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# Rival at the gate: first record of the Asian clam *Corbicula fluminea* Müller, 1774 (Bivalvia: Corbiculidae) in Greece

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**Abstract –** This contribution presents the first record of the Asian clam *Corbicula fluminea* Müller, 1774 in Greece. The species was collected in Erythropotamos River (Reka Luda Луда река), one of the main tributaries of Evros River (classical name of the Maritsa river), thus being the southernmost record of this invasive bivalve in the Balkan Peninsula. The most likely entry source of *C. fluminea* in Greece is through active or passive downstream drift through the Bulgarian part of Erythropotamos River, which is shared between Bulgaria and Greece. Special attention is now required to assess the invasion extent of the Asian clam in the entire Evros basin and adjacent river basins of Bulgaria and the Aegean basins of Greece and Turkey.

**Keywords:** Invasive / alien species / bioinvasion / dispersion / freshwater

**Résumé – Rival à la porte : Première mention de la palourde asiatique *Corbicula fluminea* Müller, 1774 (Bivalvia : Corbiculidae) en Grèce.** Cette contribution présente le premier signalement de la palourde asiatique *Corbicula fluminea* Müller, 1774 en Grèce. L'espèce a été récoltée dans la rivière Erythropotamos (Reka Luda Луда река), l'un des principaux affluents de l'Evros (nom classique de la rivière Maritsa), constituant ainsi la mention la plus méridionale de ce bivalve envahissant dans la péninsule balkanique. La source d'entrée la plus probable de *C. fluminea* en Grèce est la dérive active ou passive vers l'aval de la partie bulgare de l'Erythropotamos, que se partagent la Bulgarie et la Grèce. Une attention particulière est maintenant nécessaire pour évaluer l'étendue de l'invasion de la palourde asiatique dans tout le bassin de l'Evros et les bassins fluviaux adjacents de Bulgarie et les bassins de la mer Égée de Grèce et de Turquie.

**Mots clés :** invasion / espèces exotiques / bioinvasion / dispersion / eau douce

The Asian clam *Corbicula fluminea* Müller, 1774 is regarded as one of the most persistent invasive species in freshwater ecosystems, due to its great potential for dispersion and nuisance characteristics responsible for high ecological and economic impacts (Sousa *et al.*, 2008a, 2008b). It can dominate macroinvertebrate communities, physically alter benthic habitats, and disrupt regulating ecosystem services (Sousa *et al.*, 2008b, 2014). Despite policy and management efforts to reduce invader spread, *C. fluminea* continues to spread across freshwater systems (Caffrey *et al.*, 2016). The rapid growth, high fecundity, early sexual maturity, short life span, and its association with human activities make *C. fluminea* a non-indigenous invasive species likely to colonize new environments (Sousa *et al.*, 2008a). It has undergone a massive global range expansion since the 1940s, spreading from its native range in Southeast Asia, to North, Central and South America, and then to Europe (Crespo *et al.*, 2015).

*C. fluminea* has spread to most European rivers including the Rhine, Garonne, Loire, Seine, and Rhone (Marescaux *et al.*, 2010) and in the entire Danube Basin (*e.g.* Csányi, 1999; Bij de Vaate and Hulea, 2000). It has also spread to river basins in the Iberian and Italian Peninsula (Cianfanelli *et al.*, 2007; Pérez-Quintero, 2008) and in hydrologically unconnected watersheds including the UK (Elliott and zu Ermgassen, 2008) and Ireland, where it was recorded for the first time in 2010 (Lucy *et al.*, 2012). Expansion of invasive range and

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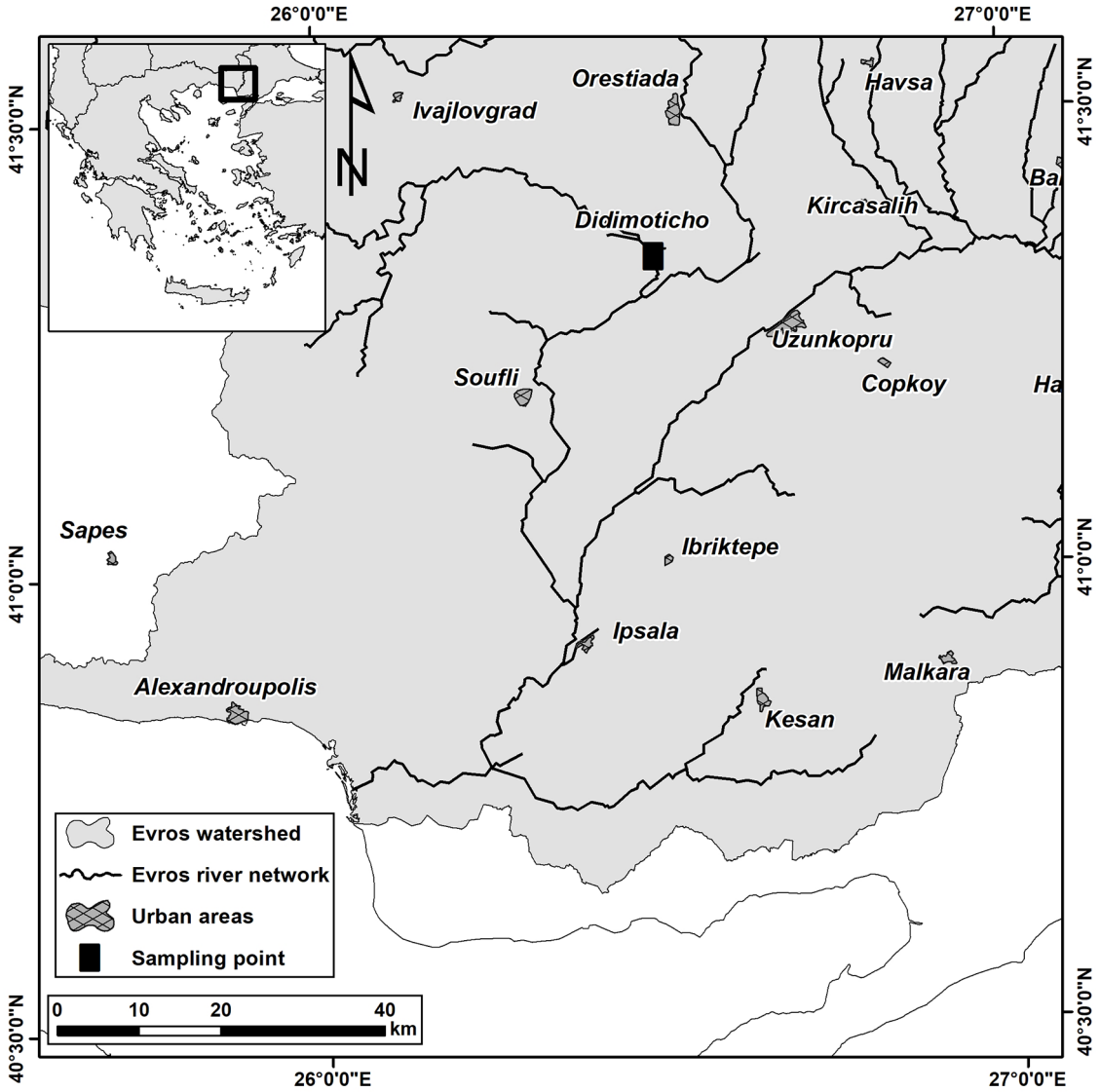


**Fig. 1.** Map showing the sampling site of *Corbicula fluminea*.

flourishing populations are also recently reported in Poland (*e.g.* Oder river; Cebulska and Krodkiewska, 2019) and western Turkey (Serdar, 2018).

In this contribution, *C. fluminea* is reported for the first time in Greece, being the southernmost record of the species in the Balkan Peninsula. The Asian clam was found in the Evros River Basin, the second largest river in southeastern Europe (after the Danube), and the main river in the Balkan Peninsula, with a total length of about 530 km and a drainage area of 53,000 km$^2$. This transboundary river is shared among Bulgaria, Turkey, and Greece.

On the 8th of September 2018, 12 individuals of *C. fluminea* were found during a routine EU Water Framework Directive monitoring in the lower part of the Erythropotamos river, only 2.5 km from its confluence with the main-stem of the Evros river (Fig. 1); specimens were photographed alive on-site and re-released into the water. Benthic conditions are dominated by silt, and there are also gravel and sandy substrates with some woody debris. Margins are rich in aquatic macrophytes (including floating plants, such as *Trapa natans*) and fringed with helophytes (*e.g. Sparganium* sp., *Typha* sp.). The sampled site is rich in fish; 17 species have been recorded in recent years. Due to low river waters creating exposed river beds, the living clams were incidentally located in the shallows. Physicochemical parameters of the sampling location were measured with a GPS Aquameter from Aquaread Ltd.

Due to the taxonomic uncertainties involving the *Corbicula* genus, the identity of the species was verified by genetic analysis. Whole genomic DNA was extracted from small tissue pieces preserved in 96% ethanol, using a standard high-salt protocol (Sambrook *et al.*, 1989), from 2 individuals. A fragment of the cytochrome oxidase c subunit I gene (COI) was amplified by PCR using universal primer modified versions, *i.e.*, LCO22me2 and HCO700dy2 (Walker *et al.*, 2006, 2007). PCR conditions are described in Froufe et al. (2014) with an annealing temperature of 48 °C. Amplified DNA templates were purified and sequenced (forward and reverse) by the commercial company Macrogen, using the same primers. The sequences obtained were compared with

**Table 1.** Physicochemical parameters and habitat characteristics of *C. fluminea* collection site.

| PARAMETERS (Units) | |
|---|---|
| pH | 8.22–8.45 |
| Water temperature (°C) | 24.15 |
| Conductivity (μS/cm) | 793 |
| TDS (mg/l) | 397 |
| Salinity (ppt) | 0.39 |
| Turbitity (NTU) | 14.9 |
| D.O. (%) | 158.46 |
| D.O. (mg/l) | 13.35 |
| BOD (mg/l) | 20.5 |
| Cl (mg/l) | 83.83 |
| Si (mg/l) | 5 |
| N-$NO_2$ (mg/l) | 0.078 |
| N-$NO_3$ (mg/l) | 4.372 |
| N-$NH_4$ (mg/l) | 0.024 |
| DIN (mg/l) | 4.474 |
| TN (mg/l) | 4.692 |
| P-$PO_4$ (mg/l) | 0.005 |
| TP (mg/l) | 0.014 |
| Mud (0.0625–2 mm) (%) | 50 |
| Silt & Clay (<0.0625 mm) (%) | 50 |
| Aquatic vegetation (%) | 30 |
| Canopy cover (%) | 20 |
| Air temperature (°C) | 30 |
| Physicochemical quality | Good |

sequences present in GenBank using BLAST (Altschup *et al.*, 1990) and with the BOLD database (Ratnasingham and Hebert, 2007). No indels and no stop codons were observed, after translating all sequences to amino acids. The identification of the newly sequenced individuals (a single haplotype: GenBank SUBMITTED) as *C. fluminea* was confirmed by both BOLD (99.8% match) and BLAST (99.6%).

The physicochemical characteristics of sampling locality are presented in Table 1. Overall, the physicochemical quality of the sampling site was good, as classified according to the physicochemical quality index (Skoulikidis *et al.*, 2006); however, fish-based assessments of the lower Erythropotamos water body have indicated moderate to poor conditions in recent years, mainly due to degradation from water abstraction, hydromorphological changes and seasonal eutrophic conditions that affect natural fish assemblages (Zogaris *et al.*, 2018). Although the potential use of *Corbicula* species for biomonitoring purposes has been suggested (*e.g.* Doherty, 1990), *C. fluminea* is considered generally intolerant to severe pollution (*e.g.* Ilarri *et al.*, 2010). Europe's Directives on improving ecological quality may have facilitated invasion by providing better environmental conditions for all life stages of *C. fluminea* (Karatayev *et al.*, 2007) as this seems to be correlated with the reinvasion of zebra mussel in some European rivers (Jantz and Neumann, 1992) and other invasive species in the Laurentian Great Lakes of North America (Mills *et al.*, 2003).

In most cases dealing with biological invasions, it is very difficult to trace the introduction vectors and pathways and the subsequent dispersion. In Europe for example, inland waterway connectivity facilitates the spread of invasive non-autochthonous species (Leuven *et al.*, 2009; Panov *et al.*, 2009). European floodplains (*i.e.* low altitude rivers) are the main corridor routes consisting of an interlinked network of 30 main canals with more than 100 branches, and more than 350 ports (Panov *et al.*, 2009; Galil *et al.*, 2008). An increasing number of dams have also facilitated many lentic alien and translocated species (Koutsikos *et al.*, 2019) and large river systems such as the Evros have experienced recent developments, particularly in the Evros river basin's Bulgarian section.

We speculate that *C. fluminea* reached the Greek waters of Erythropotamos river most possibly through passive downstream transport from Bulgaria. The Evros river basin has been called an "open-door" for invasive alien biota (Ozulug *et al.*, 2018). This refers to human-assisted aquatic species dispersal which disperses downstream of Bulgarian entry points. In this way, the north-south running Evros and its tributaries such as the Erythropotamos, are pathway gates for species entering the Aegean basins of Greece and Turkey from Bulgaria's Danubian and Black Sea basin invasive alien biota (Zogaris *et al.*, 2019). In recent years this transport could have been either facilitated by human activities, such as increased hydroelectric dam-building, fishing and various recreational activities or by bird transfer (Crespo *et al.*, 2015; Coughlan *et al.*, 2017) or naturally by downstream drift. *C. fluminea* has found to be capable of floating after being exposed to gentle water currents produced by an aquarium filtration system (current speeds, 10 to 20 cm/sec; Prezant and Chalermwat, 1984). The first record of *C. fluminea* in Bulgaria was from the Danube River at Vetren in 2001 and after 11 years the species has occupied the entire Bulgarian stretch of the Danube, from Vrav to Vetren, where it reached densities up to 16,560 ind/$m^2$ (Hubenov *et al.*, 2013). Recently, the species was also found in lentic waters, two reservoirs and one sand-pit lake, located at altitudes of up to 525 m a.s.l. (Hubenov *et al.*, 2013). Based on the Bulgarian invasion experience with the species, it is anticipated that the entire Evros River Basin is or soon will be occupied by the clam. Thus, more detailed surveillance is urgently needed in order to assess the degree of *C. fluminea* establishment in the basin and adjacent freshwater bodies.

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