



## Защита Растений Plant protection

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### Molecular Analysis of Parasitoid Flies Tachinidae

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**Abstract.** The parasitoids from Tachinidae family have important role in biological control; nevertheless, the phylogenetic relationships of supra genera groups are poorly studied. Here, we present phylogenetic analyses of the family based on molecular data. 73 species of parasitoid flies belonging to 30 tachinid genera, including the four currently recognized subfamilies (Dexiinae, Exoristinae, Phasiinae, Tachininae) and 20 tribes were analyzed in the molecular study. The Tachinidae are reconstructed as a monophyletic assemblage based on morphological data and with four nonhomoplasious apomorphies (synapomorphies). Monophyly is well supported by a bootstrap value. Our morphological analysis generally supports the subfamily grouping Dexiinae + Phasiinae, while Tachininae + Exoristinae is not supported as one group, and with only the Exoristinae and the Phasiinae reconstructed as monophyletic assemblages. The Dexiinae, which were previously considered a well-established monophyletic assemblage (except for few studies), are reconstructed as polyparaphyletic with respect to the Phasiinae. The Tachininae are reconstructed as a paraphyletic grade, while monophyly of Exoristinae was recovered except genus *Admontia* Brauer & Bergenstamm, which arose within subfamily Tachininae. In contrast to molecular analysis, all subfamilies are polyparaphyletic groups in which they interact with each other, with the exception of Phasiinae, which includes most of its taxa in a monophyletic group.

**Keywords:** Analysis, Molecular, Morphology, Tachinidae

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**Author's contribution.** The author of this research paper has directly participated in the planning, execution, or analysis of this study. The author has read and approved the final version submitted.

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## Introduction

Tachinidae are characterized as among the most diverse families of Diptera in characters, colors and behavior, in addition to the hosts. This family counts about 10 000 species [1] and is composed of four subfamilies (Phasiinae, Dexiinae, Tachininae and Exoristinae) including nearly 60 tribes [2, 3]. At least 15 orders of Arthropoda are hosts by all known tachinid species that considered as internal parasitoids of insects or other arthropods [1]. However, the most of tachinid species parasitize holometabolous insect larvae (Lepidoptera, Coleoptera, and Hymenoptera) or adult beetles, as well as true bugs [1, 2]. As enemies of these primarily phytophagous groups, tachinids play important role in biological control.

## Materials and methods

Tachinids were obtained by hand collecting and malaise trap from Russia and Egypt, and one species from Panama. Tachinidae were sampled to provide representative specimens (A total of 120 specimens were analyzed) of the four subfamilies (Dexiinae, Exoristinae, Phasiinae, Tachininae), and 30 genera of 20 tribes, and out-group based on previous works we selected the studied taxa [2, 4–7].

We collected nearly all the sequences of COI gene (mDNA) for tachinid species that opposite the genera which we brought from Russia and Egypt. They were composed of 73 nucleotide sequences collected from NCBI with Accession numbers (table 1). We used COI gene in this study to compare its ability as a traditional marker for solving the cladistic relationships within the radiative family Tachinidae with the recent studies that used new markers, which revealed more of these relationships for this family. COI gene was used only once but very few species of Tachinidae family were used for phylogeny. Alignment Sequences and analysis were performed by MEGAX program [8].

Table 1

### Nucleotide sequences that collected from NCBI<sup>1</sup> and analyzed in this study

Species	Accession number	Number of base pairs	Link (URL)
<i>Estheria petiolata</i>	KX844428	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844428.1/">https://www.ncbi.nlm.nih.gov/nuccore/KX844428.1/</a>
<i>Rondania nr. dimidiata</i>	KX843735	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX843735">https://www.ncbi.nlm.nih.gov/nuccore/KX843735</a>

<sup>1</sup> Nucleotide. Search database. <https://www.ncbi.nlm.nih.gov/nuccore/HM417303>

Continuation of the table 1

Species	Accession number	Number of base pairs	Link (URL)
<i>Athrycia cinerea</i>	KR 395891	585 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KR395891">https://www.ncbi.nlm.nih.gov/nuccore/KR395891</a>
<i>Thelaira nigripes</i>	KX844342	622 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844342">https://www.ncbi.nlm.nih.gov/nuccore/KX844342</a>
<i>Thelaira americana</i>	HM417100	654 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/HM417100">https://www.ncbi.nlm.nih.gov/nuccore/HM417100</a>
<i>Thelaira solivaga</i>	KX844207	620 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844207">https://www.ncbi.nlm.nih.gov/nuccore/KX844207</a>
<i>Voria erasmocoronadoi</i>	MF325369	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/MF325369">https://www.ncbi.nlm.nih.gov/nuccore/MF325369</a>
<i>Voria sp.</i>	KR 386101	579 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KR386101">https://www.ncbi.nlm.nih.gov/nuccore/KR386101</a>
<i>Admontia degeerioides</i>	JN 302070	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JN302070">https://www.ncbi.nlm.nih.gov/nuccore/JN302070</a>
<i>Admontia grandicornis</i>	KR 621021	555 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KR621021">https://www.ncbi.nlm.nih.gov/nuccore/KR621021</a>
<i>Bactromyia aurulenta</i>	MG475545	516 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/MG475545">https://www.ncbi.nlm.nih.gov/nuccore/MG475545</a>
<i>Prooppia crassiseta</i>	HQ581756	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/HQ581756">https://www.ncbi.nlm.nih.gov/nuccore/HQ581756</a>
<i>Drino sp.</i>	HM882180	521 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/HM882180">https://www.ncbi.nlm.nih.gov/nuccore/HM882180</a>
<i>Elodia ambulatoria</i>	KX844551	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844551">https://www.ncbi.nlm.nih.gov/nuccore/KX844551</a>
<i>Cylindromyia bicolor</i>	MN 868900	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/MN868900">https://www.ncbi.nlm.nih.gov/nuccore/MN868900</a>
<i>Cylindromyia rufipes</i>	MN 868879	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/MN868879">https://www.ncbi.nlm.nih.gov/nuccore/MN868879</a>
<i>Cylindromyia intermedia</i>	MK660720	312 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/MK660720">https://www.ncbi.nlm.nih.gov/nuccore/MK660720</a>
<i>Cylindromyia fumipennis</i>	HQ945071	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/HQ945071">https://www.ncbi.nlm.nih.gov/nuccore/HQ945071</a>
<i>Gymnosoma nudifrons</i>	KP044778	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KP044778">https://www.ncbi.nlm.nih.gov/nuccore/KP044778</a>
<i>Ectophasia crassipennis</i>	MN 868783	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/MN868783">https://www.ncbi.nlm.nih.gov/nuccore/MN868783</a>
<i>Phasia aurulans</i>	JN 310367	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JN310367">https://www.ncbi.nlm.nih.gov/nuccore/JN310367</a>
<i>Phasia mesnili</i>	KX844068	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844068">https://www.ncbi.nlm.nih.gov/nuccore/KX844068</a>
<i>Phasia pusilla</i>	MN 868790	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/MN868790">https://www.ncbi.nlm.nih.gov/nuccore/MN868790</a>
<i>Phasia punctigera</i>	HM417303	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/HM417303">https://www.ncbi.nlm.nih.gov/nuccore/HM417303</a>
<i>Phasia aurulans</i>	KM571524	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KM571524">https://www.ncbi.nlm.nih.gov/nuccore/KM571524</a>
<i>Phasia obesa</i>	JN 310368	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JN310368">https://www.ncbi.nlm.nih.gov/nuccore/JN310368</a>
<i>Leucostoma sp.</i>	KP047351	593 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KP047351">https://www.ncbi.nlm.nih.gov/nuccore/KP047351</a>

Continuation of the table 1

Species	Accession number	Number of base pairs	Link (URL)
<i>Leucostoma simplex</i>	KX843880	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX843880">https://www.ncbi.nlm.nih.gov/nuccore/KX843880</a>
<i>Leucostoma tetraptera</i>	KX843764	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX843880">https://www.ncbi.nlm.nih.gov/nuccore/KX843880</a>
<i>Leucostoma gravipes</i>	KR 520627	629 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KR 520627">https://www.ncbi.nlm.nih.gov/nuccore/KR 520627</a>
<i>Leucostoma meridianum</i>	KX843930	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX843930">https://www.ncbi.nlm.nih.gov/nuccore/KX843930</a>
<i>Macquartia nudigena</i>	KX844477	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844477">https://www.ncbi.nlm.nih.gov/nuccore/KX844477</a>
<i>Macquartia dispar</i>	JN 298651	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JN 298651">https://www.ncbi.nlm.nih.gov/nuccore/JN 298651</a>
<i>Macquartia tessellum</i>	KY 846615	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KY 846615">https://www.ncbi.nlm.nih.gov/nuccore/KY 846615</a>
<i>Macquartia viridana</i>	KX844333	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844333">https://www.ncbi.nlm.nih.gov/nuccore/KX844333</a>
<i>Mintho rufiventris</i>	KX843818	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX843818">https://www.ncbi.nlm.nih.gov/nuccore/KX843818</a>
<i>Nemoraea pellucida</i>	KX844529	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844529">https://www.ncbi.nlm.nih.gov/nuccore/KX844529</a>
<i>Peribaea setinervis</i>	KY 421538	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KY 421538">https://www.ncbi.nlm.nih.gov/nuccore/KY 421538</a>
<i>Peribaea hertingi</i>	KX844508	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844508">https://www.ncbi.nlm.nih.gov/nuccore/KX844508</a>
<i>Peribaea setinervis</i>	KX844049	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844049">https://www.ncbi.nlm.nih.gov/nuccore/KX844049</a>
<i>Peribaea tibialis</i>	KX843900	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX843900">https://www.ncbi.nlm.nih.gov/nuccore/KX843900</a>
<i>Siphona grandistylum</i>	KX844528	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844528.1/">https://www.ncbi.nlm.nih.gov/nuccore/KX844528.1/</a>
<i>Siphona hokkaidensis</i>	HM431957	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/HM431957">https://www.ncbi.nlm.nih.gov/nuccore/HM431957</a>
<i>Siphona sonorensis</i>	JF871072	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JF871072">https://www.ncbi.nlm.nih.gov/nuccore/JF871072</a>
<i>Siphona plusiae</i>	HM417418	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/HM417418">https://www.ncbi.nlm.nih.gov/nuccore/HM417418</a>
<i>Actia nr. cinerea</i>	JF271139	657 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JF271139">https://www.ncbi.nlm.nih.gov/nuccore/JF271139</a>
<i>Actia interrupta</i>	KR 395397	588 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KR 395397">https://www.ncbi.nlm.nih.gov/nuccore/KR 395397</a>
<i>Actia diffidens</i>	KR 394266	549 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KR 394266">https://www.ncbi.nlm.nih.gov/nuccore/KR 394266</a>
<i>Loewia sp.</i>	KR 393520	562 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KR 393520">https://www.ncbi.nlm.nih.gov/nuccore/KR 393520</a>
<i>Loewia foeda</i>	KR 667561	589 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KR 667561">https://www.ncbi.nlm.nih.gov/nuccore/KR 667561</a>
<i>Loewia erecta</i>	KX844484	630 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844484">https://www.ncbi.nlm.nih.gov/nuccore/KX844484</a>
<i>Loewia brevifrons</i>	KX844315	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844315">https://www.ncbi.nlm.nih.gov/nuccore/KX844315</a>

Ending of the table 1

Species	Accession number	Number of base pairs	Link (URL)
<i>Loewia adjuncta</i>	KX843701	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX843701">https://www.ncbi.nlm.nih.gov/nuccore/KX843701</a>
<i>Synactia parvula</i>	KX844364	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844364">https://www.ncbi.nlm.nih.gov/nuccore/KX844364</a>
<i>Bithia modesta</i>	MG968012	532 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/MG968012">https://www.ncbi.nlm.nih.gov/nuccore/MG968012</a>
<i>Bithia acanthophora</i>	KX844149	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844149">https://www.ncbi.nlm.nih.gov/nuccore/KX844149</a>
<i>Bithia spreta</i>	KX843739	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX843739">https://www.ncbi.nlm.nih.gov/nuccore/KX843739</a>
<i>Strongygaster celer</i>	KP046934	635 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KP046934">https://www.ncbi.nlm.nih.gov/nuccore/KP046934</a>
<i>Strongygaster sp.</i>	JF867537	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JF867537">https://www.ncbi.nlm.nih.gov/nuccore/JF867537</a>
<i>Strongygaster triangulifera</i>	HM412619	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/HM412619">https://www.ncbi.nlm.nih.gov/nuccore/HM412619</a>
<i>Nowickia alpina</i>	KX843975	590 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX843975">https://www.ncbi.nlm.nih.gov/nuccore/KX843975</a>
<i>Nowickia ferox</i>	KX844164	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844164">https://www.ncbi.nlm.nih.gov/nuccore/KX844164</a>
<i>Nowickia marklini</i>	MF836056	588 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/MF836056">https://www.ncbi.nlm.nih.gov/nuccore/MF836056</a>
<i>Nowickia sp.</i>	KM571428	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KM571428">https://www.ncbi.nlm.nih.gov/nuccore/KM571428</a>
<i>Dexiosoma caninum</i>	JN 310385	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/JN 310385">https://www.ncbi.nlm.nih.gov/nuccore/JN 310385</a>
<i>Microphthalma obsoleta</i>	HQ945084	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/HQ945084">https://www.ncbi.nlm.nih.gov/nuccore/HQ945084</a>
<i>Microphthalma disjuncta</i>	HQ583135	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/HQ583135">https://www.ncbi.nlm.nih.gov/nuccore/HQ583135</a>
<i>Microphthalma europaea</i>	KX844102	658 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX844102">https://www.ncbi.nlm.nih.gov/nuccore/KX844102</a>
<i>Calliphora vomitoria (outgroup)</i>	AY 536643	414 bp	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AY 536643">https://www.ncbi.nlm.nih.gov/nuccore/AY 536643</a>

## Results

This analysis includes 73 nucleotide sequences. 683 positions were the total of the final dataset. Phylogenetic trees were obtained by different methods (Maximum Likelihood «ML» and Neighbor Joining «NJ»).

The both analyses (ML and NJ) using COI gene showed that all subfamilies were polyparaphyletic except Phasiinae; most tribes used in this study (three from four tribes) were monophyletic; *Cylindromyiini* tribe and *Gymnosoma nudifrons* species were outside of the phasiin clade. Unlike all previous phylogenetic studies, we found that the Phasiinae were grouped as sisters to the Tachininae (in part). And all other subfamilies relationships contained diversity.

According to the ML analysis, dividing Tachinidae into two big clades was observed:

The first clade includes Tachininae (Siphonini + Nemoraeni + Macquartini + Tachinini) and Phasiinae (Phasiini + Gymnosomatini + (Leucostomatini + *Proopia crassiseta* (Aldrich & Webber, 1924))).

The second clade includes the subfamilies (remaining Tachininae, Dexiinae and Exoristinae) in addition to genus *Cylindromyia* Meigen, 1803 and species *Gymnosoma nudifrons*.

The first clade is divided into two groups:

1) Siphonini ((*Peribaea* Robineau-Desvoidy, 1863 + *Goniocera* Brauer & Bergenstamm, 1891) + *Siphona* Meigen, 1803);

2) Siphonini ((*Peribaea* + *Goniocera*) + *Siphona*) are monophyletic group except *Actia cinerea* (Macquart, 1834) and *Peribaea tibialis* (Robineau-Desvoidy, 1851), where the first is clustered with Exoristinae genera (BACTROMYIA Brauer & Bergenstamm, 1891 and *Admontia* Brauer & Bergenstamm, 1889) and Dexiinae, while the second is placed as a sister to *Nemoraea pellucida* (Meigen, 1824).

Phasiinae is divided into two groups:

The first genus composed of *Leucostoma* (Leucostomatini) + the exoristin species *Proopia crassiseta* (Erycini) that is originated within *Phasiinae*.

The second includes *Ectophasia crassipennis* (Fabricius, 1794) (Gymnosomatini) + genus *Phasia* Latreille, 1804 (Phasini).

Phasiinae are represented here by 5 genera (4 tribes), 3 tribes (Phasiini, Leucostomatini and Gymnosomatini by genus *Ectophasia* Townsend, 1912) clustered as monophyletic group. While the *Cylindromyia* (Cylindromyiini) is placed as a sister with (*Synactia* Villeneuve, 1915 and *Loewia* Egger, 1856) and *Gymnosoma* Meigen, 1803 is sister with Exoristinae (*Admontia* Brauer & Bergenstamm, 1889 and *Elodia* Robineau-Desvoidy, 1863). Genus *Phasia* (Phasini), *Leucostoma* Meigen, 1803 (Leucostomatini) and *Cylindromyia* (Cylindromyiini) are recovered (reconstructed) as monophyletic groups.

Tachininae (part) include:

1) Group genus *Nemoraea* (Nemoraeni) + Siphonin species *Peribaea tibialis*.

2) Group *Nowickia* Wachtl, 1894 (Tachinini) + *Macquartia* Robineau-Desvoidy, 1830 (Macquartini).

The genera *Macquartia* and *Nowickia* are weakly supported as sisters (29 %) and this cluster is placed as a sister to (*Nemoraea pellucida*+ *Peribaea tibialis*).

The second variable clade splits into two groups.

The first group includes two assemblages:

*Strongygaster* Macquart, 1834 (Strongegasterini) + ((*Gymnosoma nudifrons* (Phasiinae) + *Admontia blanda* (Fallén, 1820)) + *Admontia egeerioides* (Coquillett, 1895)) + *Elodia ambulatoria* (Meigen, 1824)) the latter three Exoristinae.

Tachininae (part) (*Mintho* Robineau-Desvoidy, 1830 (Minthoini)) + (*Bithia* Robineau-Desvoidy, 1863 (Leskini)), where *Estheria petiolata* (Bonsdorff, 1866) (Dexiinae) and *Voria erasmocoronadoi* Fleming & Wood, 2017 (Dexiinae) are sisters to *Bithia*.

*Gymnosoma nudifrons* (Phasiinae) (this species is a sister with *Admontia blanda*) is originated within the exoristin two genera *Admontia* and *Elodia* and both (*Gymnosoma*

*nudifrons*, *Admontia* and *Elodia*) are clustered as a sister group to *Strongegaster* (*Strongegasterini*) that is placed in unstable subfamily, where morphologically in our study was a sister with Phasiinae as in Cerreti 2014. While that in [3] was positioned within Tachininae.

The Tachininae genus *Mintho rufiventris* (Fallén, 1817) is a sister with the cluster [*Voria erasmocoronadoi* (*Voriini*) + (*Estheria petiolata* (*Dexiini*) + *Bithia*)].

The second group is composed of:

A- [*Cylindromyia* (*Phasiinae*) + (*Loewia* (*Loewini*) + *Synactia* (*Ernestini*))] latter two belong to Tachininae) and cluster is a sister to the next assemblage.

B- The two species of *Thelaira* Robineau-Desvoidy, 1830 (*vorini*, *Dexiinae*) as a sister to the following two clades:

First: *Thelaira americana* Brooks, 1945 sister to [(*Voria* sp. + *Athrycia cinerea* (*Coquillett*, 1895)) (*vorini*, *Dexiinae*) + [(*Bactromyia aurulenta* (*Meigen*, 1824) + *Admontia grandiconis* (*Zetterstedt*, 1849) (the two *Exoristinae*)) + (*Nowickia marklini* *Zetterstedt*, 1838 + *Actia cinerea*)]].

Second: [*Microphthalma europaea* Egger, 1860 + (*Microphthalma* *Macquart*, 1844 (remaining species) + *Dexiosoma*)] this clade belongs to tribe Megaprosopini (*Tachininae*).

*Cylindromyia* (*Cylindromyini*) is a sister with the two genera *Loewia* and *Synactia* belonging to subfamily Tachininae. And, this assemblage is a sister to *Dexiinae*, *Exoristinae* and *Tachininae* taxa.

*Voriini* tribe taxa, *Thelaira Americana* sister to (*Voria* sp. + *Athrycia cineria*) are clustered in one group with the two *Exoristinae* species (*Bactromyia aurulenta* + *Admontia grandiconis*) and (*Bactromyia aurulenta* (*Eryciini*) + *Admontia grandiconis* (*Blondellini*)) and *Tachininae* species (*Nowickia marklini* (*Tachinini*) + *Actia cinerea* (*Siphonini*)).

In spite of *Voriini* was clustered in one clade (first clade), it is not monophyletic group (graduated), where *Thelaira solivaga* (*Harris*, 1780) and *Thelaira nigripes* (*Fabricius*, 1794) are sisters with remaining *vorini*, *Exoristinae* and *Tachininae* taxa and *Voria erasmocoronadoi* are placed in another clade.

The tribe Megaprosopini is moderately supported (72 %) as monophyletic and represented here by two genera *Microphthalma* and *Dexiosoma* *Rondani*, 1856, where *Microphthalma europaea* is a sister with *Microphthalma* (remaining species) + *Dexiosoma caninum* (*Fabricius*, 1781), where *Microphthalma* is not clustered as monophyletic group.

NJ analysis method showed that the cluster [*Siphonini* + (*Mintho rufiventris* + (*Admontia* + *Gymnosoma nudifrons*))] is a sister to all rest of Tachinidae. *Strongygaster* + (*Thelaria* and *Elodia*) are grouped as a sister with Phasiinae and both are clustered as a sister group with the variable clade [*Tachininae* (*Ermitini*, *Loewiini*, *Megaprosopini*, *Actia cinerea* and *Nowickia marklini*)], [*Exoristinae* (*Bactromyia aurulenta* and *Admontia grandiconis*)], [*Dexiinae* (*Athrycia cinerea*, *Thelaira americana* and *Voria* sp.)] and [genus *Cylindromyia*]. This big group is placed as a sister with the remaining *Tachininae* where the two *Dexiinae* species (*Estheria petiolata* and *Voria erasmocoronadoi*) are originated within it.



## Discussion

It has long been proposed that the Tachinidae are a monophyletic clade depending on larval and adult characters [2, 9] and the current molecular studies nearly entirely reported this view [3, 5, 10, 15]. However, the works so far contain few outgroup taxa [4], unable to solve this issue.

The big split of subfamilies in recent studies grouping Phasiinae + Dexiinae and Exoristinae + Tachininae was strongly supported whether using morphological data in [2] or molecular by [3, 10, 15]. This hypothesis, first proposed by [10], reports other one differs from previous that clustered subfamilies containing (Phasiinae + Exoristinae) and (Dexiinae + Tachininae, [11]).

Our (molecular) dendrograms (figs. 2 and 3 show that all the subfamilies are poly paraphyletic except Phasiinae that is grouped as monophyletic group and confirmed by [2] and molecular by [1, 3, 10], exclusive placement of *Cylindromyia* (Fig. 2) is positioned as a sister to Tachininae taxa, weakly supported (17 % ML; 20 % NJ) and agrees with [10] in outside of this genus from Phasiinae. It is reported in [10] that the suggestion that *Cylindromyini* may not be included in Phasiinae, or that the subfamily itself may not be monophyletic group is novel. However, using greater taxon sampling in [2, 3, 15] revealed the monophyly of Phasiinae with *Cylindromyini*.

A potential solution would be to split the subfamily Phasiinae along these statistical lines and create a monophyletic Phasiinae and a new monophyletic subfamily *Cylindromyinae*. However, *Cylindromyini* shares many traits with Dexiinae and several dexiines have historically been placed in *Cylindromyini* (notably *Epigrimyia* Townsend, 1891 and *Beskia* Brauer & Bergenstamm, 1889). Before *Cylindromyini* can be elevated to subfamily status, the status of Dexiinae and its relationship to Phasiinae needs to be solidified [15].

We placed the enigmatic genus *Strongygaster* (clustered with *Thelaria* and *Elodia*) with very weak confidence as a sister to Phasiinae (NJ analysis) and originated within Tachininae and Exoristinae (ML analysis), where it had been previously placed by [13] and others [3, 15].

However, some authors [3, 14], reported *Strongygastrini* to be joined with Tachininae, *Strongygaster* is apparently similar to *Phasia* and some other phasiines, with large compound eyes and few bristles, but the biology does not couple with Phasiinae, and phallic reduction increases the confusion relationships. The morphological and molecular phylogenetic analyses [2, 15] respectively, has also been supported the position of *Strongygaster* within Phasiinae that correspond with our morphological study (fig. 1).

Relationships among the Dexiinae are among the most poorly supported in our analyses. In analyses of less informative COI gene, the few included dexiine taxa in ML are grouped in a cluster as sisters to each other and with other Tachininae and Exoristinae taxa, but not in monophyletic clade. In addition, *Estheria petiolata* and *Voria erasmocoronadoi* are clustered as sisters out of this clade. While in NJ they are widely dispersed across the tree and in both cases the relationships among them (Dexiinae taxa) are unclear. This result about Dexiinae relationships also was observed by [8] using the same marker COI but a very few Tachinidae taxa were used. However, in [3] the Dexiinae comprised a well-supported monophyletic Dexiinae, relationships among tribes within the subfamily exhibited more uncertainty.



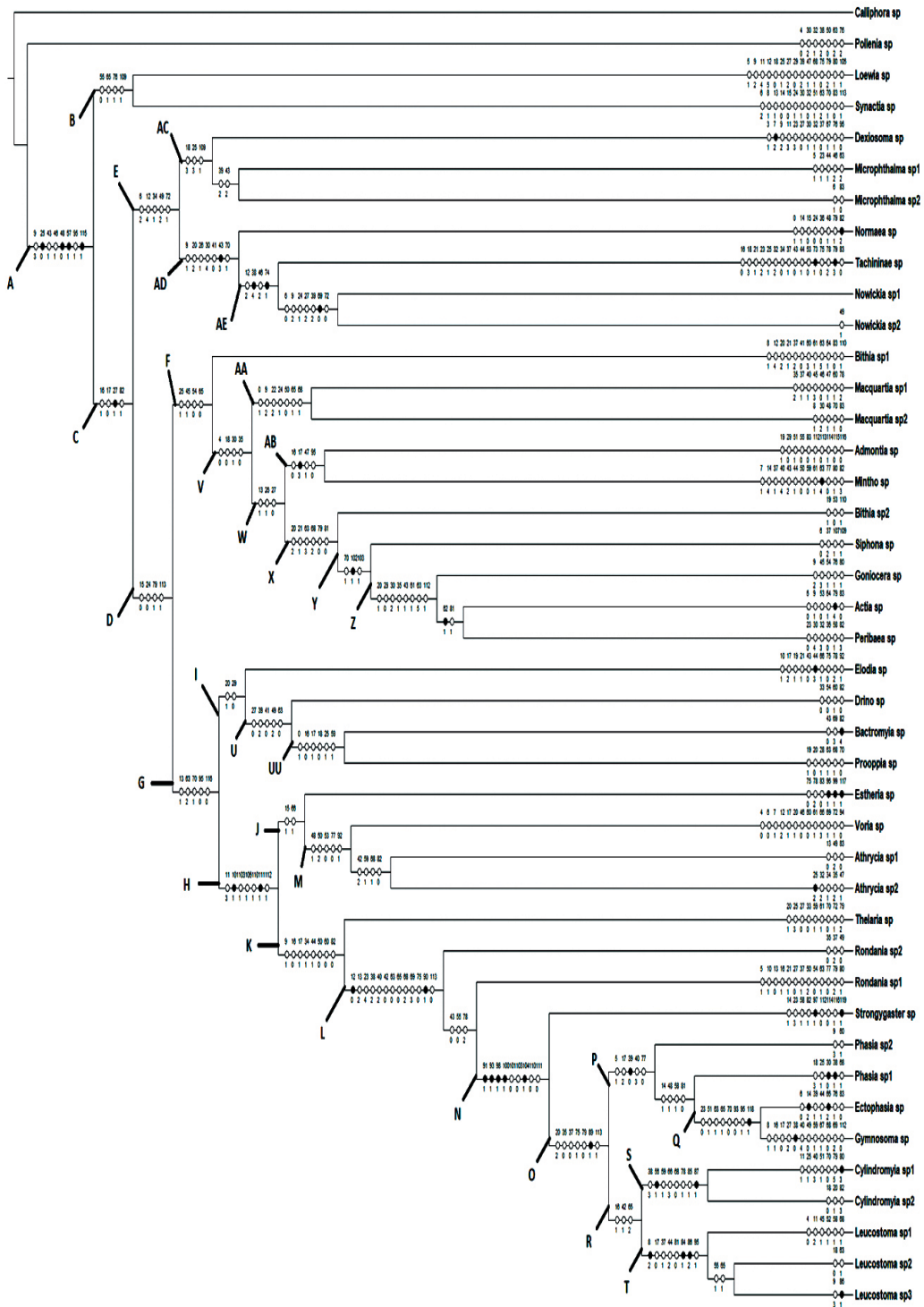
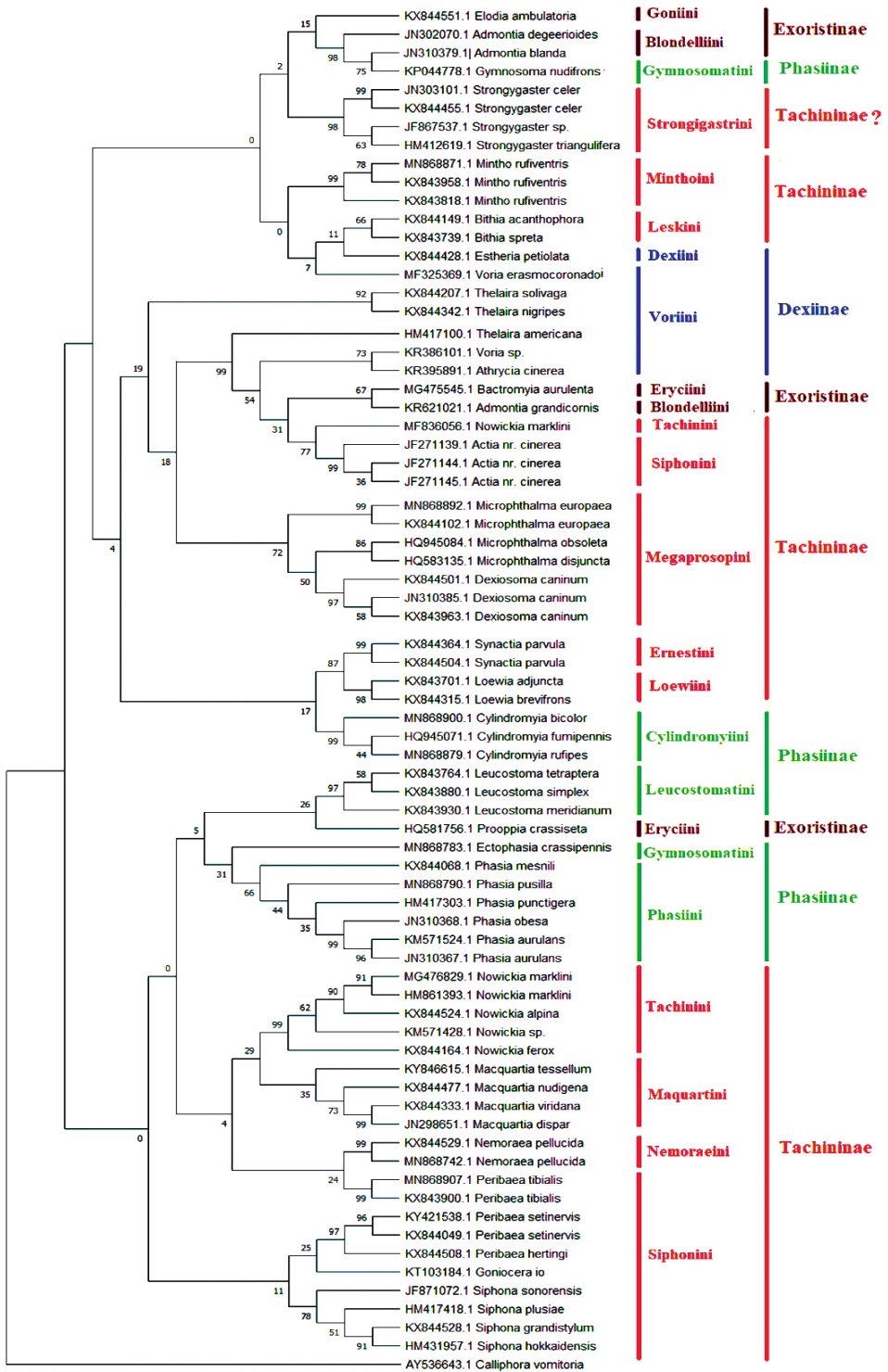


Fig. 1. Dendrogram was constructed using the Maximum Parsimony (MP) method. *Pollenia* sp. and *Calliphora* sp were used as out groups. On the branches – white circles are homoplastic (plesiomorphic) characters, and black circles are autapomorphic characters



**Fig. 2.** Maximum likelihood phylogeny based on COI gene analysis. ML bootstrap values are shown above

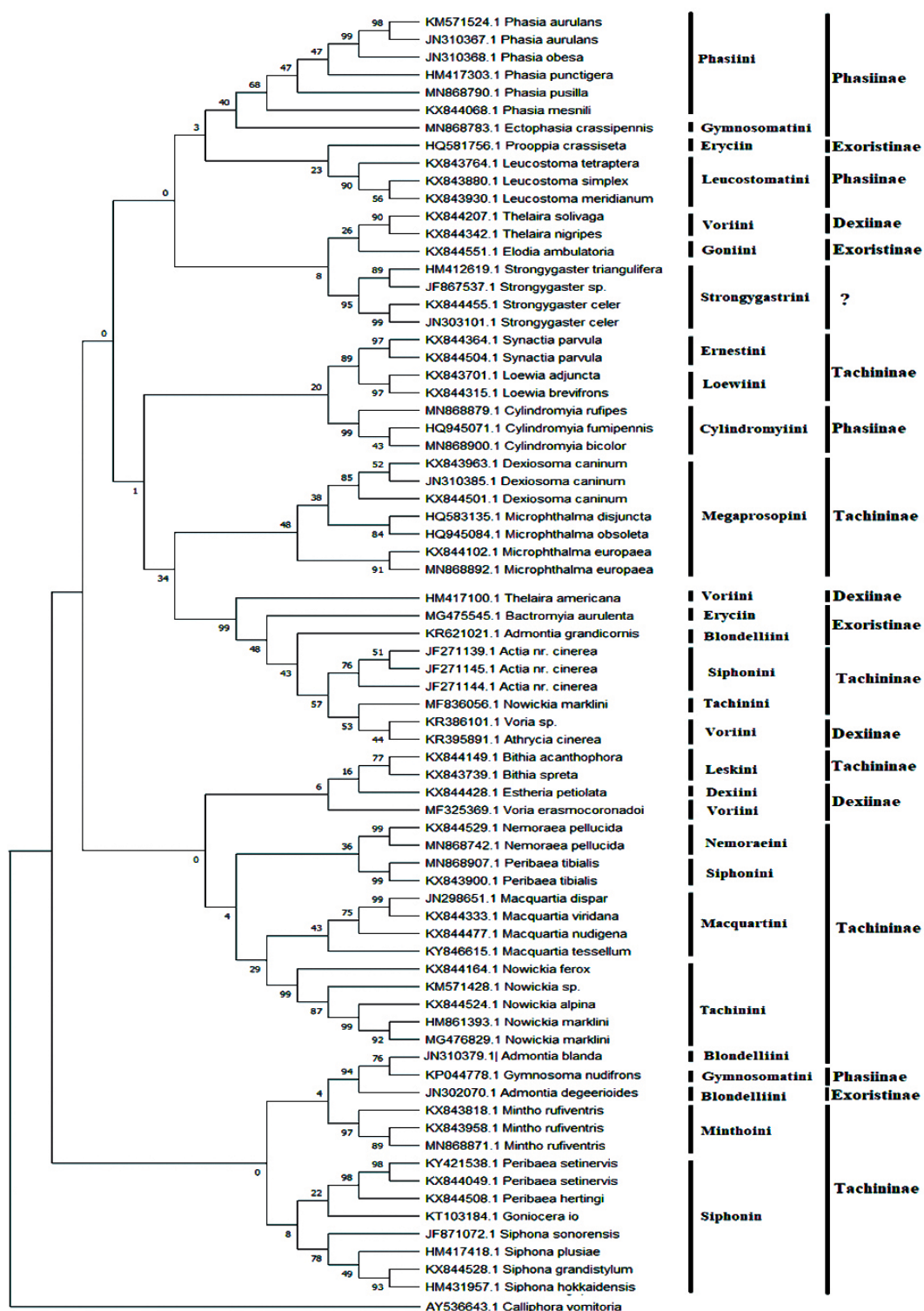


Fig. 3. The evolutionary history was reconstructed using the Neighbor-Joining method

Monophyly of the subfamily Exoristinae has been supported by all six major phylogenetic analyses of Tachinidae [2–5, 15], and it is supported here as well (*in our morphological tree*), but it was not supported in our molecular analysis, where Exoristinae taxa are scattered on the tree. *Prooppia* Townsend, 1926, is within Phasiinae, while *Bacteromyia aurulenta* and *Admontia grandicornis* are originated within tachinin and dexiini taxa. *Admontia* (remaining species) and *Elodia ambulatoria* are sisters with *Strongygaster*.

Tachininae are the most morphologically heterogeneous subfamily of Tachinidae, lacking clear morphological synapomorphies [12]. In the recent morphological analysis, [2] found the Tachininae to be polyphyletic, with clades of Myiophasiini + Palpostomatini, Macquartini and Ormiini and in molecular [3] Myiophasiini + Macquartini (none of which is represented here except *Macquartia*) were placed at the base of Tachinidae, and the subfamily being paraphyletic with respect to the Exoristinae as in our study, Tachininae is a paraphyletic group and scattered along the tree. Here, some Tachininae (Tachinini, Macquartini, Nemoraeni and Siphonini) are sisters with Phasiinae clade, while Ernestini and Loewini are sisters with Cylindromyini. In addition, Megaprosopini is a sister with *Actia cinerea* and *Nowickia marklini* with Dexiinae taxa. Moreover, Minthiini and Leskini are sisters with Dexiinae and Exoristinae. The Monophyly of Siphonini was supported in previous studies [2, 3], and it is in agreement with our study but in our analysis there is exception, where genus *Actia* and *Peribia tibialis* are out of this tribe.

In the morphological and molecular analyses (figs. 2 and 3, the relationships of some Tachininae taxa are nearly similar as following:

*Macquartia* (Macquartini) is a sister to *Nowickia* (Tachini) and both are clustered with *Nemoraea* (Nemoraeni).

Morphologically, *Loewia* and *Synactia* are positioned in the base of the tree, where, in contrast to molecular analysis, but with the same relationship, they are grouped as sisters.

In addition, Monophyly of Megaprosopini (*Microphthalma* and *Dexiosoma*) is moderately supported in both analyses. Moreover, *Mintho-leskiine* is clustered in ML (COI) and morphological analyses but it is dispersed (separated) in NJ analysis.

## Conclusion

We can conclude that additional taxa sampling and using informative markers (genes) will be necessary to resolve some questionable taxa specially the higher taxa.

However, we used few taxa for each genus and each tribe, but observed that using COI gene does not have ability to recover (reveal) the subfamilies as monophyletic and reveal its problems. While, the same marker (COI) revealed that the most of the genera and tribes are monophyletic groups:

**Tribes:** Siphonini, Megaprosopini, Strongygasterini, Leucostomatini, Macquartini, Tachinini (*Nowickia*), Phasiini, Cylindromyini.

**Genera:** *Macquartia*, *Nowickia*, *Siphona*, *Peribaea*, *Leucostoma*, *Cylindromyia*, *Phasia*, *Microphthalma*, *Strongygaster*, *Admontia*, *Bithia*, *Loewia*.

COI gene used in the study was observed to be very poor informative for solving (could not solve) relationships in the higher taxonomic tachinid taxa.

## References / Библиографический список

1. Stireman III JO, O'Hara JE, Wood DM. Tachinidae: evolution, behavior, and ecology. *Annual Review of Entomology*. 2006; 51(1):525—555.
2. Cerretti PO, O'Hara JE, Wood DM, Shima H, Inclan DJ, Stireman III JO. Signal through the noise? Phylogeny of the Tachinidae (Diptera) as inferred from morphological evidence. *Systematic Entomology*. 2014; 39(2):335—353. doi: 10.1111/syen.12062
3. Stireman III JO, Cerretti P, O'Hara JE, Blaschke JD, Moulton JK. Molecular phylogeny and evolution of world Tachinidae (Diptera). *Systematic Entomology*. 2019; 139:106358. doi: 10.1016/j.ympev.2018.12.002
4. Stireman III JO. Phylogenetic relationships of tachinid flies in subfamily Exoristinae (Tachinidae: Diptera) based on 28s rDNA and elongation factor-1 $\alpha$ . *Systematic Entomology*. 2002; 27(4):409—435. doi: 10.1046/j.1365-3113.2002.00187.x
5. Tachi T, Shima H. Molecular phylogeny of the subfamily Exoristinae (Diptera, Tachinidae), with discussions on the evolutionary history of female oviposition strategy. *Systematic Entomology*. 2010; 35(1):148—163. doi: 10.1111/j.1365-3113.2009.00497.x
6. Wood DM. Tachinidae. In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM. (eds.) *Manual of Nearctic Diptera. Volume 2*. Ottawa, Canada; 1987. p.1193—1269.
7. Tschorsnig HP, Richter VA. Family Tachinidae. In: Papp L, Darvas B. (eds.) *Contributions to a manual of Palaearctic Diptera (with special reference to flies of economic importance). Volume 3. Higher Brachycera*. Budapest: Science Herald; 1998. p.691—827.
8. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*. 2018; 35(6):1547—1549. doi: 10.1093/molbev/msy096
9. McAlpine JF. Phylogeny and classification of the Muscomorpha. In: McAlpine JF, Wood DM (Eds) *Manual of Nearctic Diptera. Volume 3. Agriculture Canada Monograph 32*; 1989. Pp. 1397—1518, 1333—1581.
10. Winkler IS, Blaschke JD, Davis DJ, Stireman III JO, O'Hara JE, Cerretti P, Moulton JK. Explosive radiation or uninformative genes? Origin and early diversification of tachinid flies (Diptera: Tachinidae). *Molecular Phylogenetics and Evolution*. 2015; 88:38—54. doi: 10.1016/j.ympev.2015.03.021
11. Shima H. Parasitic way of life in tachinid flies. *Insectarium*. 1989; 26:4—9, 46—51, 88—94, 120—126. (In Japanese)
12. Mesnil LP. 64g. Larvaevorinae (Tachininae). In: *Die Fliegen der Palaearktischen Region*. 1966; 10:881—928.
13. Herting B. Catalogue of Palearctic Tachinidae (Diptera). In: *Stuttgarter Beiträge zur Naturkunde. Serie A (Biologie)*. 1984.
14. O'Hara JE, Wood DM. Catalogue of the Tachinidae (Diptera) of America north of Mexico. In: *Memoirs on Entomology, International Volume 18*. 2004.
15. Blaschke JS, Stireman III JO, O'Hara JE, Cerretti P, Moulton JK. Molecular phylogenetics and piercer evolution in the bug-killing flies (Diptera: Tachinidae: Phasiinae). *Systematic Entomology*. 2018; 43(1):218—238. doi: 10.1111/syen.12272

## Анализ молекулярных данных паразитоидных мух семейства Tachinidae

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**Аннотация.** Паразитоиды семейства Tachinidae играют важную роль в биологическом контроле, тем не менее филогенетические взаимоотношения надродовых групп мало изучены. Здесь мы представляем



филогенетический анализ семейства, основанный на молекулярных данных. Филогенетический анализ в молекулярном исследовании, включающем 73 вида, принадлежащих к 30 родам тахинид, включая четыре признанных в настоящее время подсемейства (*Dexiinae*, *Exoristinae*, *Phasiinae*, *Tachininae*) и 20 триб. Тахиниды реконструируются как монофилетический комплекс на основании морфологических данных и с четырьмя негомозиготными апоморфиями (синапоморфиями). Монофилия хорошо поддерживается начальной загрузкой. Наш морфологический анализ в целом поддерживает подсемейство, объединяющее *Dexiinae* + *Phasiinae*, в то время как *Tachininae* + *Exoristinae* не поддерживается как одна группа, и только *Exoristinae* и *Phasiinae* реконструируются как монофилетические сообщества. *Dexiinae*, которые ранее считались устойчивым монофилетическим сообществом (за исключением нескольких исследований), реконструированы как полипарафилетические по отношению к *Phasiinae*. *Tachininae* реконструированы как парафилетический класс, в то время как монофилия *Exoristinae* была восстановлена, за исключением рода *Admontia* Brauer & Bergenstamm, который возник в подсемействе *Tachininae*. В отличие от молекулярного анализа все подсемейства представляют собой полипарафилетические группы, в которых они взаимодействуют друг с другом, за исключением *Phasiinae*, которое включает большинство своих таксонов в монофилетическую группу.

**Ключевые слова:** анализ, молекулярный, морфология, тахиниды

**Конфликт интересов:** Автор заявляет об отсутствии конфликта интересов.

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