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Molecular and morphological diversity of *Baetis braaschi* Zimmermann, 1980 (Ephemeroptera: Baetideae); with notes on species' description

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Abstract
Although mayflies are the key elements to assess the health value of freshwaters, their biodiversity in the Middle East, in particular in Iran, is poorly studied. *Baetis braaschi* belongs to the subgenus *Rhodobaetis* Jacob, 2003, with a wide distribution in Europe and Western Asia. The taxonomy of *B. braaschi* has always been source of disagreement as many species have so far been described by researchers and were synonymized later. The species, *Baetis braaschi*, is investigated here, using molecular and morphological data using collected specimens from different area in Iran and compared with its conspecific from Eastern Europe. Although Iranian specimens showed 99% molecular similarity with their conspecifics in Eastern Europe, e.g., Ukraine and Georgia, the morphological results showed that Iranian specimens of *Baetis braaschi* has significant morphological differences with conspecific specimens from Eastern Europe. Patterns of head, femora and size of the first and second teeth of mandibles in *B. braaschi* are varied from Europe to Iran.

Key words: mayflies, cox1, Iran, distribution, redescription

INTRODUCTION

Mayflies are aquatic insects belonging to the order Ephemeroptera; the order is one of the oldest orders of insects that emerged in the Carboniferous Period (Brittain, 1982). The order Ephemeroptera, as a diverse group, includes more than 3000 species, 400 genera, and 42 families (Barber-James et al., 2007; Gattolliat et al., 2012). High ecological sensitivity to freshwater pollutants, present the order Ephemeroptera as key element in health indicator of freshwaters (Gattolliat & Sartori, 2008; Resh & Unzicker, 1975). In contrast to Europe, the mayfly's fauna of the Middle East has been less studied, particularly in Iran (Bojková et al., 2018). Among identified mayflies from Iran, species of the genus *Baetis* are distributed in most rivers of Iran (Mohammadian, 2005). According to the recent checklist of mayflies of Iran, 46 species and 25 genera are present in the country, of which 12 nominal species belong to the genus *Baetis* (Bojková et al., 2018).

Baetis braaschi belongs to the subgenus *Rhodobaetis* Jacob, 2003, with a wide distribution in Europe and Western Asia (Soldán & Godunko, 2008). It is considered as a resistant species in unpleasant

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environment conditions (Sroka & Godunko, 2012). Taxonomy of the species has always been source of disagreement (e.g. Godunko et al., 2004; Sroka & Godunko, 2012). *Baetis braaschi* was first described by Zimmermann (1980), based on three larva specimens that found by Dietrich Braasch from the southern part of the Crimea (Ukraine) in 1970. In 1982, *Baetis stipposus* was described from Uzbekistan, Kazakhstan, Turkmenistan, and Tajikistan (Kluge, 1982). Novikova (1987) recorded *B. stipposus* from Ukraine and suggested the species *B. stipposus* is the junior synonym can be synonymized with *B. bisri* (Thomas & Dia, 1983) from southwestern Lebanon (Novikova, 1987). Godunko et al. (2004) redescribed the larvae, imago and subimago of *B. braaschi* using the specimens collected from Crimea and synonymized it with *B. stipposus*, giving priority to the first one. Sroka et al., (2012) studied intraspecific variability in a rich set of material of *B. braaschi* from the three distant regions (Crimean Peninsula, Eastern Ukraine and the Caucasus Mountains within Georgia) and a former study conducted by Kluge (1982). We studied *B. braaschi* in Iran and compare the molecular and morphological findings with the most recent revision by Sroka et al. (2012) and redescription by Godunko et al. (2004).

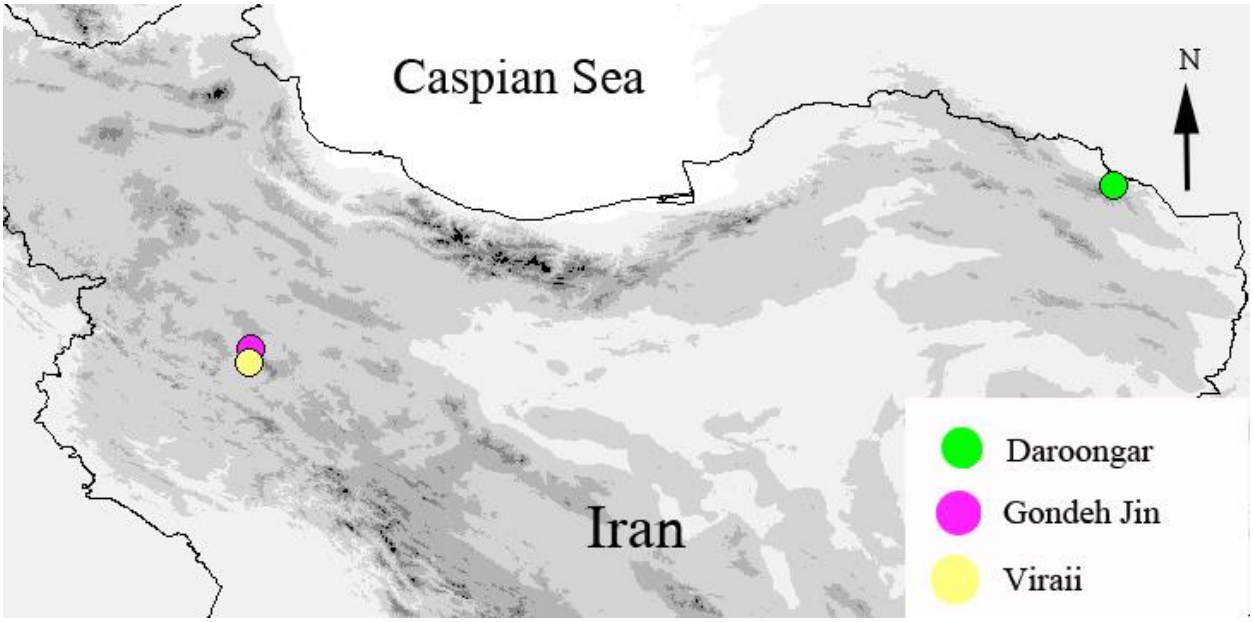

FIGURE 1. Sampling localities of *Baetis braaschi* in Iran.

MATERIAL AND METHODS

Material sampling and examination

Specimens were collected simply by hand from stony and/or sandy bottom of some selected rivers in different areas of Iran (Fig. 1). Details of sampling localities and number of specimens are provided herein: 1) **Iran**, *Khorasan Razavi Prov*. Dargaz City, Daroongar River (37°02'05"N 59°32'32"E); No. 12; 2); 5 November 2016; H. Malvandi leg. **Iran**, *Hamadan Prov*. Asadabad County, Viraii River (34°43'03"N 48°13'15"E). No. 11; and 3); 20 August 2018; A. Roohi Aminjan leg. **Iran**, *Hamadan Prov*. Bahar County, Gondeh Jin River (34°54'05"N 48°15'27"E); No. 7; 20; August 2018; A. Roohi Aminjan leg. Collected specimens were in larval stage and preserved in 80% ethanol.

Morphological study

Morphological characters were investigated in detail in lab under a stereomicroscope Olympus SZ30 and microscope Olympus BH2. Macrophotographs of larvae were taken using camera Nikon D3S. Characteristics of larvae were examined based on Sroka et al. (2012), Godunko et al. (2004), and Kluge (1982).

TABLE 1. List of DNA sequences of *B. braaschi* used for molecular analysis.

LOCALITY OF SEQUENCES	GENBANK ACCESSION NUMBER
GEORGIA	JN164278
	JN164285
	JN164295
	JN164282
	JN383392
	JN383391
	JN383390
	JN383389
	JN383388
	JN383387
	JN383386
UKRAINE	JN164280
	JN164283
	JN164284
	JN164281
	JN383383
	JN383384
IRAN	Pending

Molecular study

Three legs from one side of the larvae were separated and kept in absolute ethanol for DNA extraction. DNA was extracted using modified Puregene method (Gentra Systems). Mitochondrial gene Cox1 (cytochrome oxidase subunit I ~650 base pairs) was partially amplified with polymerase chain reaction (PCR) with the primer combination COL6 (5′-TYTCHACAAAYCATAAAGAYATYGG-3′) (Schubart, 2009) and COH6 (5′-TADACTTCDGCRTGDCCAAARAAYCAT-3′) (Schubart & Huber, 2006). The PCR conditions were as follows: initial denaturation step at 94°C for 4 min followed by 36 cycles including denaturation for 45s at 94°C, annealing for 45s at 50°C, extension for 1 min at 72°C; a final extension at 72°C for 5 min. PCR products were outsourced for sequencing to Macrogen (South Korea) using primer COL6. DNA sequences were manually edited using Chromas version 2.6.6 (https://technelysium.com.au/wp/chromas/) and aligned using program BioEdit version 7.2.5 (Hall et al., 1999). In order to fully understand the intraspecific diversity, 17 sequences of *B. braaschi*, previously used by different studies, were borrowed from the Genbank (Table 1). Haplotype network was constructed using PopArt software version 1.7 (Leigh & Bryant, 2015).

RESULTS

Systematic account

Baetis braaschi Zimmermann, 1980
Baetis stipposus KLUGE, 1982: 18 syn. n.
Baetis stipposus KLUGE: NOVIKOVA, 1987
Baethis tiposus: KISELEVA & VASYUTA, 1986 partim; KISELEVA, 1987 partim
Baethisgr steposus: KISELEVA, 1992 partim
Baethis stiposus: KISELEVA, 1997 partim
Baetis braaschi ZIMMERMANN: GODUNKO, PROKOPOV, 2000; JACOB, 2003
Baetis braachi ZIMM.: PROKOPOV, 2000
Baetis braaschi ZIMM.: PROKOPOV, 2000
Baetis braaschi: PROKOPOV, 2001
Baetis braaschi GODUNKO et al., 2004

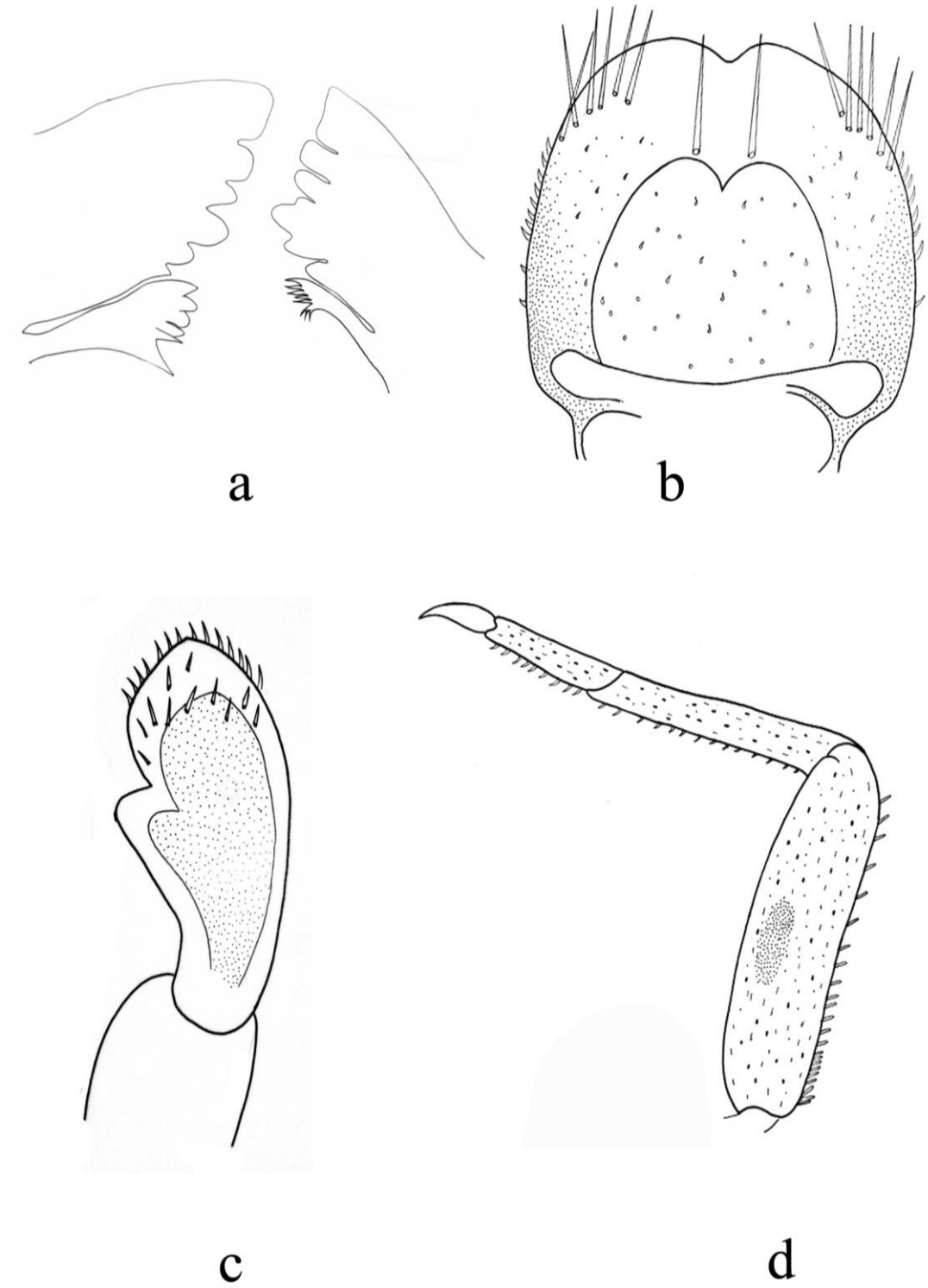

FIGURE 2. *Baetis braaschi*. larva: a, mandibles; b, labrum; c, labial palp; d, hind leg and tarsal claw.

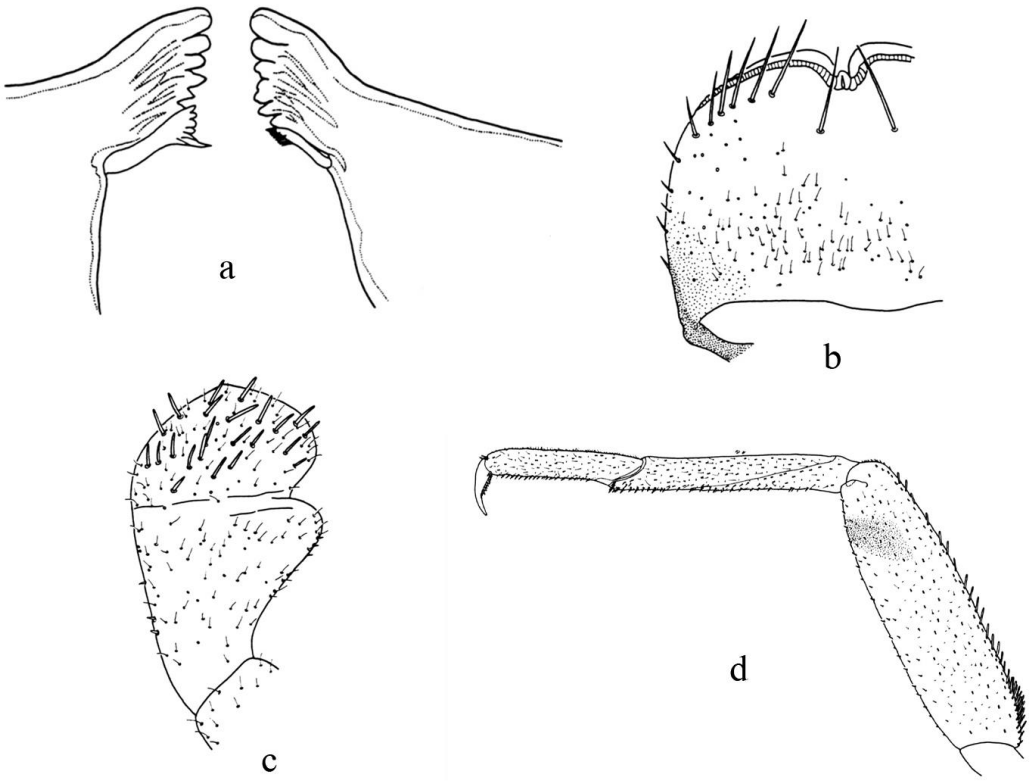

FIGURE 3. *Baetis braaschi* redescribed by Godunko (2004). a, mandibular; b, labrum; c, maxillary palp; d, hind leg

Morphological findings

Mature larva. Length: body 2.5-11 mm; cerci 2.5-4.5 mm. General color of body brown, Head dark brown without spots, antennae dark brown. Pronotum and mesonotum dark brown with clear longitudinal light brown stripes. Labrum wide (the width/length ratio less than 1.70) with 1+ 5-6 long bristles each side and few small marginal bristles. Labrum surface with numerous fine hairs (Fig 2-b). Right mandible with 7 teeth and left mandible 6 teeth. Prostheca present on left and right mandible, prostheca on both mandibles distinctly asymmetrical (Fig. 2-a). Outermost tooth on mandibles about twice the width of the second tooth (Fig. 2-a). Segment 3 of labial palps distinctly wide and slightly pointed at the apex with numerous bluntly pointed bristles (Fig. 2-c). Glossae and paraglossae relatively wide, inner margin of glossae with 9-16 bristles and paraglossae with three rows of bristles on apex. Legs uniformly light brown, femora with distinct elliptical brown band medially at center, (Fig. 2–d), tibia darker in distal and tarsus darker in apex. Bristles on upper margin of femur clearly longer than the bristles on lower edge of femur, these bristles blunt at the tip. Hairs and spines there are in both margins. External and inner margin of tibia with same spines and hairs, but hairs in external margin are more. Inner margin of tarsi with spines, while external margin with spines and hairs. Spines of inner margins distinctly longer and more than external margin of tarsi. Surface of femora, tibiae and tarsi with pointed and quadrangular bristles and many fine hairs. Tarsal claws sub-terminal without short hair bristles. Tarsal claw pointed, dark brown, darker than tarsi, with 11-20 teeth (Fig. 2-d). Abdominal tergite brown, segments I, X and IX are light and on them not seen any pattern. On abdominal terga II, III, IV, VI, VII and VIII there are two light round spots with a light stripe between them. In the tergite V spots are larger and combined. Sterna light, yellowish without spots. There are 7 pairs of gills brown on abdominal segments 1-7. Gills 1 and 7 symmetrical, with almost

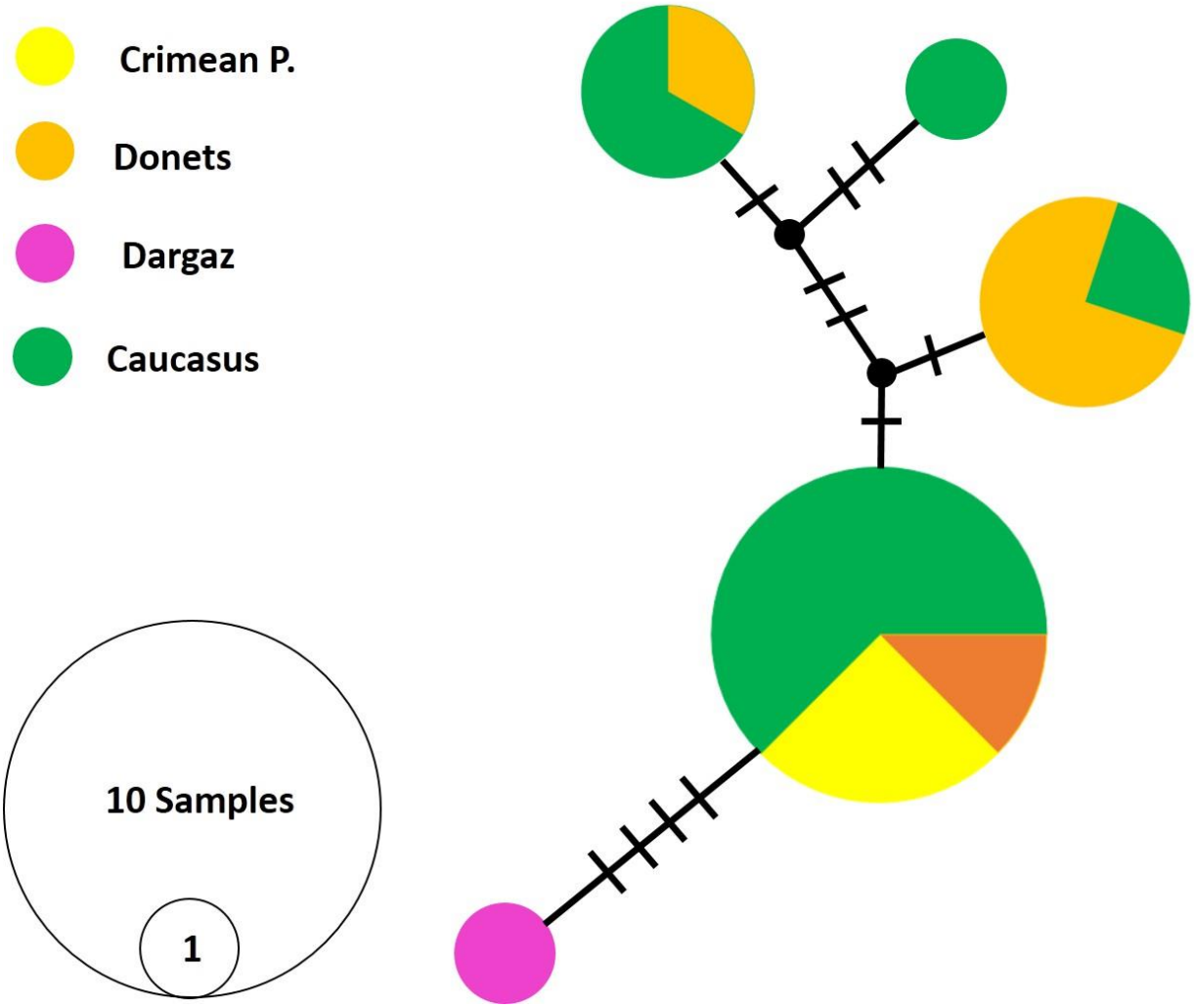

FIGURE 4. Parsimony network of *Baetis braaschi* specimens from Iran, Ukraine and Georgia

same size, gills 2-6 asymmetric and relatively large, external margin of all gills finely serrated, with fine hair, without strong spines. Cerci and terminal filament light brown, without a dark band or rings. Terminal filament slightly shorter than cerci, its length 2/3 –3/4 the length of cerci.

*Remarks***.** In most specimens the outermost tooth on mandibles is twice the width of the second tooth (Fig. 2-a), but in some specimens the first outermost tooth and second tooth mandibles have the same width and length, so width and length of the first and second tooth mandibles are variable. In species redescribed by Godunko (2004), head carries dark spot while in Iranian specimens head is without spot; moreover in type description, femora dorsally have distinct brown spot distally (Fig. 3-d), whereas in Iranian specimens it is elliptical and located medially (Fig. 2–d). Pattern of head, femurs and width and length of first and second tooth (Fig. 3-a) in *B. braaschi* can be variable from Eastern Europe to Iran and Eastern Asia.

Molecular findings

We used molecular data to understand if such morphological differences are in line with molecular finding or not? The morphological results showed that species of *Baetis braaschi* has significant morph

differences with its conspecific specimens from Ukraine and Georgia. The parsimony network indicated a high intra-specific diversity among specimens of *B. braaschi* from different localities. The Iranian specimen comprise a unique haplotype, not shared by the other haplotypes from different localities. *Baetis Braaschi* from Iran is distinctly differentiated (uncorrected p-distance, 1%) from its closest haplotype from Ukraine and Georgia. (Fig. 4).

DISCUSSION

In described specimens of *B. braaschi* by Sroka et al. (2012), head presented dark spot, while in Iranian specimens it is absent. In original description of species, mentioned that femora have distinct brown spot distally, whereas in Iranian specimens, femora have distinct elliptical brown band centrally in dorsal part of the legs (Fig. 2–d). Therefore, pattern of head, femora and size of first and second teeth in the specimens of *B. braaschi* is variable from Eastern Europe to Iran.

Due to extensive distribution of *B. braaschi* from Eastern Europe to Iran (Sroka et al., 2012), it can be suggested this species have even wider distribution to Eastern Asia and Europe. Such high intraspecific molecular variation in freshwater benthos, with almost the same distribution, is also presented in other freshwater organisms; e.g., *Potamon ibericum* (Parvizi et al., 2018). All examined specimens in the current study were collected in larval stage, therefore description of imago and subimago stages from Iran is demanded. Considering the limited study done on Iranian mayflies (Moazzen et al., 2021) and their importance in freshwater food webs and as pollution indicator in assessing the health of the freshwater ecosystems, it is proposed to conduct comprehensive study on fauna of mayflies in Iran. In addition, the region severely suffers from water shortage in last couple of decades and there has been important warning that some unique and endemic freshwater species are critically endangered (Kiabi et al., 1999; Coad, 1980).

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