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Co-occurrence of narrow-clawed crayfish (*Astacus leptodactylus* sensu lato) and noble crayfish (*Astacus astacus* L.) in the southwestern Balkans: The case of Lake Pamvotida (NW Greece)

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Abstract. Two individuals of the locally non-indigenous crayfish species *Astacus leptodactylus* (sensu lato) were collected by trapping in Lake Pamvotida (Region of Epirus, Greece). This is the first record of the species from the southwestern Balkans and particularly from the Ionian biogeographic area. It suggests that there has been a significant human-mediated expansion in *A. leptodactylus* territory across the Pindos cordillera. Moreover, four individuals of the species *Astacus astacus* (L.) were also collected from the lake. The re-occurrence of this species after at least two decades of presumed absence represents a unique known case of co-occurrence for these two species in Greece. We present a brief overview regarding the current distribution of both species (in Europe, Balkans, Greece, as well an occurrence map for Lake Pamvotida), a record of their morphometrics and a description of the main morphological characters of the captured specimens. The unexpected presence of *A. leptodactylus* in Lake Pamvotida suggests that it was probably transferred there or escaped from restaurant facilities situated around the lake and on Nisi Island. In the case of *A. astacus*, its indigenous status (i.e. specimens originating from an existing and naturally recovering population in the same lake/habitat), although questionable, cannot be disregarded until future extensive genetic work. Overall, the presence of these species in the lake could, in the long term, help to re-establish crayfishing there.

Key words: co-occurrence, biogeographical barrier, crayfish introduction, Lake Pamvotida.

Introduction

The alteration of glacial and interglacial periods during the Quaternary led to the retreat of *Astacus astacus* (L.) and *Astacus leptodactylus* (sensu lato) species in glacial refugia and subsequent recolonization had a great impact on their distribution patterns. The existence of such refugia for *A. astacus* has been documented for southeastern Europe (Black Sea basin and Western Balkans) and Germany (Schrimpf et al. 2011, 2014). Similarly, it was suggested that *A. leptodactylus* survived in at least two glacial refuges, one possibly situated on the outskirts of the Caspian Sea, and the other in the vicinity of the Black Sea (Minor Asia or the Balkan Peninsula) (Maguire et al. 2014). However, current distribution of both species has been radically shaped by human-mediated factors such as translocation, stocking, farming, habitat alterations, man-made river interconnections and introduction of non-indigenous crayfish species (NICS).

Noble crayfish *A. astacus* is the most widespread indigenous crayfish species (ICS) in Europe. The species' range covers central and northern parts of the continent, including the North Sea, Baltic Sea, Black Sea, Adriatic Sea basins and Balkan Peninsula (Holdich et al. 2009, Kouba et al. 2014). Despite substantial local and regional losses due to its high susceptibility to the crayfish plague (caused by *Aphanomyces astaci* Schikora, 1906), competition with NICS, pollution and habitat degradation, indigenous populations are still found in vast areas, mainly across the central parts of the continent from Scandinavia to the southern Balkans (Füreder et al. 2006, Holdich et al. 2006, Kouba et al. 2014). The species is the most commercially desirable ICS and widespread introduced populations exist, including insular ones in Cyprus, the UK and even in Morocco. The habitat type of this species is exclusively freshwater (Holdich et al. 2006). The availability of shelter influences its spatial distribution and is particularly important for lentic populations (Westin & Gydemo 1988). The species is sensitive to pollution and to physical damage of habitat; however it can withstand moderate organic enrichment (dissolved oxygen values of 3-4 mg l^{-1}), but it generally avoids muddy bottoms (Füreder et al. 2006, Holdich et al. 2006, Kozák et al. 2015).

The narrow-clawed crayfish *A. leptodactylus* is the second most widespread ICS in Europe (Holdich et al. 2009, Kouba et al. 2014). The presumed native range of this species is associated with the Ponto-Caspian river basins (i.e. Caspian Sea, Black Sea, lower and middle Danube, lower sections of Don, Dniester, Volga and their tributaries; Köksal 1988). It covers the north and eastern Balkans, Ukraine, Belarus, the western part of the Russian Federation up to the Caspian Sea and Turkey (both the European and the Asiatic part; Harlıoğlu & Harlıoğlu 2009). The species has been introduced into several European countries mainly for astaciculture or as a substitution to replace *A. astacus* stocks devastated by crayfish plague (Skurdal & Taugbøl 2002). Therefore its current distribution largely reflects human-aided colonization. It is considered to be the most eurybiontic species compared to other ICS, as it is less sensitive to pollution, is able to withstand wide water temperature and salinity fluctuations and is capable of surviving low dissolved oxygen levels (Füreder et al. 2006, Holdich et al. 2006, Kozák et al. 2015). *Astacus leptodactylus* may also be able to withstand chronic infection by *A. astaci* (Kokko et al. 2012, Schrimpf et al. 2012; Svoboda et al. 2012), similarly to freshwater crabs, which may serve as local reservoirs of *A. astaci* (Svoboda et al. 2014). In Greece, four species of freshwater crabs are present, of which *Potamon fluviatile* (Herbst, 1785) is present in the Kalamas, Acheron, and Arachthos river drainages close to lake Pamvotida (Maurakis et al. 2004). However, both *A. leptodactylus* and *A. astacus* are under continuous pressure from non-indigenous crayfish species (NICS), which are gradually spreading and displacing ICS populations.

As for the neighboring countries of Greece, *A. astacus* is indigenous to Albania, the Former Yugoslav Republic of Macedonia (FYROM) and Bulgaria (Holdich et al. 2006, Holdich et al. 2009, Kouba et al. 2014). In Greece, *A. astacus* is the most widespread species compared to other ICS (i.e. *A. leptodactylus* and *Austropotamobius torrentium* (Schr.)), although its current distribution range appears restricted, at least at a local scale (reviewed in detail by Koutrakis et al. 2007 and Perdikaris 2009). *Astacus leptodactylus* is particularly widespread in Bulgaria and in the European part of Turkey, but it is currently absent from the southwestern Balkans (i.e. from FYROM and Albania). The species also has a scattered (although limited) distribution in Italy, due to introductions that occurred after the 1980s (Holdich et al. 2006, Holdich et al. 2009, Kouba et al. 2014). The indigenous status of *A. leptodactylus* in Greece was proposed based on a) specimens collected during the 19th century (1893) from the River Evros and deposited in the Natürhistorisches Museum in Vienna (Machino & Holdich 2006); b) the observation of these samples by Karaman (1963) who treated the species as indigenous to both Greece and Turkey (but without specifying exact places in Greek territory) and c) recent confirmation of the species' presence in the same river close to the city of Didimotiho, based on collected samples (Perdikaris et al. 2007).

Until 1975, Lake Pamvotida (in northwest Greece) sustained one of the healthiest and commercially exploited populations of *A. astacus* in Greece (Paschos & Kagalou 1995), which died out due to pollution, eutrophication and a suspected incident of crayfish plague, which was first reported in 1982 and affected *A. astacus* populations across the entire region (Theocharis 1986, Koutrakis et al. 2007, Perdikaris 2009). The absence of the species from the system was suggested in 1996 during a field survey by Yoichi Machino (Machino & Holdich 2006). In October and November 2015 two specimens of *A. leptodactylus* were collected from Lake Pamvotida. Subsequently (December 2015) four specimens of *A. astacus* were also collected. Therefore, our current work aims to report a) the first records of *A. leptodactylus* outside its native range in Greece and b) the certain observation of *A. astacus* in the lake after at least two decades of presumed absence.

Materials and Methods

Lake Pamvotida

The lake (known also as Lake Pamvotis or Ioannina Lake) is located at 470 m a.s.l and it has been formed during the late Miocene to the Pliocene period. It has a maximum length of approximately 7.9 km, a maximum width of 5.4 km, an average depth of between 4-5 m (11 m max.) and a total surface area of 19.4 km^2 (Fig. 1). In the northern part of the lake there is a 2 km^2 island with a maximum elevation of 59 m above the lake's water level. It is bordered by the city of Ioannina to the west, Mt Mitsikeli to the northeast and cultivated land to the southeast and west. Basin hydrology is not thoroughly understood because of its karstic nature. Three fresh water springs feed the lake (Ddrabatova, Sedeniki and Kria), although most of the water input derives from the abundant rainfall of the Region of Epirus. Two small inflows drain an agricultural watershed in southeast and a watershed with mixed land uses (urban, rural, agriculture and industry) in southwest. Drainage occurs through a system of sinkholes that drain

Table 1. Selected abiotic characteristics of water quality and Trophic State Index values of Lake Pamvotida (after Nitas 2011). TSI (Chl-a), TSI (SD) and TSI (TP) represent Carlson's Trophic State Index (TSI) values based on chlorophyll-a, transparency and total phosphorus readings, respectively.

	Min	Max	Notes
Water temperature	6.1 ^{o}C (February, lake substrate)	28.5 ^{o}C (July, surface)	
Dissolved oxygen (surface)	6.5 mg l^{-1} (October)	23.7 mg l^{-1} (June)	10 mg l^{-1} (average), close to the lake substrate at 3.5 m below surface: <1 mg l^{-1} during July
pH	7.7 (substrate)	9.9 (surface)	
Total phosphorus (TP; surface)	0.1 mg l^{-1}	0.4 mg l^{-1}	
Total nitrogen (TN) (surface)	2 mg l^{-1}	3.5 mg l^{-1}	
Transparency (Secchi Disk – SD)	0.41 m (July)	1.4 m (January)	
Chlorophyll-a (Chl-a)	4.9 mg m^{-3} (April)	298.1 mg m^{-3} (Jun)	46.6 mg m^{-3} (average)
TSI (Chl-a)	49 (April)	78 (June)	
TSI (SD)	56 (January)	70 (September)	TSI scale: 0-100
TSI (TP)	64 (January)	83 (August)	

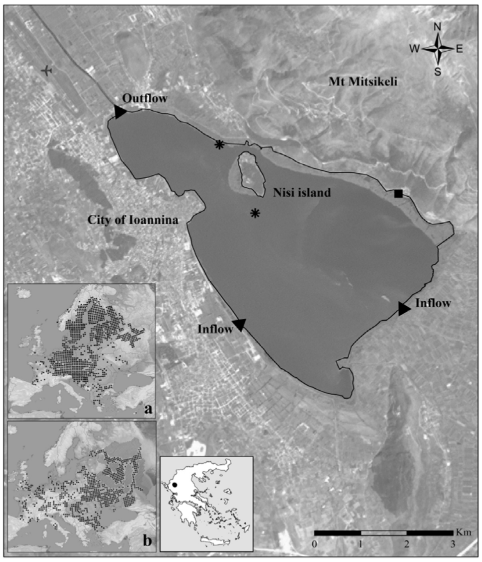

Figure 1. First records of *Astacus leptodactylus* (s.l.) and new records of *Astacus astacus* (L.) in Lake Pamvotida (NW Greece) indicated with asterisks and a square, respectively. The embedded maps (modified from Kouba et al. 2014) show (a) the distribution of *A. astacus* and (b) *A. leptodactylus* in Pan-European level.

to the rivers Arachthos, Louros, and Kalamas. Lake Lapsista (40 km^2, 1–3 m depth), formerly connected to Lake Pamvotida near the present outlet, was drained during 1959 to increase the available agricultural land. A man-made tunnel and ditch was constructed in 1960 that now drains from the outflow of Lake Pamvotida to the Kalamas River (Romero et al. 2002). The most recent report issued by the Pamvotida Lake Management Body that monitored the water quality of the lake (from June 2010 to May 2011) provided results regarding several water characteristics (Table 1 after Nitas 2011). The use of Carlson's Trophic State Index (TSI) (Carlson 1977) provided ranges for TSI based on chlorophyll-a (TSI (Chl-a), transparency (TSI (SD)) and total phosphorus (TSI (TP)) readings.

Field sampling, species identification and morphological measurements

Six specimens (i.e. two *A. leptodactylus* and four *A. astacus*) were collected by professional fishermen from Lake Pamvotida (Fig. 1) using funnel drum net fish traps (classified and abbreviated as FIX08.9.0 by the International Standard Statistical Classification of Fishing Gear (Nédélec & Prado 1990), or FPO (pots and traps) following the EU Regulation 1379/2013), locally referred to as *daoulia* or *volki*. All specimens were given to the Pamvotida Lake Management Body and were sent to the authors for identification. The keys of Füreder & Machino (2002) and Holdich et al. (2006) were used for species determination, and the morphological characteristics and measurements were taken using an electronic caliper with an accuracy of 0.01 mm (Paget Trading Ltd.). Measurements follow Füreder & Machino (2002). The morphological measurements obtained were: Cephalothorax Length (CL: the length between the tip of the rostrum and the posterior border of the carapace, in the dorsal view); Rostrum Length (RL: the length between the distal tip of the rostrum and the basal line of the rostrum's connection to the carapace); Acumen Length (AL: the length between the distal tip of the rostrum and the basal line between the marginal spines of the rostral border); Tail without Telson (TaL: the length between the posterior border of the carapace and the posterior border of the sixth abdominal segment in the dorsal view); Telson Length (TeL: the length of the terminal abdominal segment in the dorsal view); Eye Diameter (ED: the maximum diameter of the eye); Total Length (TL: the sum of the CL, TaL and TeL); Rostro-Cervical Carapace Length (CCL: the length between the distal tip of the rostrum and the distal point of the cervical suture in the dorsal view); Cephalothorax Width (CW: the maximum width of the cephalothorax in the dorsal view); Abdomen Width (AW: the maximum width of the abdomen in the dorsal view); Chela Width

Table 2. Basic data on species/code of specimens, sex and locations of collection.

Species (specimen number)	Sex	Date of collection	Lat	Lon
A. leptodactylus (specimen 1)	♂	16.X.2015	39.6832° N	20.8669° E
A. leptodactylus (specimen 2)	♂	14.XI.2015	39.6699°	20.8765° E
A. astacus (specimens 3-6)	♂	23.XI.2015	39.6721° N	20.9108 ° E

(CheW: the maximum width of the chela in the dorsal view); Chela Length (CheL: the length of the chela between the tip of the dactylus and the anterior border of the carpus in the dorsal view) and Chela Height (CheH: the maximum height of the chela (measured at the propodus palm) in the lateral view).

The specimens were photographed using a Sony Cybershot DSC-HX50 compact digital camera. The plates were created using the Google Picasa 3.9.141 software. All specimens were subsequently placed in 95% alcohol and deposited in the Zoological Museum of the University of Athens (ZMUA). Finally, GIS software (ArcMap 10.2, Esri, USA) was used to generate an occurrence map based on the sampling coordinates.

Results

The *A. leptodactylus* specimens were collected on the northern (near shore, close to Drabatova spring) and in the central part of the lake (south of Nisi Island) (Fig. 1, Table 2). The *A. astacus* specimens were collected in the southeastern part of the lake close to Durachan monastery.

Specimens 1-2 were identified as adult males of *A. leptodactylus* (specimen 1 is shown in Fig. 2). All 2 specimens exhibited the following characteristics: narrow 1st pair of pereiopods (chelipeds) and elongated chelae, propodus and dactylus with the upper parts tuberculated, propodus without an incision, sides and top of carapace covered with spines and tubercles, two pairs of post-orbital ridges with one apical spine on each, a prominent spine behind the cervical groove, almost parallel rostrum sides without spines on their borders, a long very prominent acumen with one pair of subapical lateral spines, smooth media carina (=cresta median), male gonopode 2 with talon, chelae underside pale in color.

Specimens 3-6 were also identified as adult males of *A. astacus* (specimen 3 is shown in Fig. 3). All 4 specimens exhibited the following characteristics: granulation (i.e. tubercles) on the carapace (mainly on the sides) particularly on the robust and rough claws (but less granular/coarser than in *A. leptodactylus*), two tubercles on the inner side of the propodus with a shallow incision between them, rostrum sides smooth and almost parallel, the anterior part of the acumen very pointed and triangular, the acumen with a row of spines on its central part (cresta median) giving a saw-tooth appearance from the side, two pairs of post-orbital ridges, a row of spines behind the cervical groove, no hepatic spines in front of the cervical groove, male gonopode 2 without talon, chelae underside smoother and red to dirty brown in color with a red chela joint.

Detailed morphological measurements for both species are presented in Table 3.

Discussion

The capture of *A. leptodactylus* specimens in Lake Pamvotida suggests a previously unnoticed introduction of the species outside its native range. Given that neither Machino reported any crayfish presence during his field survey in 1996 (Machino & Holdich 2006), nor subsequently during the period 2005-2009 (Koutrakis et al. 2007, Perdikaris 2009), this introduction could be a recent incident. These are the only specimens of this particular species reported from southwestern Balkans and western Greece until today. They also represent the most southerly collected samples in the Balkans and Europe, excluding the Asiatic part of Turkey. Concerning the introduction pathway, natural spread westward of the Region of Macedonia (Greece) is impossible due to the distance from native populations, geographical barriers (i.e. the Pindus cordillera) and accordingly no connection between river catchments. Moreover, the species was not available in the Greek aquarium market during a recent survey of freshwater crayfish in eleven large-size pet shops located in three major cities and in three large internet-based aquarium companies in Greece (Papavlasopoulou et al. 2014), although it is sold in the crayfish pet trade in the UK, the Netherlands, Germany and Turkey (Faulkes 2015). Therefore, given the low popularity of crayfish among Greek hobbyists, the opportunity for dumping remains limited. In the absence of propagule pressure data (i.e. the 'introduction effort' or the composite measure of the

Figure 2. *Astacus leptodactylus* (specimen 1) plate. A: dorsal view; B: ventral view; C: carapace detail (dorsal); D: rostrum (dorsal); E: chelum (ventral); F: telson (ventral). Scale: cm.

Figure 3. *Astacus astacus* (specimen 3) plate. A: dorsal view; B: ventral view; C: carapace detail (dorsal); D: rostrum (dorsal); E: chelum (ventral); F: telson (ventral). Scale: cm.

Table 3. Morphological measurements of the *Astacus leptodactylus* and *Astacus astacus* specimens collected in Lake Pamvotida.

	Astacus leptodactylus		*Astacus astacus*	
Character	Specimens n=2 (♂)		Specimens n=4 (♂)	
CL	57.95-60.59		55.59 (46.22-62.43)	
RL	14.77-16.96		17.44 (14.16-20.28)	
AL	7.23-7.80		6.69 (4.83-7.67)	
TaL	40.74-44.37		39.57 (34.57-44.93)	
TeL	15.86-16.65		15.55 (12.64-17.06)	
ED	3.95-3.96		3.34 (3.02-3.58)	
TL	112.24-120.82		110.71 (93.43-124.41)	
CCL	37.24-39.89		38.09 (32.02-42.46)	
CW	30.43-30.68		29.93 (25.01-34.25)	
AW	25.54-26.99		25.60 (21.94-28.89)	
CheW (Right – Left)	17.92 -18.24	16.56 -17.82	21.27 (17.60-26.92)	17.36 (16.59-18.23)
CheL (Right – Left)	57.64-59.29	56.32-58.28	52.26 (44.56-65.79)	43.80 (41.69-46.96)
CheH (Right – Left)	9.82-10.24	8.81-9.46	12.55 (10.75-15.74)	9.69 (9.41-9.89)

number of individuals released into a region to which they are not native; Carlton 1996), two hypotheses are proposed here as possible methods of introduction: a) translocation and release by fishermen (even due to mis-identification of the species as *A. astacus*) or b) escapes from cages submerged in the lake or from aquaria used for the temporary holding of crayfish offered as a delicacy by restaurants, which are located on Nisi Island and on the northern shore, very close to the collection locations of the species. In fact, a youtube video presents numerous specimens of the species in a glass aquarium of a restaurant on the Nisi, Lake Pamvotida (https://www.youtube.com/watch?v=tKHRDM m4kDA), together with specimens of *Pacifastacus leniusculus* (Dana).

The introduction to Lake Pamvotida and the intrastate movement of *A. leptodactylus* may not be unique in Greece. Historic evidence (during the 1980s) of unverified stocking has been reported for the River Pinios in central Greece (Koutrakis et al. 2007) and adult live individuals of the species were seen during the late 2010s by one of us (CP) in a restaurant near the artificial Lake Agra (village of Nisi, Edessa, Central Macedonia, Greece). According to the restaurant owner and the local people, these individuals originated from a population in the River Nestos (close to the village of Paradeisos in the region of Eastern Macedonia and Thrace; Perdikaris 2009). According to recent information (Perdikaris, unpub. data), the species was successfully introduced to the artificial lake of Polyphytou (Region of Western Macedonia) and one more established population exists in the River Pinios (Region of Thessally).

The current work also reports the re-occurrence of *A. astacus* in Lake Pamvotida. Historical records of *A. astacus* in Lake Pamvotida were reported in the literature for a period of 50 years (Athanassopoulos 1935; Karaman 1963; Kinzelbach 1986; Theocharis 1986). After the population crash during the 1970s (Paschos & Kagalou 1995) efforts to re-establish the population, such as the release of 100 individuals originating from the River Kalamas during 2004, were regarded as unsuccessful (Perdikaris 2009). According to the TSI (Chl-a) results, Lake Pamvotida becomes hypertrophic between June and September and eutrophic for the rest of the year. It can be also classified as hypertrophic according to the TSI (TP) and eutrophic according to the TSI (SD). Overall, these values suggest a degraded water quality during the last decades, although the construction of a waste treatment plant has prevented further deterioration. Moreover, the catchment has faced substantial urban, agricultural and industrial development over the last 40 years which has resulted in cultural eutrophication (Romero et al. 2002). Accordingly, the possibility that over this period, the crayfish could populate the limited tributaries of the lake remains low. Dissolved oxygen levels during July were measured even below 1 mg l^{-1} close to the substrate (see Table 1), which seem to be prohibitive for *A. astacus* survival, with lethal levels equal to 1.0 mg l^{-1} at temperature range of 15-18ºC (Krupauer 1982). Therefore, the origin of these specimens (i.e. whether they represent remnants of the initial indigenous population, isolated within in-lake refugia supported by spring water, recent translocation(s) or simply sporadic escapes/dumping from restaurant aquaria/cages) cannot be confirmed at this point without extensive genetic work.

On the contrary, there are no obvious environmental constraints for the establishment of *A. leptodacylus,* which has a higher saprobic valency (i.e. 2.0) compared to *A. astacus* (1.0-1.8) and *A. torrentium* (1.2) (Moog et al. 1995, Sládečková et al. 1998). This species can survive with dissolved oxygen levels as low as 0.5 mg l^{-1} in muddy and turbid habitats (Füreder et al. 2006). So when compared to *A. astacus, A. leptodactylus* appears to be similarly suited to the local climate, but better suited to environmental conditions. However, the existence of sub-optimal conditions for *A. leptodactylus*, is a possible reason for their low trapped numbers.

The absolute presence of *A. astacus* and *A. leptodactylus* males in the samples of the current study does not imply that females are non present in the lake, as the sample size was small. Therefore, there is a need for further sampling, targeting gravid females and/or juveniles, in order to provide solid evidence for the establishment of both species. Additionally, in the case of the *A. astacus*, this species will need to survive the low oxygen levels present in the summer, therefore establishment should be proposed based on inter-annual sampling data.

According to the IUCN (2000), an invasion requires the 'establishment of a new species that becomes an agent of change, and threatens native biological diversity'. Although it is premature to suggest that the current sample represents either an established population or a single release, *A. leptodactylus* belongs to these ICS able to become invasive once released. The species appears to share features with other invasive crayfish species (Tricarico et al. 2010) (e.g., size, fecundity, high population density, moderate levels of resistance to crayfish plague, probably due to slow evolutionary adaptation to the pathogen expressed by increased resistance of some host specimens or infection by less virulent strain of *A. astaci*). In order to assess and quantify the invasion risk posed by crayfish, the Freshwater Invertebrate Invasiveness Screening tool was recently used on ICS and selected NICS in Italy. *Astacus leptodactylus* was identified as a medium risk species, but with a score very close to the threshold level (Tricarico et al. 2010). The application of the same tool for Greece listed *A. leptodactylus* in the high risk category, with an identical score to the threshold value (Papavlasopoulou et al. 2014). The respective values for *A. astacus* were zero for Italy and three for Greece and in both cases they were far below the threshold level. Although such quantitative risk screening tools involve the operator's subjectivity and variability in outcomes depending on different biogeographical boundaries, presence or absence of other crayfish species and local conditions, they could assist as initial steps in proactive risk management. These results suggest that *A. leptodactylus* (and even *A. astacus*) has a rather moderate to high degree of invasion potential, particularly when *Austropotamobius* species are present. Although competitive inferiority for *A. astacus* was suggested when it was compared to *A. leptodactylus,* based on colonizing ability and aggression (Holdich 1999), there are many localities where both species coexist many years or decades without significant dominance of *A. leptodactylus* in Poland, Lithuania and Latvia (see Kozák et al. 2015 and references therein). Contrary to *A. leptodactylus*, *A. astacus* has demonstrated its ability under experimental conditions to successfully exploit and occupy hiding places (Zaikov et al. 2010). On the other hand, *A. astacus* was forced out by *A. leptodactylus* which also expanded its range along certain shorelines of the lake Ägeri, Switzerland (Stucki & Romer 2001). Accordingly, the outcome of the co-existence in lake Pamvotida is currently unclear.

Management and conservation strategies for ICS have not been drafted in Greece. From the legislative viewpoint (see the detailed review in Koutrakis et al. 2007), *A. astacus* is under the general protection provided by the EC Habitats Directive (92/43/EEC in which *A. astacus* is listed in Annex V). Greek legislation forbids crayfish fishing from the 15th of February to the 15th of May. It has also established a minimum size of 10 cm for captured individuals (Royal Decree 142/1971, FEK 49A/1971). For *A. astacus* the minimum allowable size of captured individuals is 7 cm in total length (mentioned as *A. fluviatilis* in the Ministerial Act A2-3354/05.11.2007, FEK 2207/B'). Important crayfish habitats need to be identified and protected, as Species Protection Areas (SPAs), according to article 22 of Appendix II of the EC Habitats Directive 92/43/EEC and crayfish are important indicators of surface water quality under the Water Framework Directive (2000/60EC). For Lake Pamvotida, (which is listed in the NATURA 2000 framework) the occurrence of both species increases its overall biodiversity and should be considered in local regulatory framework and management plans. Regulatory improvements should be made in order to control trapping pressure and ideally to divert it towards *A. leptodactylus*. However, the primary focus should be directed towards water quality improvement and wider environmental protection of the ecosystem. Moreover, information and sensitization of the local community about the ecological/conservational importance of ICS and the harmful impacts posed by unofficial and deliberate crayfish translocations and introductions are equally important.

The present findings represent a significant human-mediated range expansion of *A. leptodactylus* in Greece to a point outside its native range. Moreover, the specimens caught co-occurred with *A. astacus*, whose re-occurrence is reported after a long period of presumed absence from the system. The presence of *A. leptodactylus* suggests that unregulated introductions/translocations of aquatic organisms are occurring, something which may lead to negative consequences (e.g. asymptomatic carrying of crayfish plague and transmission via secondary translocations to syntopic or allotopic *A. astacus* populations in the recipient region).

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