 8 *Anevrina* species. Tree length, 2430; consistency index, 0.36; retention index, 0.36. Numbers above branches are Bayesian posterior probability values/bootstrap values (%) and those below branches are Bremer support indices.

Taxonomy

Since Brown's (1995) revision, four additional species have been described: *A. capillata* (Michailovskaya 1999), *A. wyatti* (Bänziger & Disney 2006), *A. glabrata* (Liu & Zhu 2006) and *A. microcilia* (Liu & Fang 2006). Of these, *A. microcilia* is extremely similar in genitalic structure to the Burmese *Triphleba ctenochaeta* Beyer and *T. schmitzi* Beyer (Beyer, 1958), both belonging to the *T. nipponica*-group of species. Gotô & Takeno (1983) suggested that these "*Triphleba*" species should be placed in *Anevrina*, but this suggestion has not been investigated yet. Until the proper classification of this group (which also includes *T. nipponica* Schmitz and *T. segrex* Beyer) is clarified, we omit them from our concept of *Anevrina*.

Below, a new species from Costa Rica, the first known from the Neotropical region, is described and illustrated, and an updated checklist and world key is provided. All specimens are deposited in the collection of the Instituto Nacional de Biodiversidad, Costa Rica (INBC).



FIGURES 2–5. Scanning electron micrographs, hind femora. **2–4.** *Anevrina variabilis* (Brues), anterior view, posterior view, and high magnification posterior view. **5.** *Anevrina unispinosa* (Zetterstedt), high magnification posterior view.

Anevrina neotropica Smith and Brown, sp. nov.

Holotype. ♂, COSTA RICA: Puntarenas: Estación La Casona, R.B. Monteverde, 1520m, 23–26.ii.1993, N. Obando, G. Barbosa, A. Pound. #2675.(INBC)

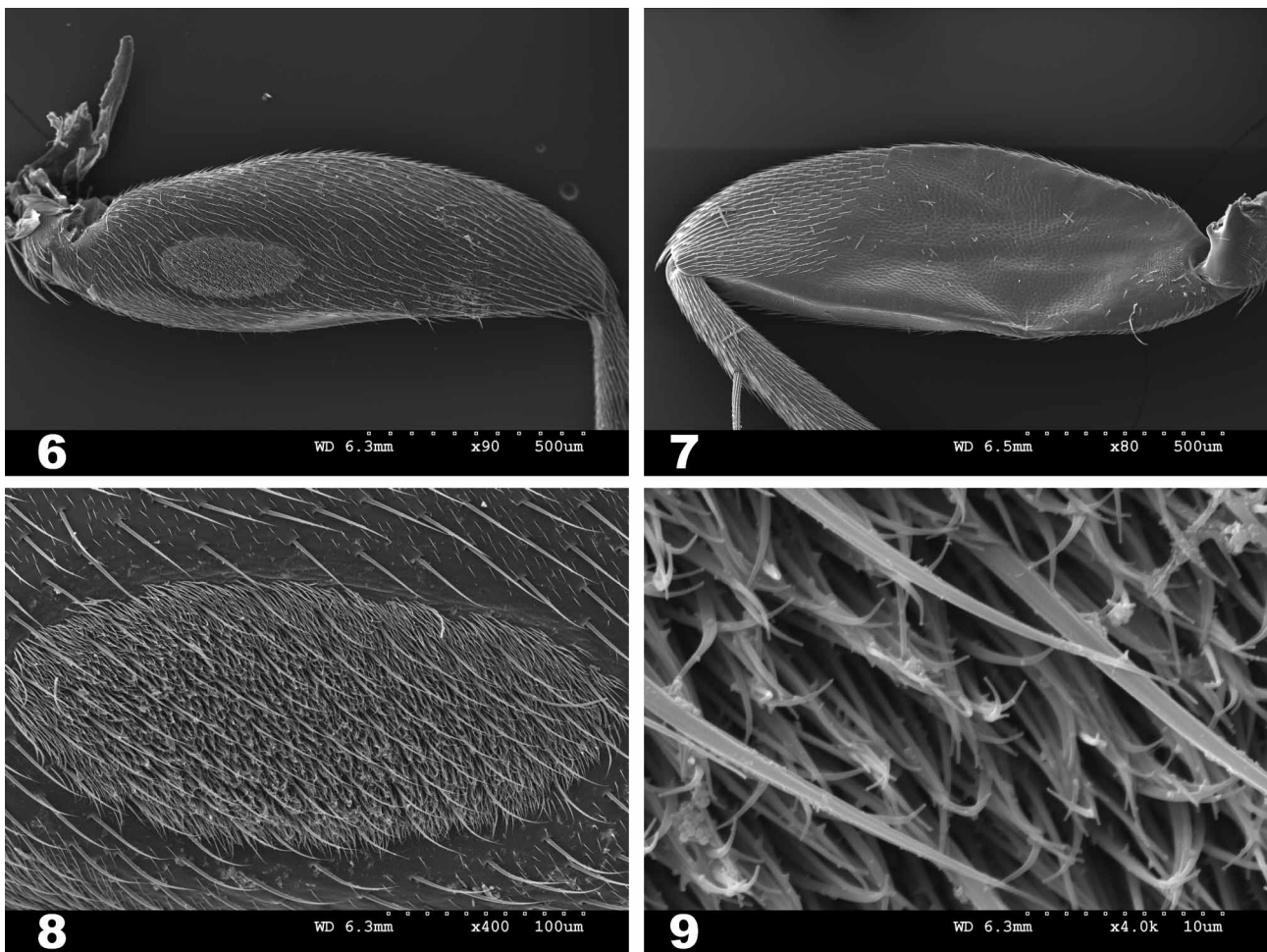
Paratypes. COSTA RICA: 2 ♀ same data as holotype except 18–22.ii.1993, 28.ii–8.iii. 1993, #2172, #2677 (INBC).

Diagnosis. This species has the setation of the hind tibia similar to that of *A. unispinosa*, but unlike *A. unispinosa*, there are 20–25 peglike setae on the inner face of the hind femur of the male.

Description. Body length 2.80–3.70 mm. Body color entirely dark brown, except tibia and tarsomeres lighter brown. Mean frontal ratio (height divided by width) 1.67. Flagellomere 1 and palpus of normal size, not enlarged. Wing with mean costal ratio 0.66. Mean costal sector ratio 5.94 : 4.13 : 1, range 4.33–8.00 : 4.00–4.44 : 1. Halter yellow. Foretibia with one dorsal seta at midlength. Midtibia with pair of basal setae and one anterior seta near base. Hind tibia with one anterobasal seta and one anteroapical seta, also small anterior seta at apex and pair of ventral spurs present. Inner face of hind femur with 20–25 peg-like setae in male (Fig. 15), absent in females. Male terminalia dark brown, with uniform setae (Figs. 16–17). Right surstylus tapered posteriorly (Fig. 17).

Phylogenetic relationships. Hind tibial setation potentially link this species with *A. unispinosa*, based on the loss of the dorsobasal seta (Brown 1995). A more compelling character, the presence peglike setae on the inner face of the hind femur of the male suggests a close relationship with *A. luggeri*, *A. macateei*, and *A. curvinervis* (Fig. 18).

Etymology. Named as the first known species from the Neotropical region.

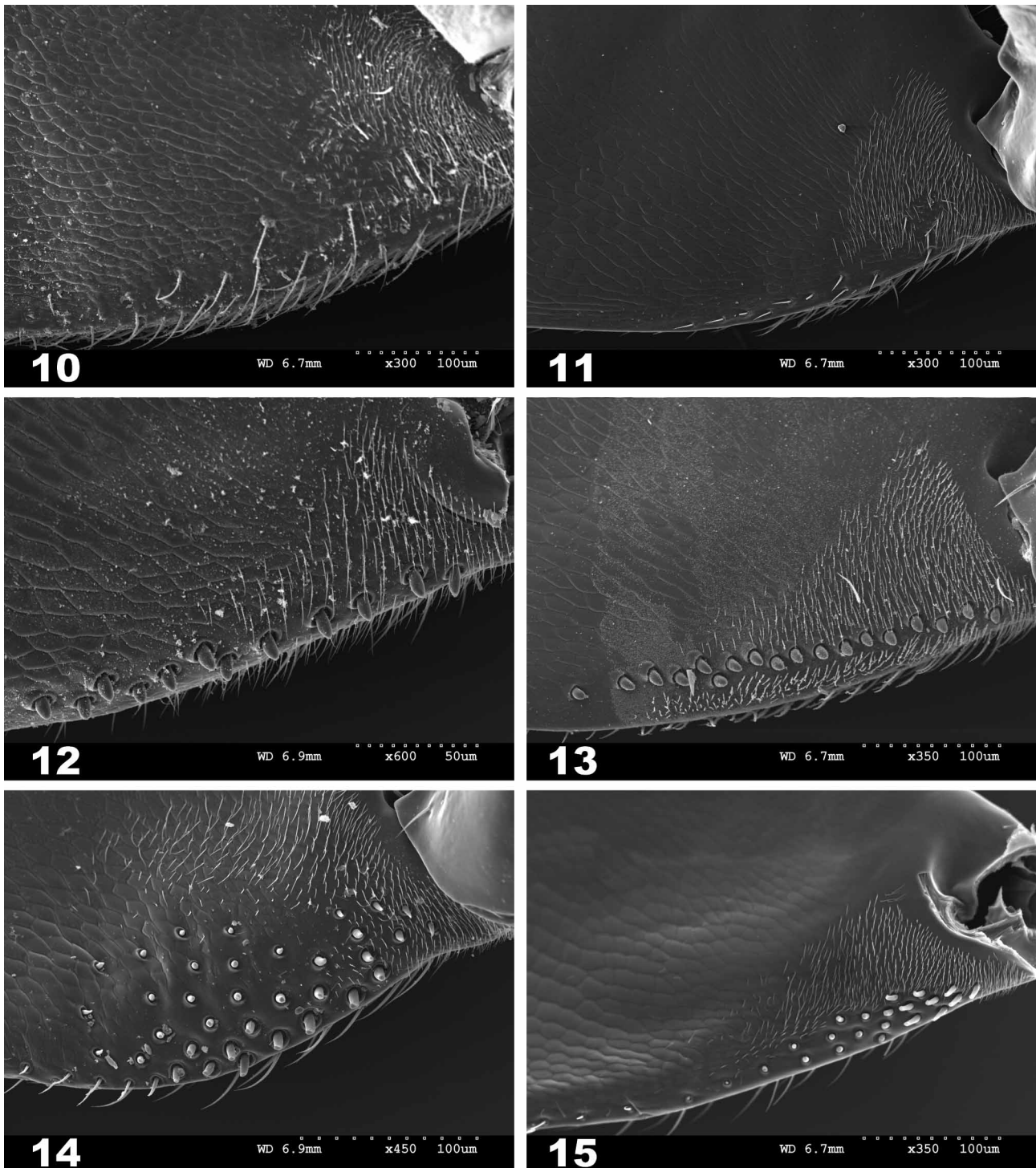


FIGURES 6–9. *Anevrina thoracica* (Meigen), scanning electron micrographs, hind femur. **6.** Anterior view. **7.** Posterior view. **8–9.** Anterior oval patch, low magnification and high magnification.

Anevrina urbana (Meigen, 1830)

Phora setigera Loew, 1874: 420. **NEW SYNONYMY**

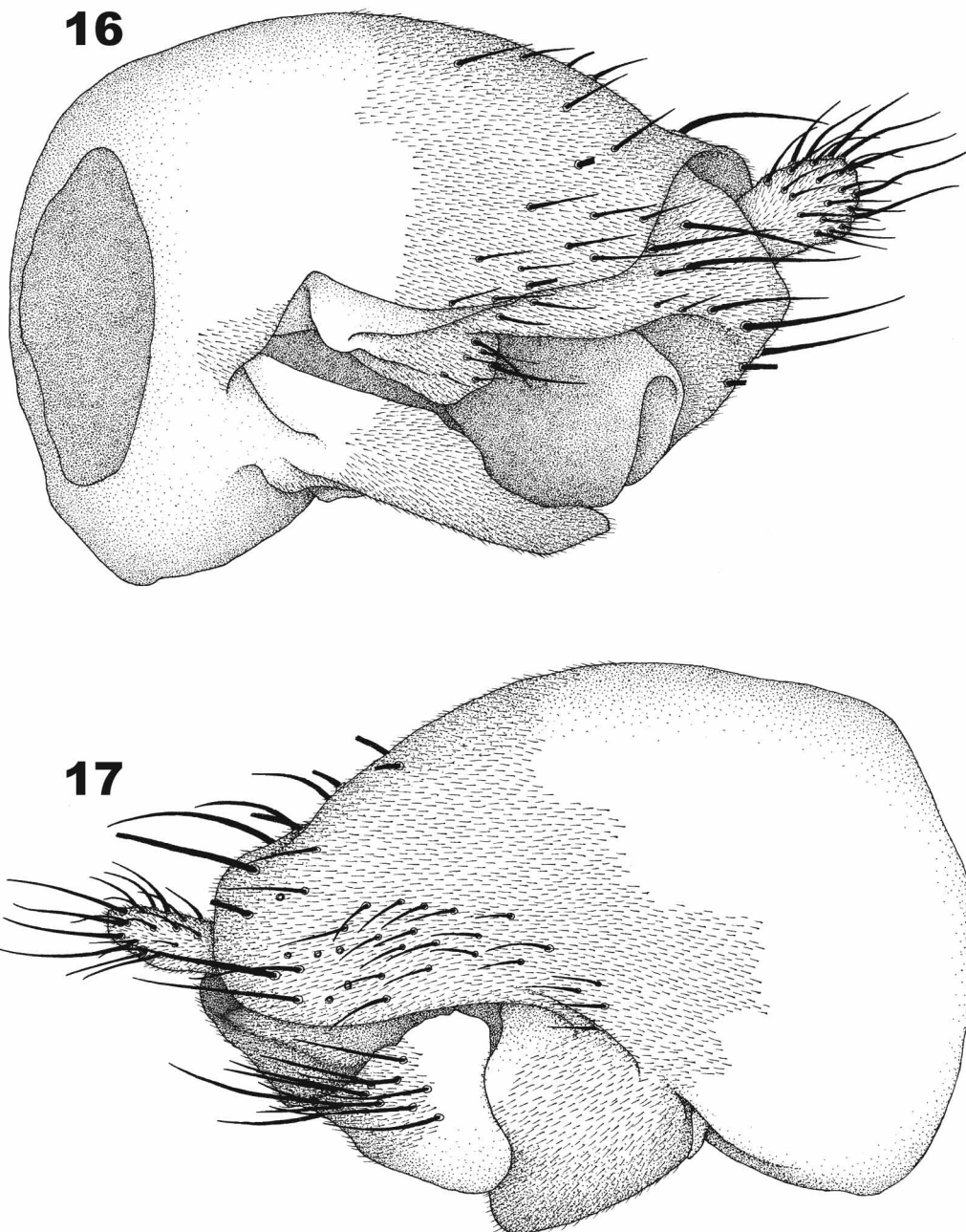
Notes on synonymy: Full synonymies of these species are given by Borgmeier (1968). Previously, Brown (1995) synonymized *A. spinipes* (Coquillett) with *A. setigera*.



FIGURES 10–15. Scanning electron micrographs, hind femora, posterior view, high magnification. **10.** *Anevrina olympiae* (Aldrich in Brues). **11.** *Anevrina urbana* (Meigen). **12.** *Anevrina macateei* (Malloch). **13.** *Anevrina luggeri* (Aldrich). **14.** *Anevrina curvinervis* (Becker). **15.** *Anevrina neotropica*, sp. n.

Some European specimens of *A. urbana* have tibial setation similar to that of *A. setigera*, with 2 setae on the foretibia, and 2 dorsal and 2 anterior setae on the midtibia. One remarkable female specimen from Belgium (LACM ENT 219118) has different setation on the left and right side of the body: left side foretibia with 2 dorsal setae, midtibia with 2 dorsal, 2 anterior, hind tibia with 5 dorsal and 2 anterior; right side foretibia with 1 dorsal setae, midtibia with 1 dorsal, 2 anterior, hind tibia with 4 dorsal and 2 anterior. Thus, the left side would key out to *A. setigera* and the right side to *A. urbana*. Examination of the male terminalia of specimens

of *A. urbana* from Slovakia and *A. setigera* from North America indicates that they are the same species (also confirmed with a specimen of *A. urbana* from Sweden by H. Nakayama, personal communication), but we have seen no males with *A. setigera*-type setation from Europe to compare. Schmitz (1941) also recorded only female specimens of *A. setigera* from Europe. In contrast, no specimens with *A. urbana*-type setation are known from North America. Possibly, North American specimens with the “*A. setigera*”-type setation are the result of a founder effect, where a small, biased sample of adults was introduced. A population genetics study on this species would be highly informative.



FIGURES 16–17. *Anverina neotropica*, sp. n., male terminalia. 16. Left side. 17. Right side.

Checklist of World *Anverina* species, with distributions

A. capillata Michailovskaya 1999— Palearctic Region

A. curvinervis (Becker 1901)—Holarctic Region

A. glabrata Liu & Zhu 2006—Oriental region

- A. kozaneki* Brown 1995—Palearctic Region
- A. luggeri* (Aldrich 1892) —Nearctic Region
- A. macateei* (Malloch 1913)—Nearctic Region
- A. olympiae* (Aldrich, in Brues 1904)—Nearctic Region
- A. neotropica* **new species**—Neotropical Region
- A. sphaeropyge* Beyer 1958— Oriental Region
- A. thoracica* (Meigen 1830)—Holarctic Region
- A. unispinosa* (Zetterstedt 1860)—Palearctic and Oriental Regions
- A. urbana* (Meigen 1830)—Holarctic Region
- A. variabilis* (Brues 1908)—Nearctic Region
- A. wyatti* Disney 2006 —Oriental Region

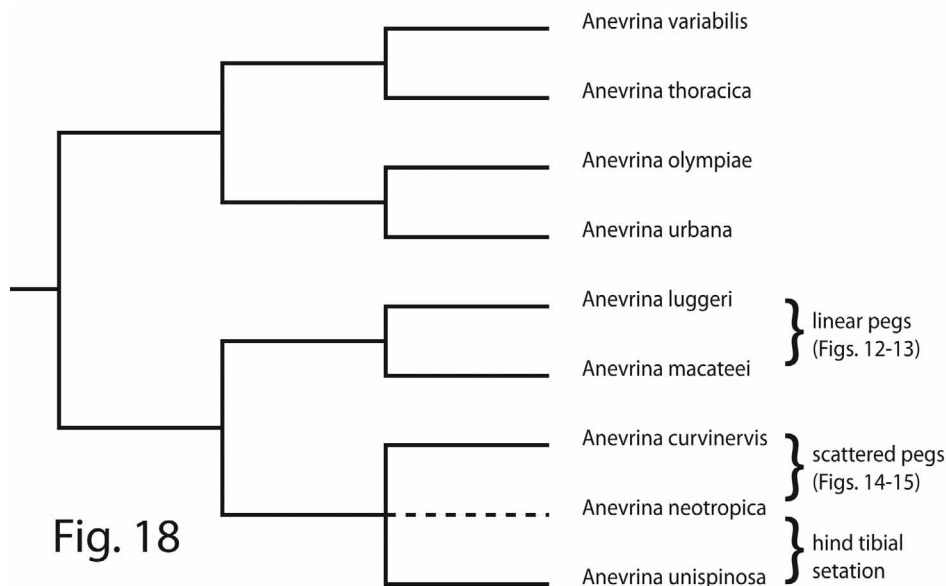


Fig. 18

FIGURE 18. Hypothesized phylogenetic placement of *Anevrina neotropica* sp. n. within the molecular phylogeny.

Key to World *Anevrina*

Note: this key should be used in conjunction with fig. 1 of Brown (1995), available at <http://www.phorid.net/phoridae/phorpub.html>

- 1 Hind tibia, aside from ventral spurs, with one anterobasal and one anteroapical seta only (fig. 1C in Brown 1995) 2
- Hind tibia with three or more setae..... 4
- 2 (1) Inner face of hind femur of male with >20 scattered peglike setae (Fig. 15); Costa Rica *A. neotropica* **new species**
- Inner face of hind femur of male without peglike setae (Fig. 5); Palearctic and Oriental Regions 3
- 3 (2) Setulae of wing vein Rs short, pale; legs yellow; thorax and abdominal tergites orange brown; only female known *A. wyatti* Disney
- Setulae of vein Rs darker, longer; more dark brown in color (but Burmese males with lighter colored legs and thorax) *A. unispinosa* (Zetterstedt)
- 4 (1) Aside from ventral spurs, hind tibia with only one seta present distinctly below mid-level (figs. 1D–G, Brown 1995) 5
- Hind tibia with more than one setae present distinctly below mid-level (figs. 1B, H–L, Brown 1995) 10
- 5 (4) Halter brown 6
- Halter yellow..... 7

- 6 (5) Hind tibia with dorsobasal seta subequal to and higher than anterobasal seta (fig. 1F, Brown 1995); inner face of hind femur of male with a linear arrangement of <20 peg-like setae (Fig. 12) *A. macateei* (Malloch)
- Hind tibia with dorsobasal seta distinctly longer and lower than anterobasal seta (fig. 1E, Brown 1995); inner face of hind femur of male with a scattered arrangement of >20 peg-like setae (Fig. 14) *A. curvinervis* (Becker)
- 7 (5) Hind tibia with only one dorsal seta, such that setation consists of anterobasal, dorsobasal, and anteroapical setae only (fig. 1D, Brown 1995) 8
- Hind tibia normally with more than one dorsal setae and thus more than three setae (fig. 1G, Brown 1995) 9
- 8 (7) External face of midtibia with distinct naked patch; inner face of hind femur of male not examined *A. glabrata* Liu & Zhu
- External face of midtibia without distinct naked patch; inner face of hind femur of male with scattered hairs (Fig. 10) *A. olympiae* (Aldrich)
- 9 (7) Foretibia with one seta; anterior face of hind femur with distinct oval patch of small setae (Figs. 6–9) *A. thoracica* (Meigen)
- Foretibia with more than one seta; anterior face of hind femur without distinct oval patch .. *A. sphaeropyge* Beyer
- 10 (4) Hind tibia with only three to four setae: one or two basal setae and two apical setae at same level (figs. 1B, I, Brown 1995) 11
- Hind tibia with more than four setae (figs. 1H, J, K, Brown 1995), or if only four setae present, dorsoapical seta more basal than anteroapical seta (fig. 1L, Brown 1995) 12
- 11 (10) Hind tibia with pair of basal setae (figs. 1 I, Brown 1995); inner face of hind femur of male with a linear arrangement of <20 peg-like setae (Fig. 13); halter yellow *A. luggeri* (Aldrich)
- Hind tibia with one basal seta only; inner face of hind femur lacking peglike setae; halter brown *A. kozaneki* Brown
- 12 (10) Hind tibia with two dorsal setae, one at one-third, one at two-thirds length of tibia; external face of hind femur of male with distinct sulcus (Fig. 2); inner face of hind femur with long hairs (Fig. 4); female with abdomen bright orange, contrasting with dark brown remainder of body *A. variabilis* (Brues)
- Hind tibia with three or more dorsal setae; male femur without sulcus; inner face of hind femur various; female abdomen not bright orange 13
- 13 (12) Inner face of male hind femur with long hairs (as in Fig. 3); far eastern Russia *A. capillata* Michailovskaya
- Inner face of male hind femur bare; Europe, North America *A. urbana* (Meigen)

Note: Females of these two species have similar tibial setation, and cannot be reliably separated at this time. Distribution and association with males may provide some information, however.

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