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NEW RECORDS OF EMPIDIDAE AND HYBOTIDAE (INSECTA: DIPTERA) FROM HYRCANIAN FORESTS IN IRAN AND AZERBAIJAN

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Summary. The genus *Dolichocephala* Macquart, 1823 of the family Empididae is recorded for the first time from Iran and Azerbaijan, including one species *D. monae* Joost, 1981. A neighbour-joining tree based on genetic divergences on the CO1 gene within nine species of *Dolichocephala* is provided. In addition, the genus *Oropeza* Collin, 1926 of the family Hybotidae (one species *O. sphenoptera* (Loew, 1873)) and two species of the subgenus *Empis* s. str. of the genus *Empis* Linnaeus, 1758 (*E. (E.) earina* Collin, 1960 and *E. (E.) temryukiensis* Kustov et Shamshev, 2013) of the family Empididae are reported for the first time from the territory of Iran. The female of *E. (E.) temryukiensis* is described for the first time.

Key words: Diptera, Empididae, Hybotidae, barcoding, fauna, new records, Hyrcanian forests, Iran, Azerbaijan.

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Резюме. Род *Dolichocephala* Macquart, 1823 семейства Empididae впервые указывается из Ирана и Азербайджана, включая один вид *D. monae* Joost, 1981. Обобщены данные по сиквенсам гена CO1 девяти видов рода *Dolichocephala*. Кроме того, род *Oropeza* Collin, 1926 семейства Hybotidae (один вид *O. sphenoptera* (Loew, 1873)) и два вида подрода *Empis* s. str. рода *Empis* Linnaeus, 1758 (*E. (E.) earina* Collin, 1960 и *E. (E.) temryukiensis* Kustov et Shamshev, 2013) семейства Empididae впервые найдены на территории Ирана. Впервые описана самка *E. (E.) temryukiensis*.

INTRODUCTION

The families Empididae and Hybotidae include around 4000 and 2000 described species worldwide (Yang *et al.*, 2007). Yet, these flies remain poorly studied in Central Asia. For

instance, only 23 species of Empididae and Hybotidae have been recorded from Iran (Daugeron, 2000; Shamshev & Grootaert, 2005; Raffone, 2007; Kazerani *et al.*, 2014), while 141 species of these families were recorded in Turkey (Özgül & Civelek, 2013; Barták & Kubík, 2018).

The Hyrcanian region is a mountainous humid zone in northern Iran and southern Azerbaijan, ranging from -20 meters in the southern coastal plain of the Caspian Sea to 2500 meters (m.a.s.l.) on the northern slopes of the Alborz Mountains. This region is covered with temperate deciduous forests, dominated by *Fagus orientalis* above 1500 m a.s.l. The fauna of Empididae and Hybotidae in the Hyrcanian region remains almost unknown. Our paper includes new records of empidoidean taxa in a Hyrcanian temperate rain forest in Iran and Azerbaijan.

MATERIAL AND METHODS

Our study is based on material collected in the Hyrcanian forests during a project on saproxylic Diptera in Iran (Gilan, Mazandaran and Golestan) (Fig. 1) and on the Empididae collections (material from Azerbaijan) of the Zoological Museum of Moscow University, Moscow (ZMUM), and the Zoological Institute of the Russian Academy of Sciences, St. Petersburg (ZISP).

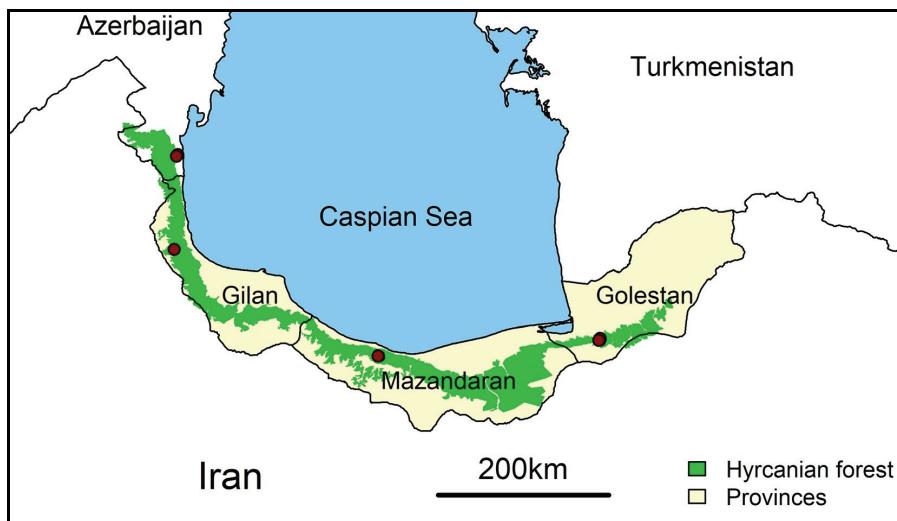


Fig. 1. Map of collecting sites where the Empidoidea species found in Hyrcanian Forests.

Specimens were collected during 2017–2018 with pan traps (filled with water and 1–2 drop dishwashing) and sweep-netting and preserved in 75% ethanol in glass vials. Pinned specimens of holotype and some paratypes are deposited in Arthropods Collection of Forests and Rangelands of Iran (ACFRI). Specimens deposited in ZISP and ZMUM were collected by sweep-netting. To facilitate determination, the terminalia were macerated in cold 10% KOH, then put for a short period in 85% lactic acid and immersed in glycerine. Terms used for adult structures primarily follow those summarised by Cumming & Wood (2009). The photos were produced using a Nikon SMZ25 stereomicroscope equipped with a Sony TM digital camera. The repository of each type is given in parentheses.

Genomic DNA was extracted using a single leg removed using sterile forceps following the Qiagen DNEasy Tissue Protocol (Qiagen, Hilden, Germany) using the manufacturer's instructions. We obtained COI barcode sequences of *ca.* 500 bp lengths from the collected specimen. The mitochondrial cytochrome oxidase 1 gene (CO1-5P-region) was amplified using LCO1490 and HCO2198 primers (Folmer *et al.*, 1994 as described in Su *et al.* (2008). Evolutionary distances were computed using p-distance pairwise deletion method (Nei & Kumar, 2000). Intra- and interspecific distances were calculated in MEGA X (Kumar *et al.*, 2018). The molecular data, corresponding sequence chromatograms and specimen metadata underpinning the analyses reported in this paper, are deposited in the BOLD database (www.boldsystems.org – Ratnasingham & Hebert, 2007) under the DOI: [dx.doi.org/10.5883/DS-DOLIWAG](https://doi.org/10.5883/DS-DOLIWAG).

RESULTS

Order Diptera Linnaeus, 1758

Superfamily Empidoidea Latreille, 1804

Family Empididae Latreille, 1804

Subfamily Clinocerinae Collin, 1928

Genus *Dolichocephala* Macquart, 1823

REMARKS. *Dolichocephala* includes 57 described species distributed almost worldwide, except southern South America, Australia, and New Zealand (Sinclair & Plant, 2017). Eleven species are known in the Afrotropics, 19 – in the Palaearctic, 20 – in the Oriental, 2 – in the Australasia and 7 – in the Nearctic (Liu *et al.*, 2014; Yang *et al.*, 2007; Sinclair & Plant, 2017). Members of the genus *Dolichocephala* are characterized by a narrow stalk connecting head and thorax, the position of occiput in relation to head, usually white spots or irrorations on the wings, and a distinct lobe beyond the clasping cercus in which the subepandrial sclerite of the male genitalia is usually extended (Sinclair & Evenhuis, 2005).

Dolichocephala monae Joost, 1981

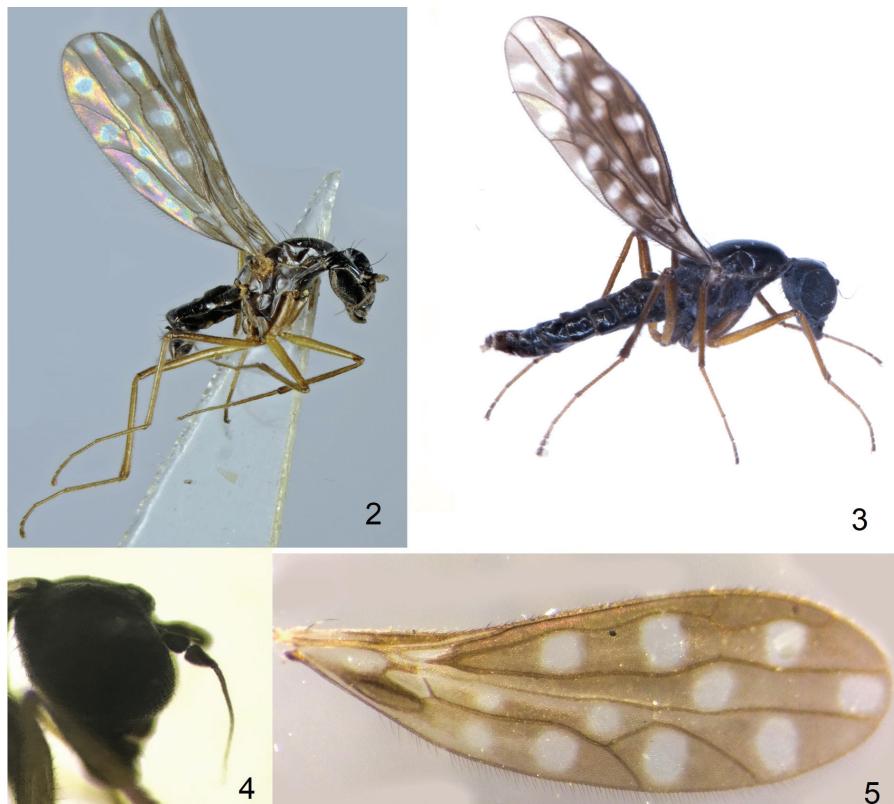
Figs 2–5

MATERIAL EXAMINED. **Iran:** Golestan province [Shast-Kola forests], 36°43'10" N, 54°24' 17" E, 1320 m, sweeping net, 10.VI 2017, 3♂, leg. F. Kazerani [ACFRI]; [Shast-Kola forests], 36°43'00.7" N 54°23'13.7" E, 1271 m, pan traps, 12.VII 2017, 6♀, leg. F. Kazerani [ACFRI]; Gilan province [Shafarood forests], 37°40'15.1"N, 48°45'09.6"E, 1138 m, pan traps, 15.VII 2018, 1♀, leg. F. Kazerani [ACFRI]. **Azerbaijan:** Lankaran [Khanbulan], 38°40' N 48°48' E, 20.V 2008, 1♂, leg. N. Vikhrev [ZMUM]; Hirkan-Burjeli env., 38°39' N 48°47' E, 15–22.V 2009, 1♀, leg. K. Tomkovich [ZMUM]; Hirkan-Burjeli env., 28.V 2009, 1♂, leg. K. Tomkovich [ZMUM]; Hirkan-Burjeli env., 15–18.V 2009, 6♂, 4♀, leg. I. Grichanov & K. Tomkovich [ZISP].

DIAGNOSIS. This is a dark species distinguished by extensively yellowish legs, brown halters and eleven round white wing spots. Male terminalia: hypandrium cone-shaped, with several short setae; phallus slender and sclerotized basally; epandrium rounded; clasping cercus deeply divided into two subequal in length processes; surstyli slender, tapered, apex pointed (Figs 2–5).

REMARKS. First record for Iran. Up to now, *Dolichocephala monae* was known after the type specimens only from Armenia (Joost, 1981; Sinclair & Shamshev, 2014).

In the collected specimens of *D. monae* from Hyrcanian forests, we faced a different pattern on the wing so that it has eleven spots and this is a fixed character in all collected specimens from Iran and Azerbaijan while in the original description mentioned wing with seven white rounded spots. However, the structures of the male terminalia, including the shape of the clasping cercus and the phallus, are quite identical to the holotype (R. Wagner, pers. comm.). The wing pattern of *D. monae* is variable in specimens collected from Georgia (B.J. Sinclair, pers. comm.). Therefore, we considered that the above-mentioned specimens belong to *D. monae*.



Figs 2–5. Habitus and wing images of *Dolichocephala monae*. 2 – male, lateral view; 3 – female, lateral view; 4 – male head, lateral view; 5 – wing.

In Europe, based on the species collected in emergence traps placed near water and wet areas, it is supposed that larva of the genus *Dolichocephala* are aquatic (Wagner & Gathmann, 1996; Meyer & Filipinski 1998; Sinclair & MacDonald, 2012; Wolton *et al.*, 2014).

DISTRIBUTION. Palaearctic: Armenia, Azerbaijan, Georgia, Iran.

MOLECULAR DELINEATION WITHIN *DOLICHOCEPHALA*. The barcodes obtained during this study were used to calculate the evolutionary distances, shown on a neighbour-joining tree (Fig. 6) which comprises seven well-separated clusters, representing nine species

of *Dolichocephala*. Comparison of the CO1 DNA barcode data, as well as p-distances to conspecifics, revealed a mean interspecific distance of 7.9% between *D. monae* and *D. thomasi* (Fig. 6). The latter species has wing with ten white rounded spots and clearly different structure of the male terminalia (Wagner, 1983). The COI barcode data showed slight difference between *D. guttata* and *D. oblongoguttata*. These species are distinguishable only by details of the male terminalia (Drake & Chandler, 1997).

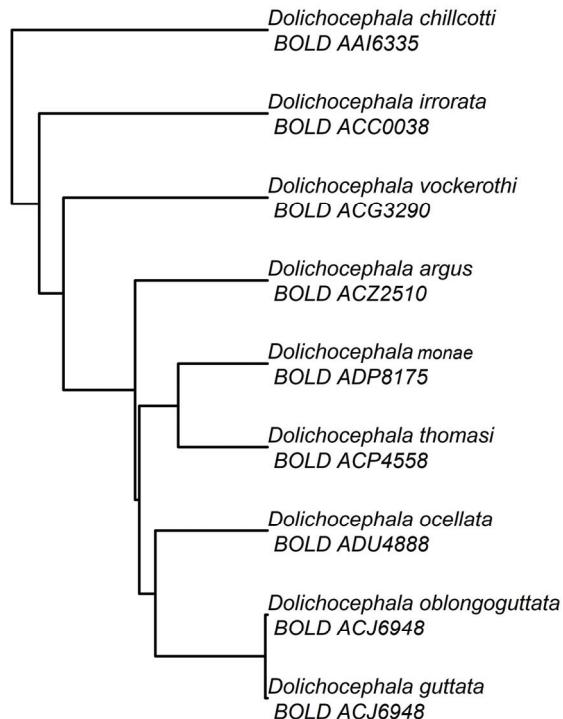


Fig. 6. Neighbor-joining tree based on genetic divergences on the CO1 gene within the genus *Dolichocephala* Macquart created in BOLD. Corresponding BIN (Barcode Index Numbers – www.boldsystems.org) are highlighted within species names.

Subfamily Empidinae Schiner, 1862

Genus *Empis* Linnaeus, 1758

REMARKS. The genus *Empis* remains purely studied on the territory of Iran. Kazerani *et al.* (2014) provided the first survey of the group including 13 species from different subgenera, except *Empis* s. str. In the Palaearctic, the nominate subgenus of the genus *Empis* includes about 130 species known mostly from Europe and the Mediterranean region. However, up to now, only one species, *E. (E.) eupeza* Loew, 1874, was recorded from Iran (described from “Astrabad”, now Gorgan). Two additional species are recorded herein. It is evident that the subgenus is much more diverse on the territory of Iran.

***Empis (Empis) earina* Collin, 1960**

MATERIAL EXAMINED. **Iran:** Tabriz, 23.IV 1914, 2♂, leg. Andriewsky [ZMUM]; the same locality, 05.V 1914, 6♂, 4♀, leg. Andriewsky [ZMUM].

DISTRIBUTION. Palaearctic: Jordan, Iran, Israel.

REMARKS. First record for Iran. Up to now, *E. earina* was known only after the type specimens taken from Jordan and Israel (Collin, 1960). According to label data, the species was recorded in February – March in Jordan and Israel as well as at the end of April and the beginning of May in East Azerbaijan Province of Iran.

***Empis (Empis) temryukiensis* Kustov et Shamshev, 2013**

MATERIAL EXAMINED. **Iran:** Tabriz, 06.IV 1914, 5♂, leg. Andriewsky [ZMUM]; the same locality and collector, 05.IV 1914, 1♂, [ZMUM]; the same locality and collector, 23.IV 1914, 1♂, 1♀, [ZMUM].

DESCRIPTION. **Female** (hitherto unknown). Similar to male (Kustov & Shamshev, 2013), except as follows. Eyes dichoptic, with ommatidia equally small. Frons broad, densely greyish pollinose, bearing several marginal setulae; occiput and ocellar tubercle with stronger setae than in male. Legs somewhat shorter, more robust; fore and hind basitarsi slender, hind tibia almost uniformly slightly flattened (except extreme base). Fore tibia with somewhat flattened dorsal setae on about apical 2/3; fore tarsomeres with shorter setae of subapical circlet. Mid femur with long, pinnate, dorsal setae over entire length and similar anteroventral setae on about apical 2/3; mid tibia with short, somewhat flattened, anterodorsal setae almost over entire length (absent near base and apex) and similar ventral setae on about basal 1/3. Hind femur with pinnate, long anterodorsal and short posterodorsal setae almost over entire length (except short subapical portion) as well as with long pinnate anteroventral setae on about apical half; mid tibia with moderately long anterodorsal setae almost over entire length (except extreme base and apex) and similar setae on about basal half ventrally. Abdomen mostly covered with short setae; cercus long, slender, with minute setae.

REMARKS. First record for Iran. Up to now, *Empis temryukiensis* was known only after the type specimens taken from Taman Peninsula of Russia (Krasnodarsky Krai) (Kustov & Shamshev, 2013; Shamshev, 2016). According to label data, it is an early spring species recorded from the beginning of April till the beginning of May in Tabriz of Iran and at the end of April in environs of Temryuk of Russia.

DISTRIBUTION. Iran (East Azerbaijan Province), Russia (Krasnodarsky Krai).

Family Hybotidae Meigen, 1820

REMARKS. Only four species of Hybotidae were recorded from Iran: *Hybos vagans* Loew, 1874, *Ocydromia glabricula* (Fallén, 1816), *Platypalpus longiseta* (Zetterstedt, 1842), and *Tachydromia parva* Chvála, 1970 (Shamshev & Grootaert, 2005; Shamshev *et al.*, 2015).

Subfamily Ocydromiinae Schiner, 1862

Genus *Oropezella* Collin, 1926

***Oropezella sphenoptera* (Loew, 1873)**

MATERIAL EXAMINED. **Iran:** Mazandaran province [Kheirroud forests], 36°34'36.23" N, 51° 34'37.94" E, 722 m, sweeping net, 15.VII 2018, 1♂, leg. F. Kazerani. [ACFRI].

REMARKS. First record for Iran. Chvála & Vonicka (2008) mentioned *O. sphenoptera* is an abundant species in the lowlands and foothills, occurring for a long period from May to September in Central Europe. Hövemeyer & Schauermann (2002) during their studies on saproxylic diptera, found this species as a zoophagous on the dead beech wood in Gottingen.

DISTRIBUTION. Single Palaearctic species of the genus; widespread in Europe (except northern parts), known from North Africa (Algeria), recently recorded from Turkey (Barták & Kubík, 2018).

DISCUSSION

The flies of the families Empididae and Hybotidae are marked elements of the biota of many ecosystems. Adults of Empididae and Hybotidae are predators or flower visitors. However, the habits of immatures of these flies are poorly understood, the larvae of a few groups (e.g., the Empididae subfamilies Clinocerinae and Hemerodromiinae) are mainly aquatic (Dyte, 1967), while many other Empididae and, especially, Hybotidae are terrestrial, occurring in forests, grasslands and agricultural fields, or are associated with various semi-aquatic habitats (e.g., edges of ponds, bogs, marshes, fens, etc.) (Cumming & Sinclair, 2008). Recently there has been considerable interest in the diversity of saproxylic Empidoidea communities and their role in the ecology of forest ecosystems (Ulyshen, 2018; Cumming *et al.*, 2018). Cumming *et al.* (2018) stated that decaying wood found in deciduous temperate forests is an important ecological niche for the immature stages of Empidoidea.

It is obvious that Empididae and Hybotidae fauna is still poorly investigated in Iran and based on the rich fauna of Hyrcanian forests further studies are necessary. Also, our knowledge of Saproxylic diptera in Hyrcanian forests is in the early stages. This study adds to the overall knowledge of Empididae and Hybotidae fauna of Iran by expanding the number of recorded species, as well as with new distributional data. In the future, more specialized studies are likely to result in additional species being known.

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