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The record of *Ametropus fragilis* Albarda, 1878 (Ephemeroptera, Ametropodidae) from Georgia

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Abstract

The representatives of the genus *Ametropus* (Insecta, Ephemeroptera) were recorded 80 years ago in Georgia. Due to the taxonomic uncertainty, the recorded specimens were considered potentially new taxa. Since then, no other record of *Ametropus* has been documented from the whole South Caucasus and its nearby regions further south. In the present contribution, we provide a new record of *Ametropus* from the Alazani River (Kura River Basin, Georgia). Morphological and DNA barcoding of the COI gene fragment showed that the species belongs to the widespread Holarctic species *A. fragilis*. Morpho-anatomical characteristics also confirmed the high degree of similarity between the newly recorded specimens and those once recorded 80 years ago. In addition, the COI barcode showed a strikingly closer relationship between Caucasian *A. fragilis* and North American *A. neavei* (3.54% divergence) compared to Caucasian and European *A. fragilis* populations (4.3% divergence). The observed pattern clearly indicates the need for further and more thorough revision of the *Ametropus* species complex in the Holarctic region.

Key words

Alazani River, CaBOL, faunistics, mayfly, South Caucasus, Vashlovani National Park

Introduction

The mayfly (Insecta, Ephemeroptera) fauna of Georgia is relatively well known thanks to recent renewed interest (Sroka 2012; Sroka and Godunko 2012; Sroka et al. 2012; Kluge et al. 2013; Godunko et al. 2015; Martynov et al. 2016; Martynov and Godunko 2017). A comprehensive overview of Georgian mayflies published in the 2018 check list (Gabelashvili et al. 2018) reported 75 species belonging to 22 genera and 12 families. Two more species, *Epeorus* (*Caucasiron*) *bicolliculatus* Hrivniak, 2017 (Heptageniidae) and *Centroptilum volodymyri* Martynov, Godunko and Palatov (2022) (Baetidae), were recently added to the Georgian mayfly fauna after Hrivniak et al. (2017) and Martynov et al. (2022). Gabelashvili et al. (2018) indicated that the records of *Ametropus* sp. for Georgia reported by Sadovsky (1946, 1948) from the Kura River were doubtful because of the absence of voucher material and exact locality data. Thus, as no additional data for this genus is available in the literature, the presence of any *Ametropus* in the South Caucasus was questionable. In the present contribution, we provide evidence of the existence of this genus in Georgia with a short overview of its habitat preferences, distribution, and the history of *Ametropus* studies in the region.

Materials and methods

Sampling

Benthic samples were collected from three plots (with a 30 meter distance) on April 17, 2022, at Mijniskure, Vashlovani National Park in Georgia (N41.11188, E46.64954, 95 m a.s.l.), in the shallow and murky waters of the Alazani River (Fig. 1). The 240-km-long Alazani River originates from the southern slope of the Greater Caucasus Mountains

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Figure 1. Mijniskure, Alazani River (Vashlovani National Park, Georgia). Sampling site of *Ametropus fragilis*.

at an altitude of about 2800 m a.s.l. It is the largest left tributary of the Kura River, and its lower reach represents the border between Georgia and Azerbaijan. At the investigated sites, the river is flowing relatively slowly (typical for a lowland river), and its bottom is composed basically of clay and muddy silt and sand from place to place. The water is less transparent in the sampling area, which is represented by a negligible amount of algae, poor species composition, and homogeneous but dense benthic communities.

Samples were collected using a kick-net with a mesh size of 500 µm according to the AQEM sampling method (Hering et al. 2004) within a depth range 15-80 cm. Benthic materials were sorted out, and specimens were preserved in 96% ethanol and then stored in a freezer under -22 °C at the scientific collections of Ilia State University under CaBOL (the Caucasus Barcode of Life). For the identification of mayfly specimens, we used the key provided by Eiseler (2005), Sadovsky (1940), and Bauernfeind and Soldan (2012).

Photos of the preserved specimen were taken using a Canon EOS 60D camera with a Canon EF-S 60mm f/2.8 Macro USM Lens. Digital images were prepared using Zerene Stacker image stacking software and Adobe Photoshop CS6.

DNA processing

Genomic DNA was extracted from tissue samples using the Quick-DNATM Miniprep PlusKit (Zymo Research) (for 25 mg of tissue). Partial sequences of cytochrome oxidase subunit I (COI) were amplified by polymerase chain reaction (PCR) using the primer pairs LCO1490-JJ and HCO2198-JJ (Astrin and Stüben 2008). Thermal conditions included denaturation at 95 °C for 1 min, followed by the first cycle set (15 cycles): 94 °C for 30 sec., annealing at 55 °C for 1 min (–1°C per cycle), and extension at 72 °C for 1:30 min. The second cycle set (25 cycles): 94 °C for 35 sec., 45 °C for 1 min., 72 °C for 1:30 min., followed by 1 cycle at 72 °C for 3 min., and the final extension step at 72 °C for 5 min. PCR amplicons were visualized on 1% agarose gels using 1.7 µl of PCR product. Sequencing of the unpurified PCR products in both directions was conducted at the Beijing Genomics Institute (Hong Kong, CN) by using the amplification primers. Barcode sequence analysis was performed using Geneious Prime 2022.1.1 (http://www.geneious.com). Extracted DNA was deposited in the scientific collections of Ilia State University, Tbilisi, Georgia, while the sequences have been submitted and checked out against the Barcode of Life Data System (BOLD) database (Ratnasingham and Hebert 2007). The BOLD System then automatically identified the Barcode Index Number (BIN) (Ratnasingham and Hebert 2013) for submitted sequences, and these were obtained using the BOLD analytical tools within BIN divergence and distance to the nearest neighbor BINs.

Results and discussion

Among the mayfly samples collected in the Alazani River, five specimens were sorted out from the single sample initially identified as *Ametropus* sp. The nymph morphology of this genus is unique among mayfly genera and cannot be confused (Wang et al. 2013, Ćuk et al. 2015). Based on the morphological characteristics, the collected mayfly specimens were further identified as *Ametropus fragilis* Albarda, 1878 (Fig. 2), given the descriptions provided by Sadovsky (1940), Bauernfeind and Soldan (2012), and Eiseler (2005). The general coloration is yellowish-brown, with slender and hairy bodies. Antennae with 16 segments. Legs are slender with long claws; the forelegs are much shorter than the middle and hind. Fore coxae with a base-attached spinous patch. There are seven pairs of single, lateral plate-like gills, each with long setae on their margins. Maxillary and labial palps are three-segmented and covered with dense hair. Given

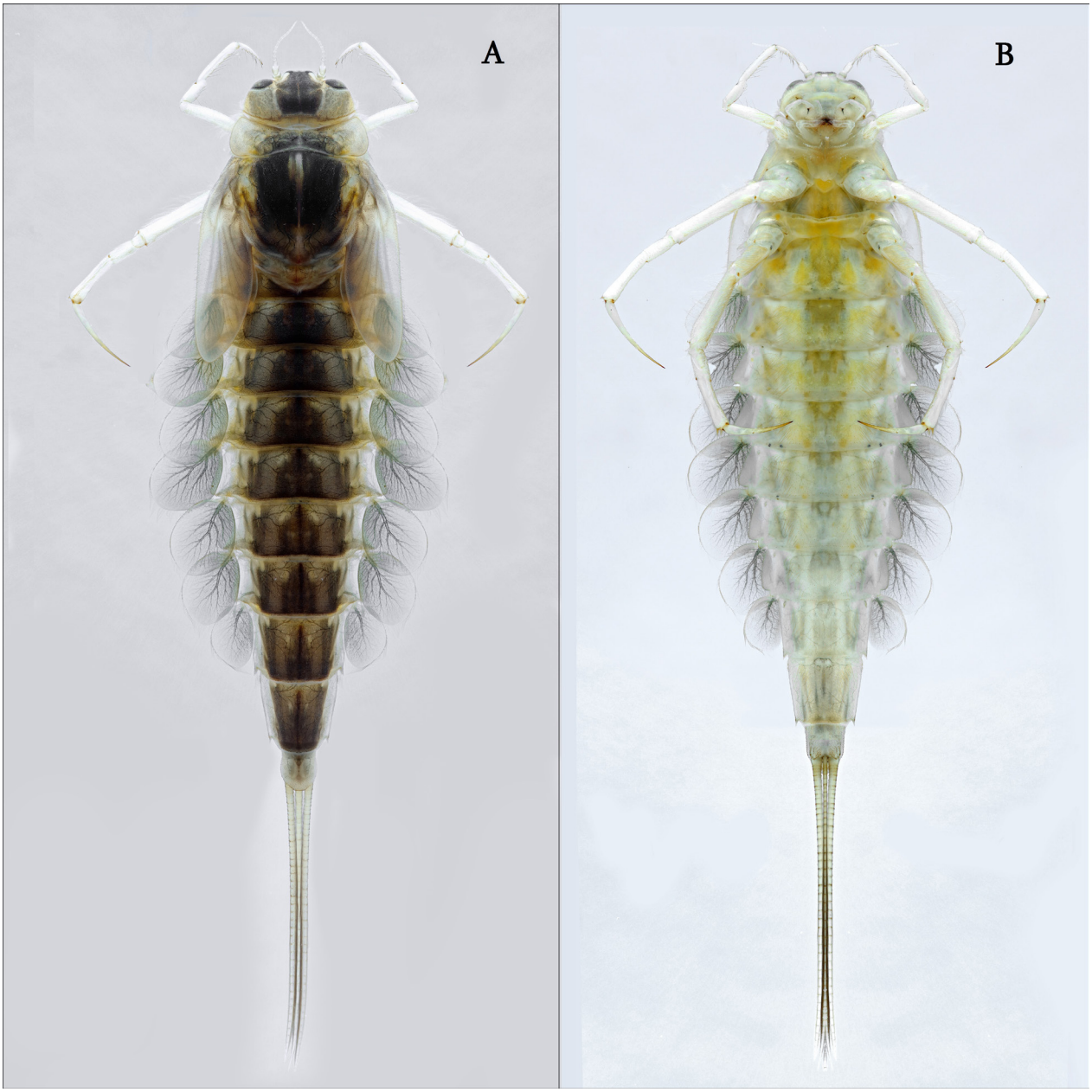

Figure 2. *Ametropus fragilis* (voucher: CaBOL-ID 1025425): **A** – Dorsal view; **B** - ventral view.

these characters, the studied individuals are morphologically highly similar to *Ametropus* sp., described and illustrated by Sadovsky (1940), in all respects (Fig. 3). All *Ametropus* larvae are psammophilous (sand-dwelling) and usually remain hidden in the muddy or sandy substratum (semi-burrowers) that perfectly matches our sampling site. However, they may be discovered on submerged logs or leaf packs in big rivers and smaller alpine streams with shifting sand substrates (Jazdzewska 1973; Bauernfeind and Soldan 2012).

We obtained three COI barcode sequences from three specimens with 626 nucleotide base pairs free from indels or stop codons. Sequences were uploaded to BOLD Systems (processing IDs EPGEO001-23, EPGEO002-23, and EPGEO003-23). The BOLD System identified these sequences under a new BIN: BOLD: AFB6526. Our newly obtained DNA barcodes were nearly identical (0.15–0.61% uncorrected p-distance). The closest taxon after BOLD System matching was the Nearctic species *Ametropus neavei* (Walker 1849) from Canada (BOLD: AAW1858, mean p-distance 3.54%). A larger genetic distance (4.3%) was found between our specimens and the single sequence of *A. fragilis* from Slovakia (BOLD: AEV8713; private barcode). The genetic relationship is also depicted in the Neighbor-Joining tree given in Figure 4.

From the family Ametropodidae, a single species (*Ametropus fragilis*) is considered to occur in Europe. The distribution of this species lies within Germany, Italy, Croatia, Poland, Austria, Slovakia, Hungary, Bulgaria, Romania, Estonia, Latvia, and Lithuania (Thomas and Belfiore 2013). The species was also recorded in Russia (Jakobson and Bianki 1905), China (Wang et al. 2013), Belarus (Lewandowski and Moroz 2001) and France (Cozilis and Chovet 2010). According to Jacob (2006), *A. fragilis* also occurs in North America, as he considered North American *A. albrighti* Traver, 1935, a synonym of *A. fragilis*. Thus, it can be considered a Holarctic species.

In the Caucasus, the presence of *Ametropus* sp. was first reported from unclear localities by Eaton (1885). In partic-

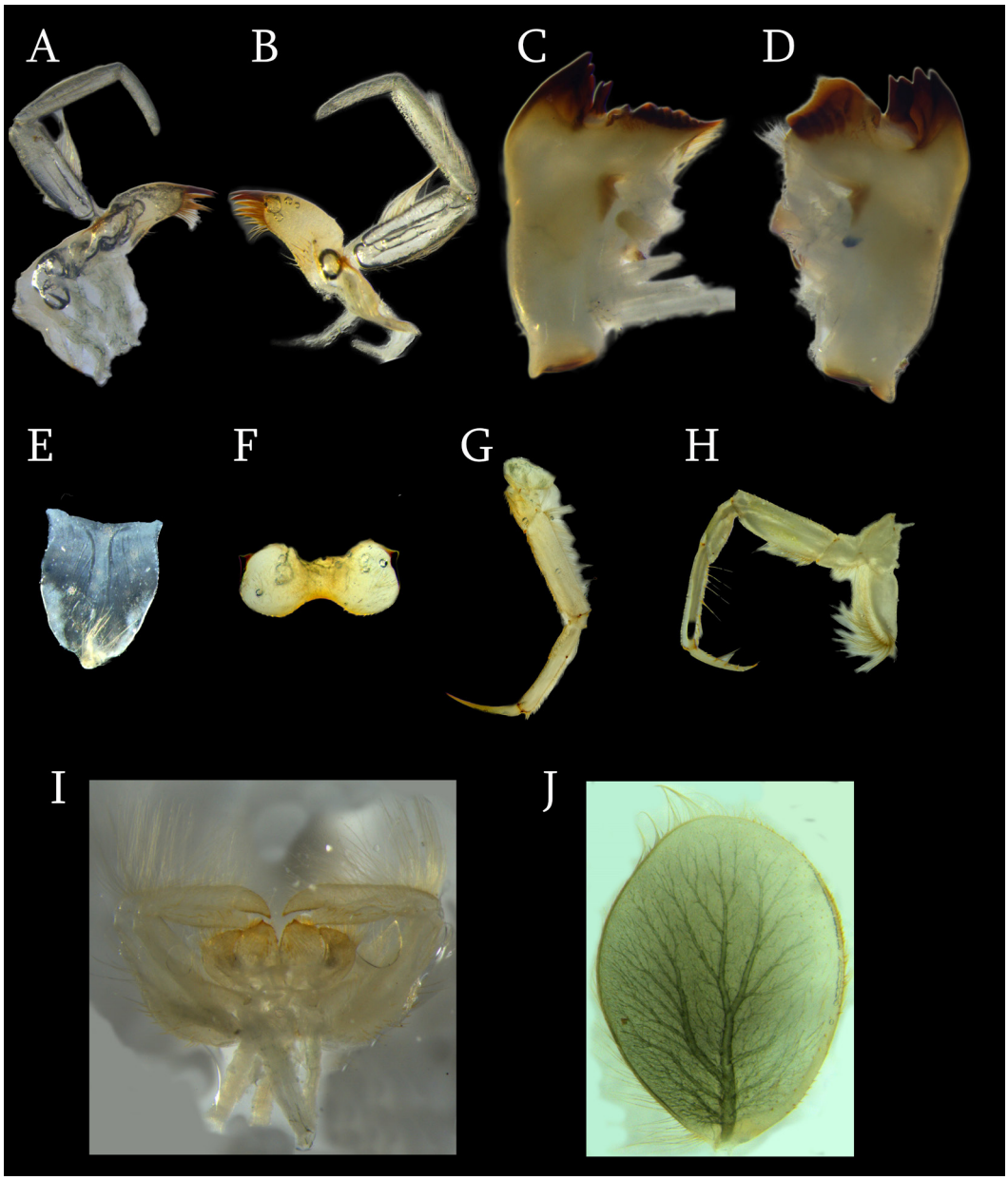

Figure 3. Larval structures of *Ametropus fragilis*: **A** – right maxilla; **B** – left maxilla; **C** – right mandible; **D** – left mandible; **E** – additional thoracic gill; **F** – labrum; **G** – left hind leg (dorsal view); **H** – left foreleg (ventral view); **I** – labium; **J** – gill plate I (dorsal view).

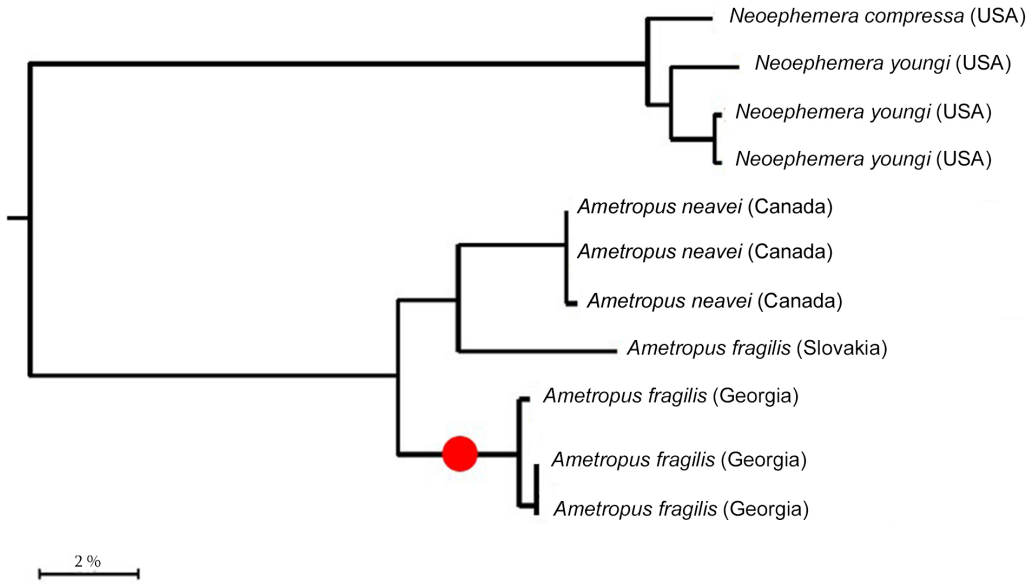

Figure 4. Neighbor-Joining tree based on COI barcode region of the *Ametropus* representatives constructed by BOLD Systems.

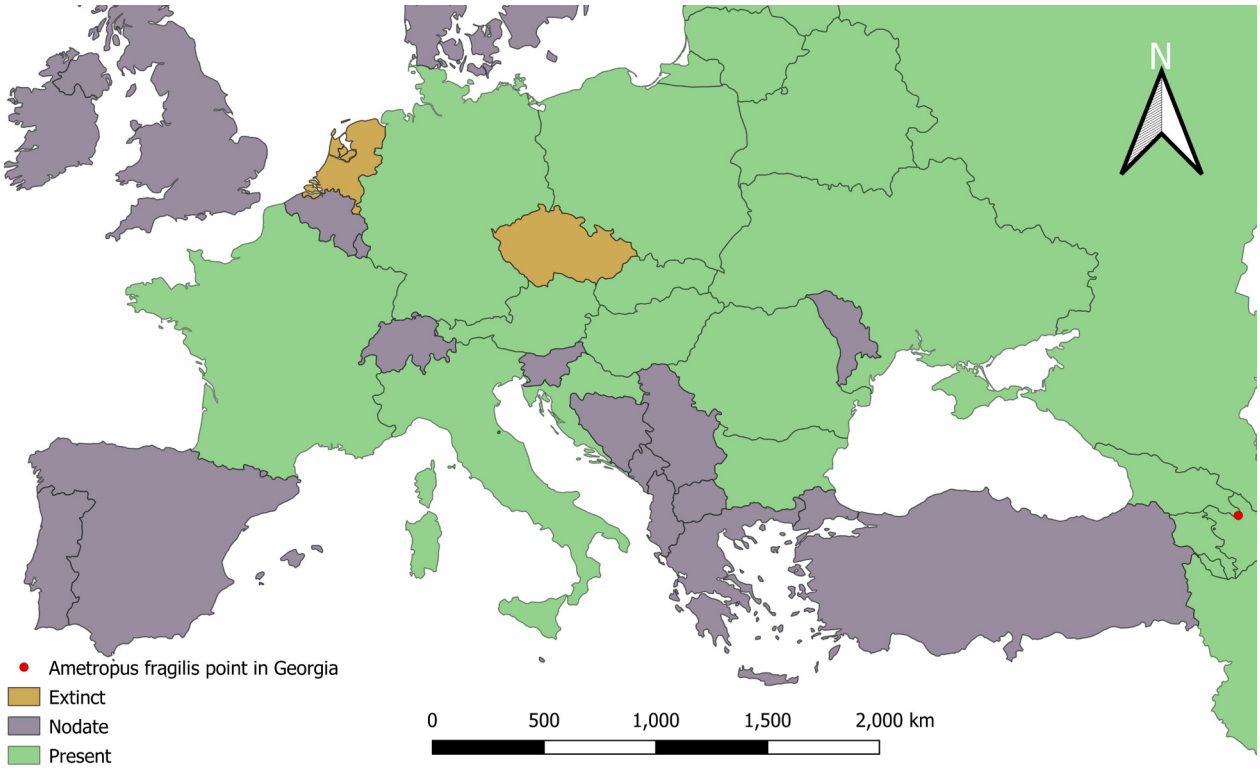

Figure 5. Distribution of the mayfly *Ametropus fragilis*, in European countries (green colored) with the location of the new record in Georgia (red spot). Countries where *A. fragilis* became extinct are shown in brown, and countries where data is not available are shown in gray.

ular, A. E. Eaton reported that specimens of *Ametropus* sp. were found in the mix of samples collected in "Russia, the Caucasus, and Armenia". Later, Sadovsky (1940) also collected *Ametropus* sp. from the Kura River in Georgia, which was supposed to be *A. fragilis* by Palatov and Chertoprud (2016). No other records of this species are known from Georgia or other South Caucasian countries. The species belonging to *Ametropus* were also recorded from the north Caucasus (North Ossetia) (Jakobson and Bianki 1905) and Iran (Braasch 1981; Bashti and Ostovan 2014). However, the later record was reported with insufficient details on the sampling locality or species identity.

Ametropus fragilis is usually rare throughout its distribution range, and its population density is very small while its distribution area is very large. Taking this into consideration, large genetic variation, i.e., around 5% divergence of the COI barcode region between populations, is therefore expected. However, the genetic closeness of Georgian populations with North American populations (named *A. neavei* in BOLD) compared to Georgian and Slovakian populations makes it difficult to interpret the genetic variation. The most intuitive explanation of the observed pattern is the recent translocation and gene flow. On the other hand, given the population characteristics and life-style peculiarities of *Ametropus*, the observed pattern can also be indicative of the existence of multiple divergent lineages of Eurasian and North American *Ametropus* and the need for taxonomic revision of the genus. Based on the state of the art of *Ametropus* taxonomy and our results, we can conclude that the Georgian populations belong to the widespread species *Ametropus fragilis*. Furthermore, since the Alazani River, where the specimens have been collected, lies right on the border between Georgia and Azerbaijan, we can say that the species also occurs in Azerbaijan. Taking the old literature information into consideration, the *Ametropus* specimens observed in Iran and Armenia most probably also belong to the same species. Accordingly, in Figure 5, we provide an updated map developed by Ćuk et al. (2015) showing the current known distribution of *A. fragilis* in Europe and the neighboring area.

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