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Filling the blank spot: first report on the freshwater crayfish distribution in Albania

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Abstract – The knowledge on the distribution of native and non-native crayfish species in Europe has substantially advanced during last two decades. However, data on the exact distribution of European crayfish are still lacking in some regions of the Balkan Peninsula, *e.g.*, in Albania. Out of five European crayfish taxa, *Astacus astacus* and *Austropotamobius torrentium* are considered to occur in Albanian freshwaters. However, until now the only confirmed localities of the occurrence of any crayfish species in Albania are a single place in the Mat River drainage and Prespa Lake. Five crayfish individuals have been collected recently from two localities in Albania: Ohrid Lake and upper Devolli River (Seman basin), as a result of a repeated sampling in all main Albanian river drainages. All specimens were morphologically and molecularly identified as the noble crayfish, *A. astacus*, providing confirmation of its presence in this Balkan country. The present paper summarizes historic and current distribution records of the crayfishes in Albania and neighbouring countries as well as provides insight into genetic variability of Albanian *A. astacus*.

Keywords: *Astacus astacus* / *Austropotamobius torrentium* / Ohrid Lake / DNA barcoding / Balkan Peninsula

Résumé – Combler une lacune : premières données sur la distribution des écrevisses en Albanie. Les connaissances sur la répartition des espèces d'écrevisses natives et non autochtones en Europe ont considérablement progressé au cours des deux dernières décennies. Cependant, des données sur la répartition exacte des écrevisses européennes manquent encore dans certaines régions de la péninsule balkanique, par exemple en Albanie. Parmi les cinq taxa européens d'écrevisse, *Astacus astacus* et *Austropotamobius torrentium* sont considérés comme présentes dans les eaux albanaises. Cependant, jusqu'à présent, les seules localités confirmées de présence d'espèces d'écrevisses en Albanie sont un endroit unique dans le bassin de la rivière Mat et dans le lac Prespa. Cinq spécimens d'écrevisses ont été collectés récemment dans deux localités en Albanie : le lac d'Ohrid et la Haute-Devolli (bassin du Seman), à la suite d'un échantillonnage répété dans tous les principaux bassins hydrographiques albanais. Tous les spécimens ont été identifiés morphologiquement et génétiquement comme étant l'écrevisse noble, *A. astacus*, confirmant sa présence dans ce pays balkanique. Le présent document résume les rapports de distribution historiques et actuels des écrevisses en Albanie et dans les pays voisins, ainsi que des informations sur la variabilité génétique d'*A. astacus* albanais.

Mots-clés : *Astacus astacus* / *Austropotamobius torrentium* / Lac Ohrid / code barres ADN / Péninsule des Balkans

The European continent is inhabited only by five crayfish taxa from two genera: *Astacus* and *Austropotamobius* (as in Kouba *et al.*, 2014). These include: the noble crayfish *Astacus astacus*, the thick-clawed crayfish *Astacus pachypus*, the narrow-clawed crayfish *Astacus leptodactylus* as well as the white-clawed crayfish *Austropotamobius pallipes* and the stone crayfish *Austropotamobius torrentium* (Kouba *et al.*, 2014); with the three latter taxa considered to possibly form species complexes (*e.g.*, Grandjean *et al.*, 2002; Klobučar *et al.*, 2013; Maguire *et al.*, 2014; Jelić *et al.*, 2016). Similarly to other parts of the world, the European crayfish fauna has been subjected to considerable population declines (Richman *et al.*, 2015), caused mainly by habitat degradation, as well as introduction of more competitive North American crayfish species and the crayfish plague pathogen, *Aphanomyces astaci* carried by them (Holdich *et al.*, 2009). The extent of these losses is especially worrying as

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freshwater crayfish are important keystone species with a substantial effect on the function and structure of whole ecosystems (Reynolds *et al.*, 2013).

The Balkan Peninsula is characterized by a high genetic diversity for many aquatic species (Bănărescu, 2004; Sket *et al.*, 2004), stemming from its role as one of the most important Pleistocene glacial refugia in Europe (Hewitt, 1999). Likewise, the populations of some European crayfish species occurring in the Balkans were observed to exhibit higher genetic variation compared with the rest of Europe (Trontelj *et al.*, 2005; Klobučar *et al.*, 2013; Schrimpf *et al.*, 2014), and hence forming genetically unique stocks of conservation importance on the continental scale. However, in contrast to most European countries, the detailed information on exact distribution of crayfish species in the Balkans is still scarce (Kouba *et al.*, 2014) and has only been recently updated for some regions: Bosnia and Herzegovina, Croatia, Serbia and Montenegro (Simić *et al.*, 2008; Maguire *et al.*, 2011; Trožić-Borovac, 2011). However, although out of four European crayfish species present in the Balkan Peninsula, two were considered to occur in Albania: *A. astacus* and *A. torrentium*, to the best of our knowledge no studies on their distribution have been published (*e.g.*, Holdich, 2002). The assumption about their presence in this country has been mainly based on the records from the transboundary waterbodies outside Albania (Karaman, 1929, 1962, 1963; Albrecht, 1982; Subchev, 2011). The aim of the present paper is to summarize historic and current distribution records and our own investigations of European crayfish occurrence in Albania, and contribute to the understanding of crayfish distribution on the Balkan Peninsula.

From 2004 to 2015, during several ichthyological surveys covering comprehensively the watercourses of all main Albanian rivers, we searched also for the presence of crayfish species. Crayfish were found only in 2015 at two locations: upper Devolli River in Seman basin (4 individuals; N 40°42′37″ E 20°51′49″, 828 m a.s.l.) and Ohrid Lake (1 indiv.; N 40°59′01″ E 20°38′23″, 690 m a.s.l.). All crayfish individuals were preserved in 96% ethanol and deposited in the invertebrate collection of National Museum, Prague, Czech Republic (accession numbers P6E 4180–4184). They were morphologically identified as *A. astacus* (Füreder and Machino, 2002). The DNA barcoding of the mitochondrial gene for the cytochrome *c* oxidase subunit I (COI; as in Mrugała *et al.*, 2015) confirmed morphological identification and revealed the presence of three haplotypes (fragments' length 635 base pairs (bp), GenBank accession numbers: KY682289-91). There are no shared haplotypes between the localities and the haplotypes differ by up to three mutations. The mitochondrial network for COI was constructed by TCS 1.21 (Clement *et al.*, 2000) using statistic parsimony. The analyses revealed that the Albanian *A. astacus* haplotypes are distinct from the ones published by Schrimpf *et al.* (2011, 2014; Fig. 1) for the great part of native distribution of this species.

In addition to our observations in Ohrid Lake and upper Devolli River, *A. astacus* population is apparently present in the Albanian part of Prespa Lake, from where 54 individuals were recently purchased from a local fisherman (Đuretanović *et al.*, 2017). Moreover, the early studies repeatedly reported its presence in transboundary waterbodies in neighbouring countries and from several drainages in a close vicinity to Albania. It was mentioned as *Potamobius fluviatilis balcanicus* in Karaman (1929); *Astacus colchicus balcanicus* in Karaman (1962); *Astacus astacus balcanicus* in Karaman (1963) and Albrecht (1982) in Ohrid Lake and rivers of the Vardar system in the Former Yugoslav Republic of Macedonia (FYROM) and in the Sitnica River (Danube drainage) in Kosovo. The presence of *A. astacus* (as *Potamobius astacus*) in Ohrid Lake was also mentioned by Stanković (1960) and reported from the Zeta River (Ohrid-Drin-Skadar basin) in Montenegro (Simić *et al.*, 2008). Finally, it is also widespread in the north-western and central regions of Greece (including the Kalamas River near the southern Albanian border; Koutrakis *et al.*, 2007).

Austropotamobius torrentium occurs throughout the Balkans (Kouba *et al.*, 2014) and its presence was recently confirmed in Albania (Subchev, 2011). A voucher specimen sampled in the northern part of the country in 2003 (River Fani i Madh, Mat River basin, N 42°04′59″ E 20°03′36″, 615 m a.s.l.) is deposited in the collection of the Natural History Museum in Budapest, Hungary (Subchev, 2011). The studies of Karaman (1929), Karaman (1962, 1963) and Albrecht (1982) indicated also that *A. torrentium* (as *Potamobius torrentium macedonicus* – Karaman (1929), *Austropotamobius torrentium* natio *macedonicus* – Karaman (1962), *Austropotamobius torrentium* – Albrecht (1982)) is present in FYROM, including Ohrid Lake as well as the Drim and Vardar river systems. Moreover, its occurrence was also suggested for Skadar Lake and its tributaries in Albania and Montenegro (Karaman, 1962). Presence in the Montenegrin part of the Skadar Lake basin was confirmed from the Rijeka Crnojevića (rather small direct inflow of Skadar Lake; Trontelj *et al.*, 2005; Petrović *et al.*, 2013). Furthermore, this crayfish species was recorded in the Morača River (main inflow of Skadar Lake) in Montenegro (Simić *et al.*, 2008). It is also widely present in central and southern part of Serbia (including Morava, Timok and Drina rivers; Simić *et al.*, 2008), as well as in northern Greece, where its western distribution extends to the area around the city of Kastoria, close to Albanian border (Koutrakis *et al.*, 2007).

Austropotamobius pallipes occurs on the Dalmatian coast with its southernmost distribution limit in the Zeta River in Montenegro, a part of the Ohrid-Drin-Skadar system (Machino and Ďuriš, 2004; Rajković *et al.*, 2012; Kouba *et al.*, 2014). In contrast, *A. leptodactylus* inhabits only the north-eastern part of the Balkan Peninsula (Kouba *et al.*, 2014). It was suggested that it might be recorded in Albania with more intensive sampling (Holdich, 2002), nevertheless, its presence has not been confirmed yet. The distribution of *A. leptodactylus* in neighbouring countries is limited to the central part of Serbia (Danube basin including Sava, Morava and Timok rivers; Karaman, 1929; Simić *et al.*, 2008), as well as to the Evros River drainage in eastern Greece (Koutrakis *et al.*, 2007). In addition, two non-native crayfish species of North American origin are present in the Balkans: the spiny-cheek crayfish, *Orconectes limosus* (reported from the Serbian stretch of the Danube; Simić *et al.*, 2008) and the signal crayfish, *Pacifastacus leniusculus* occurring at several localities in north-eastern Croatia as well as in northern Greece in Lake Agra in the Aliakmon River basin (Koutrakis *et al.*, 2007; Kouba *et al.*, 2014).

Our observations confirm *A. astacus* presence in Albania and demonstrate its occurrence also in the Albanian part of Ohrid Lake. Furthermore, although *A. torrentium* was not recorded during our surveys, it is present in the northern part of Albania and may be also expected to inhabit other freshwaters, especially close to Macedonian, Montenegrin and Greek

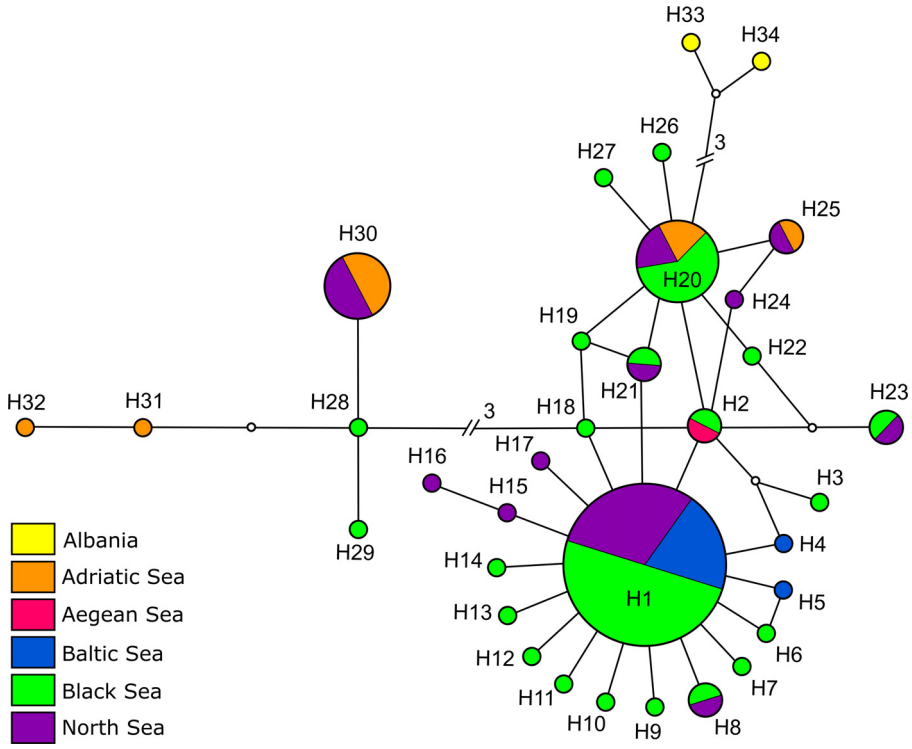

Fig. 1. The network of COI haplotypes that includes *A. astacus* haplotypes from Albanian localities (Ohrid Lake and Devolli River, both Adriatic Sea basin; obtained in the present study) and from five sea basins: Adriatic Sea (Croatia, Montenegro), Aegean Sea (Kosovo), Black Sea (Austria, Bulgaria, Croatia, Germany, Hungary, Romania), Baltic Sea (Germany, Poland), North Sea (Belgium, Czech Republic, Germany; published in Schrimpf *et al.*, 2011, 2014). The size of the circles is proportional to the frequency of the represented haplotypes at the country level in all five sea basins. Only two haplotypes from Albania are presented (from Ohrid Lake and Devolli River); due to the shorter length of the published *A. astacus* sequences (350 base pairs [bp]) the diversity of haplotypes from the Devolli River was lost (based on full length 635 bp, three haplotypes were found). The white dots indicate the median vectors. The number of base pair changes is given (no number indicates a change of 1 bp). The details on the haplotypes published in Schrimpf *et al.* (2011, 2014) are provided as a Supplementary Material.

borders. As recent reports of the IUCN (International Union for Conservation of Nature) highlight declines of both crayfish species in Ohrid Lake and its tributaries most likely caused by water pollution (Edsman *et al.*, 2010; Füreder *et al.*, 2010), it should not be neglected that it may be the case also for the other Albanian waterbodies. The natural expansion of *A. pallipes* from the Zeta River in Montenegro (Rajković *et al.*, 2012) to Albania, as well as of the two North American crayfish species from neighbouring countries seems unlikely. However, the distribution ranges of crayfish species in Europe are nowadays strongly shaped by deliberate or accidental anthropogenic introductions, *e.g.*, through the use of crayfish as fishing bait, their illegal stocking or releases of unwanted ornamental pets (Holdich *et al.*, 2009; Peay 2009). Therefore, only long-term and intensive sampling may provide the data on the exact distribution of both already recorded native European crayfish species in Albanian freshwaters, as well as may reveal the presence of any other non-native crayfish species.

Supplementary Material

Table S1. The codes and GenBank accession numbers of COI haplotypes published in Schrimpf *et al.* (2011, 2014) used for network analyses in the present study.

The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2017024/olm.

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