First records of two formerly overlooked Ponto-Caspian amphipods from Turkey: *Echinogammarus trichiatus* (Martynov, 1932) and *Dikerogammarus villosus* (Sovinsky, 1894)

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1. Introduction

The Ponto-Caspian basin, composed of the Black, Azov, and Caspian seas, is one of the most important hotspots of gammarid diversity in the Palearctic (Väinölä et al., 2008). The basin is a residue left after the regression of the Neogene epicontinental Sea of Paratethys (Dumont, 2000). The long geological history and prolonged periods of isolation of the basin have resulted in high endemism (up to 80%) of the local aquatic animal species. Among them, there are more than a hundred endemic crustacean species, of which Amphipoda are the most prominent group, forming the so called “Ponto-Caspian complex” (sensu Stock, 1974). Some of these amphipods are very successful invaders in Western Europe and the Great Lakes of North America (Jazdżewski, 1980; Ricciardi and MacIsaac, 2000; Grabowski et al., 2007a). Their range extension is driven mainly by connection of formerly isolated river basins through man-made channels as well as intentional or accidental introductions (Bij de Vaate et al., 2002; Rewicz et al., 2014). Species such as *Dikerogammarus villosus* or *Echinogammarus trichiatus* (Martynov, 1932) continuously and successfully colonize new water bodies outside the Ponto-Caspian region (Borza, 2009; Boets et al., 2012; Rachalewski et al., 2013a; Rewicz et al., 2014). The former species is well known for effective competition with or even eradication of native amphipod species (Dick et al., 2002; MacNeil and Platvoet, 2005; Rewicz et al., 2014). The ecology and impact of the latter has not been studied yet (Borza, 2009; Boets et al., 2012). Such success in range expansion can be related to features such as opportunistic feeding (Krisp and Maier, 2005), high fecundity (Grabowski et al., 2007b), wide range of physiological tolerance to water temperature (Wijnhoven et al., 2003), and conductivity (Grabowski et al., 2009) displayed by most of these Ponto-Caspian colonizers.

Turkey is a country encompassing vast areas of the coastline and drainage basin of the Black Sea and several studies have already been carried out on the distribution of Ponto-Caspian amphipod species in the Turkish inland waters. Mordukhai-Boltovskoi (1964) documented the presence of some species in the lakes Apolyont, İznik, Sapanca, and Manyas, situated along the southern and eastern coasts of the Sea of Marmara. Later, Stock (1974) described *Obesogammarus turcarum*, a new species belonging to the Ponto-Caspian complex, from Ağrı Province near the Turkish–Armenian border. Similarly, Mateus and Mateus (1990) identified another new species, *Dikerogammarus gruberi*, from Lake Sapanca, Western
Anatolia. Kocatas et al. (2003) reported Pontogammarus maeoticus (Sowinsky, 1894) as a new species for Turkey. Özbek et al. (2004a) revealed the existence of another pontogammarid species, Pontogammarus aestuarius (Derzhavin, 1924), in Lake Taşkısqlı, northwestern Anatolia. Then Özbek et al. (2004b) reported another amphipod representative of the Ponto-Caspian complex, Chelicorophium maeoticum (Sowinsky, 1898), for the first time from Turkey. In the same year, Özbek et al. (2004c) documented the distribution of Malacostraca species in the lakes Apolyont and İznik. Later Özbek and Ustaoğlu (2005) reported the presence of some amphipod species belonging to the Ponto-Caspian complex in water bodies located in Turkey’s Lake District. Özbek and Özkan (2010, 2011) recorded two new Ponto-Caspian amphipods from Turkish inland waters: Amathillina cristata as a new record for Turkey and Dikerogammarus istanbulensis Özbek & Özkan (2011) as a new species for science. As a result, the Ponto-Caspian amphipod fauna of Turkey is rather rich with 13 species recorded so far (summarized by Özbek and Ustaoğlu (2005) and Özbek (2011)). In this study, we present the first records of two Ponto-Caspian amphipods new for Turkey as well as locality data for five other previously reported species.

2. Materials and methods
Lake Durusu (Lake Terkos, Durugöl Liman) is one of the most important tap water sources of İstanbul. It plays also an important role in protecting the migrating water birds (Nankinov, 1996), which enables amphipod transport over short distances (Rachalewski et al., 2013b). The lake formerly was a bay and then was isolated from the sea by a narrow beach. Nowadays, the lake has a slightly brackish character (Güher, 2002). Amphipods were collected from the lake near the village of Balaban in September 2007 (Figure 1). The sample was gathered using benthic hand-net from all available littoral habitats (sand, mud, submerged macrophytes) at depths from 0.05 to 0.5 m. The collected specimens were preserved in 96% ethanol directly in the field and identified in the laboratory to species level based on the available literature (Mordukhay-Boltovskoj et al., 1969; Pinkster, 1993; Eggers and Martens, 2001; Özbek and Özkan, 2011). All the identified material is in the collection of the Department of Invertebrate Zoology & Hydrobiology, University of Łódź, Poland.

Individuals of E. trichiatus used for scanning electron microscope (SEM) documentation were dehydrated in an ethanol series, critical point dried, and sputter-coated with gold (10 nm). Pictures were produced with a PHENOM PRO X SEM in the Department of Invertebrate Zoology and Hydrobiology, University of Łódź.

Additional verification of the morphological identification of E. trichiatus from Lake Durusu was done with molecular markers. Total DNA from 6 individuals of E. trichiatus was extracted using the standard phenol–chloroform protocol, and ca. 640-bp-long fragments of mitochondrial cytochrome oxidase subunit I (COI) were amplified following the protocol detailed by Mamos et al. (2014). The two acquired 585-bp-long COI haplotype sequences were deposited in GenBank with the accession numbers KR007308 (5 ind.) and KR007309 (1 ind.). Then the haplotypes were compared with other COI sequences of E. trichiatus and Echinogammarus ischnus available in GenBank (Table 1). The sequence of Gammarus fossarum from locus typicus (GFOS-loctyp, GenBank number JF965886) was used as an outgroup. A phylogenetic tree was constructed using the haplotype data in MEGA 5.05 (Tamura et al., 2011) using the neighbor-joining method (Saitou and Nei, 1987) based on the p-distance (Nei and Kumar, 2000) with a bootstrap test performed on 10,000 replicates (Felsenstein, 1985). Mean Kimura 2-parameter (K2P) genetic distance between E. trichiatus sequences, with standard error (S.E.), was also calculated in Mega 5.05.

3. Results and remarks
Our sampling in Lake Durusu revealed the presence of 7 amphipod species (Table 2), all of them belonging to the so called “Ponto-Caspian complex” (sensu Stock, 1974). Five of them (Dikerogammarus haemobaphes, Echinogammarus ischnus, Pontogammarus robustoides, Amathillina cristata, and Chelicorophium curvispinum) were already known to occur in Turkey. Two (Dikerogammarus villosus and Echinogammarus trichiatus) have not been reported from this country previously.

Family Pontogammaridae Bousfield, 1977
Dikerogammarus villosus (Sovinsky, 1984)
Specimens examined: Turkey, Lake Durusu, near the village of Balaban N 41.3163, E 28.62055, 01.09.2007, 187 ind., leg M. Grabowski, K. Bącela-Spychalska.
**Diagnosis:** Large species, males 12–22 mm, females 8–14 mm. Flagellum of antenna II in males with numerous tufts of long setae, peduncle sparsely armed. In females tufts of setae sparse, with only some long setae. Propodus of gnathopod I and II with long dense setae, as long as or longer than the width of the propodus. High dorsal tubercles present on urosome segments I and II and usually armed with 2–5 spines. The diagnostic features are illustrated in Figure 2. Özbek and Özkan (2011) published a key to all known *Dikerogammarus* species. Other useful sources for identification of *D. villosus* are, e.g., Cărăuşu (1943), Cărăuşu et al. (1955), Mordukhaj-Boltovskoj et al. (1969), Eggers and Martens (2001), and Konopacka (2004).

**Table 1.** Set of cytochrome oxidase I (COI) sequences of *Echinogammarus trichiatus* and *Echinogammarus ischnus* used in the study.

<table>
<thead>
<tr>
<th>Species Haplotypes</th>
<th>GenBank accession number</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. trichiatus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etri-1</td>
<td>KR007308</td>
<td>Lake Durusu (Turkey); this study</td>
</tr>
<tr>
<td>Etri-2</td>
<td>KR007309</td>
<td>Lake Durusu (Turkey); this study</td>
</tr>
<tr>
<td>Etri-3</td>
<td>AY529051; KM024679; KM009053</td>
<td>Danube Delta (Romania); Netherlands; Western Europe</td>
</tr>
<tr>
<td>Etri-4</td>
<td>AY529050</td>
<td>Dniester Liman (Ukraine)</td>
</tr>
<tr>
<td><em>E. ischnus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eisc-1</td>
<td>AY326118</td>
<td>Southern Bug Liman (Ukraine)</td>
</tr>
<tr>
<td>Eisc-2</td>
<td>AY326119</td>
<td>Southern Bug Liman (Ukraine)</td>
</tr>
<tr>
<td>Eisc-3</td>
<td>AY326117</td>
<td>Dniester Liman (Ukraine)</td>
</tr>
<tr>
<td>Eisc-4</td>
<td>AY326120</td>
<td>Danube Delta (Romania)</td>
</tr>
<tr>
<td>Eisc-5</td>
<td>AY326121</td>
<td>Danube Delta (Romania)</td>
</tr>
<tr>
<td>Eisc-6</td>
<td>AY326115; DQ889147; FJ581620-FJ581623</td>
<td>lower River Rhine (Netherlands); no locality; Quebec (Canada)</td>
</tr>
<tr>
<td>Eisc-7</td>
<td>AY326116</td>
<td>Dniester Liman (Ukraine)</td>
</tr>
<tr>
<td>Eisc-8</td>
<td>AY326122</td>
<td>River Irpen (Ukraine)</td>
</tr>
<tr>
<td>Eisc-9</td>
<td>AY326123</td>
<td>Kiev Reservoir (Ukraine)</td>
</tr>
<tr>
<td>Eisc-10</td>
<td>AY326124</td>
<td>middle River Dniester (Ukraine)</td>
</tr>
<tr>
<td>Eisc-11</td>
<td>AY326125; AY326126</td>
<td>Volga Delta (Russia)</td>
</tr>
</tbody>
</table>

**Table 2.** Records of amphipod species in Lake Durusu, Turkey. Bold indicates newly recorded species.

<table>
<thead>
<tr>
<th>Location</th>
<th>Latitude, °N</th>
<th>Longitude, °E</th>
<th>record date</th>
<th>Dvil</th>
<th>Dhae</th>
<th>Eisc</th>
<th>Etri</th>
<th>Prob</th>
<th>Acri</th>
<th>Ccur</th>
<th>Collectors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Durusu</td>
<td>41.3163</td>
<td>28.62055</td>
<td>01.09.2007</td>
<td>187</td>
<td>43</td>
<td>3</td>
<td>17</td>
<td>6</td>
<td>10</td>
<td>16</td>
<td>M. Grabowski, K. Bącelska-Spychalska</td>
</tr>
</tbody>
</table>

Remarks: *D. villosus* is a fourth representative of the genus in the Turkish fauna, along with *D. haemobaphes* (Eichwald, 1984), *D. istanbulensis* Özbek and Özkân, 2011, and *D. gruberi* Mateus and Mateus, 1990. In its native range, *D. villosus* inhabits lower courses of large rivers like the Dnieper, Dniester, Danube, Don, and Volga, as well as limans and coastal lagoons in the Ponto-Caspian area (Rewicz et al., 2014). Its preferable habitats are stones and boulders; however, juveniles occur often among macrophytes and submerged tree roots (Devin et al., 2003). This species, commonly known as the ‘killer shrimp’, is regarded as one of the worst 100 invasive species in Europe (DAISIE, 2009). Invasion of that species into Western Europe progressed along the southern (via the Danube, Rhine, and Mittellandkanal) as well as along the central (via the Dnieper, Bug, and Vistula) invasion corridors (sensu Bij de Vaate et al., 2002). In last 20 years it has successfully invaded most of the big rivers, channels, and lakes of Western Europe, with the most recent episode of colonization in the inland waters of Great Britain in 2010 (summarized by Rewicz et al. (2014)). Our new finding extends the known distribution of the species to water bodies in the southern part of the Black Sea coast. Taking into account that this first record of the species in Turkey comes from the Ponto-Caspian region, we may conclude that this species is native to Lake Durusu and its presence there was formerly overlooked.

Family **Gammaridae** Latreille, 1802

**Echinogammarus trichiatus** (Martynov, 1932)
syn. **Chaetogammarus trichiatus** Martynov, 1932, syn. **Chaetogammarus tenellus major** Carausu, 1943

**Specimens examined:** Turkey, Lake Durusu, near the village of Balaban N 41.3163, E 28.62055. 01.09.2007, 17 ind., leg M. Grabowski, K. Bącela-Spychalska.

**Diagnosis:** Medium large species, males 12–15.5 mm, females 10–13 mm. In males, antenna II and pereiopod III densely covered with long, curled setae. Such setation is also typically present on rami of uropod III, yet they were absent in all the examined individuals from Lake Durusu (Figure 3). In females the setation is less dense and predominantly straight. Urosome segments I and II with four groups of spines (two dorso-lateral and two lateral), usually with 3–4 spines in each group. On the third urosome segment only two lateral groups are present; dorsolateral groups are reduced to two dorso-median spines only. The diagnostic features for the species are illustrated in Figure 4. The species may be identified based on the following literature: Cărăuşu (1943), Cărăuşu et al. (1955), Mordukhaj-Boltovskoj et al. (1969), Eggers and Martens (2001), and Konopacka (2004).

**Molecular identification:** Due to the lack of dense and curly uropod setation in individuals from Lake Durusu, which is one of the key features for *E. trichiatus*, we used an mtDNA COI gene for their additional molecular
identification. The two obtained haplotypes (Etri-1 and Etri-2) clustered (with 100% bootstrap value) within one clade with GenBank obtained sequences of *E. trichiatus* from Ukraine, Romania, and Western Europe (Table 1; Figure 5). Additionally, the mean K2P distance between all the compared haplotypes of *E. trichiatus* was 0.008
(±0.003 S.E.), which is way below the 0.03 upper threshold for the intraspecific genetic distance defined by Costa et al. (2009) and Hebert et al. (2003). In conclusion, despite the lack of one important key feature, the individuals can be unambiguously identified as *E. trichiatus*. Thus, our finding extends the known morphological variability of the species.

**Remarks:** So far, eight species of the genus *Echinogammarus* have been reported from Turkish inland and estuarine waters (Özbek and Ustaoğlu, 2005). Yet, after *E. ischnus* (Stebbing, 1899), *E. trichiatus* is the only other *Echinogammarus* species belonging to the Ponto-Caspian complex (*sensu* Stock, 1974) reported from the Turkey. It is important to remark that often in the literature the species occurs under its synonymic names, *Chaetogammarus trichiatus* Martynov, 1932 or *Chaetogammarus tenellus major* Caras, 1943. This taxonomic confusion has been discussed in detail by Rachalewski et al. (2013a). *Echinogammarus trichiatus* is indigenous to the basins of the Black and Azov seas. It occurs in the lower course of the Danube River and its delta as well as in fresh and slightly brackish limans and coastal lakes along the northwestern Black Sea coast. The most eastern record of the species came from the river Khosta on the Caucasian Black Sea coast, which is the locus typicus for that species (Martynov, 1932). The preferable habitat of *E. trichiatus* is hard substrate such as gravel and pebbles, but it was found also in reeds and among macrophytes (unpublished data). The species invaded inland waters of Western Europe via the southern invasion corridor (*sensu* Bij de Vaate et al., 2002). Surprisingly the first record of *E. trichiatus* outside its native range came from the upper Danube in Germany. Thus it may be expected that the species' spread was due to human-mediated transport. Subsequently, the species was found in France, Belgium, Netherlands, and recently in Poland. The species' invasion history and its present distribution in Europe are summarized by Boets et al. (2012) and Rachalewski et al. (2013a). Taking into account that this first record of the species in Turkey comes from the Ponto-Caspian region, we may conclude that, as in the case of *D. villosus*, *Echinogammarus trichiatus* is native to Lake Durusu and its presence there was previously overlooked.

**4. Discussion**

Previous studies reported the presence of only two species, the Ponto-Caspian *Amathillina cristata* and the cryptogenic *Orchestia cavimana*, from Lake Durusu (Özbek and Özkan, 2010; summary by Öztek, 2011). Our finding adds six more species to that short list. As a result,
nearly 50% of all the Ponto-Caspian amphipod species known from Turkey occur in that water body, making it a regional hotspot. It is also likely that further studies would reveal the presence of other overlooked species. The first records of Dikerogammarus villosus and Echinogammarus trichiatus from Turkey expand the national checklist of Ponto-Caspian amphipods to 15 species. The proximity of Lake Durusu to the Istanbul agglomeration and potentially associated touristic pressure may facilitate human-mediated transport of aquatic invertebrates to other water local bodies (Bącela-Spychalska et al., 2013). In Turkey, the General Directorate of State Hydraulic Works (DSI) is the main body responsible for the construction and management of reservoirs as well as the introduction of fish species into them. During their activities, some invertebrate species or aquatic plants have been introduced into the reservoirs intentionally or unintentionally. A case report was published recently about the introduction of another Ponto-Caspian amphipod, Pontogammarus robustoides (Sars, 1894), to three artificial reservoirs in Turkey (Özbek, 2011). Thus, we cannot exclude more such unintentional introductions into other Turkish reservoirs and lakes, altering the native amphipod fauna. Taking into account that both Dikerogammarus villosus and Echinogammarus ischnus are known to be successful invaders, Lake Durusu can be an important species donor on a regional scale.

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References


