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Review

Review and new records of non-indigenous freshwater invertebrates in the Ebro River basin (Northeast Spain)

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Abstract

This paper is the first attempt to compile a list of the non-indigenous aquatic invertebrate species currently known to occur in the Ebro River basin (Northeast Spain). A total of 23 exotic aquatic species have been recorded in this area, and one more (the branchiobdellida *Xironogiton victoriensis*) may also be present in this Iberian basin. Due to the negative impacts that biological invasions cause to ecosystems and to human activities, it is important to state the introduction pathways and prevent the spread of already introduced species. In order to fight against biological alien species invasions, development of national strategies on invasive alien species and international cooperation are considered essential.

Key words: invasive species, invertebrates, Ebro River basin, Iberian Peninsula

Introduction

Together with habitat destruction or degradation, biological invasions represent one of the major threats to biodiversity (Clavero and García-Berthou 2005; Ding et al. 2008). This negative effect has been estimated to be greater in freshwater than in terrestrial ecosystems (Sala et al. 2000), so that freshwater ecosystems are experiencing a decline in biodiversity and modifications that more often are associated with environmental degradation and major disturbances (Conlan 1994; MacNeil et al. 2004). This higher vulnerability of inland waters to species introductions and their subsequent spread is both anthropogenic and natural; on one hand it is due to the effects of intensive human usage and hydromorphological changes to aquatic resources (Ricciardi 2001), and on the other hand, due to natural catchment linkages among streams and lakes, the effects of downstream transport, and the dispersal capability of aquatic organisms (Gherardi et al. 2008). The rate of freshwater invasions is increasing in several countries due mainly to increased global transport, with more shipping and canalization (e.g. Minchin 2007; Roll et al. 2007; Semenchenko et al. 2009).

In addition to environmental impacts, alien species can also create serious economic and health impacts (Hallegraeff 1998; Pimentel et al. 2005; Gherardi et al. 2008). Moreover, as more pollution-tolerant invaders can often replace sensitive native species, metrics used for the assessment of the ecological status of aquatic ecosystems for the implementation of the European Water Framework Directive (WFD) may be affected by the change of benthic assemblages resulting from biological invasions (Arndt et al. 2009; MacNeil and Briffa 2009). Due to all of these factors, studies on the presence and distribution of invasive species are important in order to assist freshwater ecosystem conservation and management, especially important in regions with high biodiversity and a high number of endemic species. Moreover, inventories of non-indigenous species could help to describe patterns of invasion at both global, regional and river basin scales. Ultimately, an understanding of the mechanisms of previous invasions may help protect aquatic ecosystems from the impacts of future invaders.

The Iberian Peninsula is a hotspot of biodiversity (Médail and Quézel 1999) and knowledge of the invasive species inhabiting it is essential for conservation and environmental management. Although naturalized exotic vertebrates and plants in this region have received considerable attention, invasive invertebrates are very poorly studied and there are very few available reviews of selected taxa of invertebrate invaders in the Iberian Peninsula (García-Berthou et al. 2007). Although probably many people in wildlife studies, game wardens, and water quality monitoring samplings have records of some alien species, this information is just left in their minds or lost in reports, and only a part of these records are published in local or international journals. Fortunately, in the last few decades scientists and environmental agencies have become increasingly interested in biological invasions (Gherardi 2007a; Hulme et al. 2008), and the introduction and establishment of invasive species are considered of a transboundary (within and across country) nature and of increasing global concern (Chandra and Gerhardt 2008).

In order to improve the knowledge of alien invertebrates in the Iberian Peninsula, this study reviews the species composition and distribution of aquatic non indigenous invertebrates established in the Ebro River basin (Spain), according to published scientific literature and to data obtained from different monitoring studies carried out by the authors during the last five years.

Materials and methods

The Ebro River basin is located in the Northeast of the Iberian Peninsula (Western Europe). This basin extends to the North to the Pyrenees Mountains, to the Southwest to the Iberian Mountains and the east with the Coast-Catalonian Mountains, discharging into the Mediterranean Sea near Tortosa. The drainage area of the basin is 85.362 km^2 and there are 347 main rivers (with length of approximately 12000 km).

Data about the invertebrate alien species presence in the Ebro River basin were obtained from different monitoring studies (mainly fish community surveys by electrofishing and biological water quality assessment by macroinvertebrate hand-netting) carried out the last five years. Additional data were also extracted from different published papers and studies carried out in the Ebro River basin.

Results and discussion

Table 1 provides a summary of all the invertebrate alien species that have been recorded in the Ebro River basin, according to our sampling data and to the bibliography. Annex 1 summarizes the invertebrate alien species records according to the published bibliography and Annex 2 shows new records in the Ebro River basin. In some little-studied invertebrates groups (e.g. in parasitic Platy-helminthes) our knowledge about alien species is very limited to some specific areas or basins. It is possible that more alien species within these groups could be present in Ebro River basin, as increasing extent and rapidity of anthropochore invertebrate and fish movements are causing increased concern related to awareness of their potential and known capacity for disseminating parasites (Kennedy 1993). The aquatic invertebrate non indigenous species recorded at this time (2009) in the Ebro River basin are listed and commented below.

Craspedacusta sowerbii Lankester, 1880

This species is a freshwater jellyfish that prefers standing water and are not generally seen in fast flowing rivers. It is usually found in calm, freshwater reservoirs, lakes or impoundments, although has also been recorded in river systems. The species is indigenous to the Yangtze River valley in China, but nowadays has colonized all continents except Antarctica (Dumont 1994). It was probably transported with ornamental aquatic plants from its native region in China or from other colonized regions. The life cycle of *C. sowerbii* includes both a polyp and a medusa stage. Polyps are capable of budding to produce hydromedusae, as well as either daughter polyps that remain attached to the parent, forming a colony, or frustule larvae which move to new locations before metamorphosing into new polyps, whereas mature hydromedusae reproduce sexually by broadcasting gametes into the water (Sasaki 1999), although sexual reproduction is relatively rare because most populations of jellyfish are strictly all male or all female (Pennak 1989). The impact of this jellyfish is unclear or remains insufficiently studied. The

Table 1. Non-indigenous aquatic invertebrates species recorded in the Ebro River basin (Unint.: Unintentional; Int.: Intentional).

GROUP/Species	Indigenous distribution	Mode of arrival	Pathway	Date of first record	Reference
CNIDARIA					
Craspedacusta sowerbii Lankester, 1880	Asia	Unint.	Ornamental trade?	2009	URS and ACA, pers. comm.
PLATYHELMINTHES, TURBELLARIA					
Dugesia tigrina (Girard, 1850)	North America	Unint.	Ornamental trade	1982	Baguña et al. 1982
PLATYHELMINTHES, MONOGENEA					
Pseudodactylogyrus anguillae (Yin and Sproston, 1948)	Asia and Australia	Unint.	Stocking	2005	Maillo et al. 2005
PLATYHELMINTHES, TREMATODA					
Phyllodistomum folium (Olfers, 1816)	Ponto-Caspian?	Unint.	Shipping/Stocking	2006	Peribañez et al. 2006
NEMATODA					
Anguillicola crassus Kuwahara, Niimi and Itagaki, 1974	Asia	Unint.	Stocking	2005	Maillo et al. 2005
MOLLUSCA, GASTROPODA					
Gyraulus chinensis (Dunker, 1848)	Asia	Unint.	Culture (crops)?	1995	Welter-Schultes 2009
Melanoides tuberculata (Müller, 1774)	Africa and Asia	Unint.	Ornamental trade	1995	Álvarez Halcón 1995
Physella acuta (Draparnaud, 1805)	North America	Unint.	Ornamental trade	1920	Bofill and Haas 1920
Pomacea canaliculata (Lamarck, 1819)	South America	Unint.	Culture (crops)?	2009	GEIB 2009
Potamopyrgus antipodarum (Gray, 1843)	New Zealand	Unint.	Shipping/Stocking?	1967	Vilella 1967
MOLLUSCA, BIVALVIA					
Corbicula fluminea (Müller, 1774)	Asia, Africa and Australia	Unint.	Shipping/Stocking?	1997	Lopez and Altaba 1997
Dreissena polymorpha (Pallas, 1771)	Ponto-Caspian	Unint.	Shipping/Stocking	2001	Oscoz et al. 2006
ANNELIDA					
Xironogiton victoriensis (?) Gelder and Hall, 1990	North America	Unint.	Stocking	2008	This study
CRUSTACEA, BRANCHIOPODA					
Artemia franciscana (Kellogg, 1906)	North, Central and South America	Unint.	Stocking/Aquaculture?	2007	Amat et al. 2007
CRUSTACEA, OSTRACODA					
Dolerocypris sinensis Sars, 1903	Asia	Unint.	Culture (crops)	1986	Fores et al. 1986
Cypris subglobosa Sowerby, 1840	America, Africa and Asia	Unint.	Culture (crops)	1986	Fores et al. 1986
Isocypris beauchampi (Paris, 1920)	Africa	Unint.	Unknown	1996	Baltanas et al. 1996
Stenocypris major (Baird, 1859)	Circumtropical	Unint.	Culture (crops)	1986	Fores et al. 1986
Strandesia vinciguerrae (Masi, 1905)	Africa	Unint.	Culture (crops)	1986	Fores et al. 1986
Tanycypris sp.	Asia	Unint.	Culture (crops)	1986	Fores et al. 1986
CRUSTACEA, COPEPODA					
Lernaea cyprinacea Linnaeus, 1758	Asia	Unint.	Stocking	1995	Sterling et al. 1995
CRUSTACEA, DECAPODA					
Cherax destructor (Clark, 1936)	Australia	Int.	Stocking	1984	Bolea 1996
Pacifastacus leniusculus (Dana, 1852)	North America	Int.	Stocking	1986	Muez and Muez 1988
Procambarus clarkii (Girard, 1852)	North America	Int.	Stocking	1978-1979	Alonso et al. 2000

impact of freshwater medusa on the plankton community was considered to be insignificant, but the impact may dramatically increase with high medusa densities, and they could shape zooplankton composition indirectly by selectively predation (Spadinger and Maier 1999). They also could increase consumption of dissolved oxygen when medusa densities explode affecting fish communities and other aquatic organisms.

Our knowledge of its distribution in the Iberian Peninsula is unsatisfactory, perhaps because the polyp is rarely encountered and hydromedusae are produced only sporadically.

They have been recorded in different provinces of Spain (Pérez-Bote et al. 2006), and recently (September 2009) it has been recorded in the Ebro River basin in the Montcortes lake (in the province of Lleida) (URS & ACA, pers. comm.).

Dugesia tigrina (Girard, 1850)

This freshwater planarian is native to North America, where it is widely distributed. It was introduced into Europe in the 1920s (firstly in Germany and in England), expanding southwards and eastwards during the next decades, and reaching France, Spain and Italy in the late 1960s to early 1970s. However Ribas et al. (1989) and Benazzi (1993) suggested that *D. tigrina* spread from several independent introductions from the native area. This species was probably introduced as aquarium inhabitants by means of imported water plants (Van Der Velde 1975). Although while generally it has not caused any significant effects where introduced, displacement of native flatworms populations by *D. tigrina* has been reported in a Welsh lake (Reynoldson 1985). On the other hand, the considerable overlap in the diet composition of *D. tigrina* and native flatworm species suggest the potential for severe inter-specific competition for food (Gee and Young 1993). This species has been recorded in the lower stretch of the Noguera Ribagorzana and Ebro rivers (Baguña et al. 1982; Ribas et al. 1989; Vila-Farré et al. 2008) where it coexists with other native planarians.

Pseudodactylogyrus anguillae (Yin and Sproston, 1948)

P. anguillae is a monogenean parasite of anguillid eels indigenous to Asia and Australia. They were first detected in Europe in the late 1970s, brought from the Far East with juvenile Japanese eel, *Anguilla japonica* (Temminck and Schlegel, 1847). Different authors have reported this parasite from European eel (*Anguilla anguilla* (Linnaeus, 1758)), in cultured and wild populations (e.g. Nie and Kennedy 1991; Maillo et al. 2005). European eel is more susceptible to this parasite than their original hosts, but under favorable natural conditions it apparently does not cause the death of the new host, although in eel farms it has caused great economic losses (Koie 1991). This species has been recorded in different provinces of Spain, and it has been recorded in the Ebro delta parasitizing European eel (Maillo et al. 2005).

Phyllodistomum folium (Olfers, 1816)

This species is an endosymbiont of Zebra mussel (*Dreissena polymorpha* (Pallas, 1771)) but adult forms parasitize some fish species (Nie and Kennedy 1991; Karatayev et al. 2000). Apparently it is native from the Ponto-Caspian region (as its host). Larval stages of this helminth have been recorded recently in two mussels of the Ebro River (Peribañez et al. 2006).

Anguillicola crassus Kuwahara, Niimi and Itagaki, 1974

This nematode is native to Southeast Asia and its natural host is the Japanese eel. It probably arrived in Europe with eels imported from south-eastern Asia in about 1980 (Køie 1991). It successfully colonized most European countries throughout the 1980s, especially in freshwater environments (Ashworth and Blanc 1997). Uncontrolled movement of infected eels by man has been identified as the main vector for the continued spread of *A. crassus* in Europe. Adverse effects on wild and cultured eels may occur when the level of infestation is high or eels are under stress, and under unfavorable environmental conditions it may lead to their death (Molnár 1993; Kirk 2003). This species has been recorded in the Ebro delta parasitizing European eel (Maillo et al. 2005).

Gyraulus chinensis (Dunker, 1848)

G. chinensis is a small freshwater snail of small water bodies (e.g. swamps, rice fields) native to South and East Asia. It has been recently reported from swamps, rice fields, thermal springs and greenhouses in several European countries (Beran and Glöer 2006), although it has been also recorded from rivers (Soler et al. 2006). This species has been recorded in the Ebro delta by J. Hartmann (Welter-Schultes 2009).

Melanoides tuberculata (Müller, 1774)

The red-rimmed melania (*M. tuberculata*) gastropod snail is native to subtropical and tropical areas of northern and eastern Africa and southern Asia. This species has become established outside of its natural range in large part through the activities of aquarists (Duggan 2002), and it has been introduced in America, New Zealand and Europe including Spain (Neck 1985; Vidal Abarca and Suárez 1985; Duggan 2002; Santos et al. 2007; Gherardi et al. 2008). Red-rimmed melania has been reported to displace native gastropods where introduced. Moreover, it serves as transmission vector for several dangerous parasites to humans and it is also a host for a trematode parasite, which has been found to infect some fish species. This species has been recorded from the Ebro River basin in thermal waters from Alhama de Aragón, near Jalon River (Álvarez Halcón 1995).

Physella (Costatella) acuta (Draparnaud, 1805)

Although this mollusc has sometimes been called European Physa, its geographical origin was problematic and doubtful. This species was first described in Europe in 1805, from the Garonne river and its presence in North America was not reported until the 1990s (García-Berthou et al. 2007). Nowadays, three types of evidence indicate, however, that *P. acuta* is indigenous to North America and not to Europe: (1) the lack of records of *Physella* shells from European sediments older than the 18th century (Lozek 1964); (2) recent studies using internal morphological comparisons (Anderson 2003) and reproductive isolation experiments (Dillon et al. 2002); and (3) some historical data of the cotton trade between France and the United States in the 18th century that could explain the arrival of this species to the River Garonne, where it was first observed (Anderson 2003). The species seems to have first spread through Mediterranean regions and then more slowly into northern Europe, Africa, Asia and Australia (Dillon et al. 2002; Sinha et al. 2003; Soler et al. 2006; Semenchenko et al. 2008). This species has been widely recorded in the Iberian Peninsula and the Canary Islands (Frank 1987; Vidal Abarca and Suárez 1985; Ibáñez and Alonso 2003; Soler et al. 2006), including the Ebro River basin. This species can survive well under temporary harsh conditions (extreme temperature and water pollution) and it has even been found in sewage treatment plants (Larraz et al. 2007).

Pomacea canaliculata (Lamarck, 1819)

The golden apple snail (*P. canaliculata*) is a freshwater snail native to South America with a voracious appetite for water plants. It was introduced in south east-Asia purposely to provide additional protein for people, but it has become a serious rice pest in most South-east and East Asian countries, because it damages the young rice seedlings (Halwart 1994), and also it is the primary route of infection of the nematode *Angiostrongylus cantonensis* (Chen, 1935). Golden apple snail is one of the 100 world's worst invasive alien species (Lowe et al. 2000). It is considered to be introduced in most of southern, eastern and south-east Asia, Hawaii, Guam, Papua New Guinea, the Dominican Republic and USA. It may adversely affect not only agriculture, fisheries and human health but also the native ecosystem (Maezono and Miyashita 2003). It has been found recently in the Ebro delta (GEIB 2009).

Potamopyrgus antipodarum (Gray, 1843)

The New Zealand mud snail, *Potamopyrgus antipodarum*, is an aquatic snail native to New Zealand, and it is the most widespread non-indigenous mollusc in the world. It has spread widely and has become naturalized and an invasive species in Australia, Tasmania, Asia, Europe and North America (Ponder 1988; Cejka et al. 2008; Davidson et al. 2008; Naser and Son 2009), most likely due to inadvertent human intervention. It was introduced into Europe (in the Thames River estuary, England) at the end of the 19th century in drinking water barrels on ships from Australia (Ponder 1988). The snails were probably liberated while washing or filling water barrels or tanks and, because they can survive in brackish water, they could probably survive liberation into estuarine areas such as the Thames River. It can inhabit a wide range of ecosystems, including rivers, reservoirs, lakes, and estuaries. Mud snail is able to withstand desiccation and a variety of temperature regimes and it can spread quickly because of its ability to cling to anything from floating leaves to wading anglers. Effects of mud snail can include direct effects by displacement of native invertebrates or indirect effects on the trophic dynamics or physical characteristics of an ecosystem, and it can also play a role in the transmission of trematode parasites (Morley 2008; Son 2008). However, Múrria et al. (2008) found that in a small Mediterranean stream this species had relatively high abundances with relatively low impact on macroinvertebrate community structure, suggesting low abilities of *P. antipodarum* to attain higher densities in the harsh hydrologic condition of Mediterranean streams characterized by seasonal droughts and floods. Mud snail has been widely recorded in the Iberian Peninsula (Frank 1987; Vidal Abarca and Suárez 1985; Soler et al. 2006), and it is also widely distributed in the Ebro River basin (Vilella 1967; Altimira and Balcells 1972; Larraz 1986; Larraz and Equisoain 1993).

Corbicula fluminea (Müller, 1774)

The Asian clam (*C. fluminea*) is a freshwater bivalve inhabiting Asia, Australia and Africa, but since the last century it has dispersed in North America and Europe (Araujo et al. 1993; Brancotte and Vincent 2002). It is considered one of the most important non indigenous species in aquatic ecosystems. The introduction and subsequent dispersion of Asian clam in aquatic ecosystems is probably a result of various human activities (e.g. ballast water transport, utilization as fish bait, aquarium releases, transport of specimens as a tourist curiosity or the juvenile byssal attachment to boat hulls) (Sousa et al. 2008), but it has extensive capacities for natural dispersion since the pediveliger and juveniles are passively transported by fluvial or tidal currents, being also transported on the feet or feathers of aquatic birds (Brancotte and Vincent 2002; Sousa et al. 2008). The introduction of this species is a threat to the native biodiversity and ecosystem functioning with potential repercussions in food webs, biogeochemical cycles and human economy (Araujo et al. 1993; Sousa et al. 2008). Ecologically, this species can alter benthic substrates, compete with native mussel species for food and space, bioaccumulate contaminants and be a vector of introduction of new parasites and diseases. In terms of economic impacts, Asian clam is a known biofouler in power plant and industrial water systems, and has also caused problems in irrigation canals and pipes.Since the first Iberian Peninsula record for the Asian clam at the beginning of the 1980´s, it has experienced an exponential increase up until 2008, and Asian clam is now present, at least, in more than six great Iberian basins (Pérez-Quintero 2008). In the Ebro River basin it was found first in the Ebro delta (Lopez and Altaba 1997), and in the lower stretch of the Ebro River (Oscoz et al. 2006). However the Asian clam was found in irrigation canals from Navarra (Araujo 2004) and hence expanded in different rivers of Navarra (Oscoz et al. 2008). In the 2009 macro-invertebrate samplings new records in the Ebro River basin were found downstream from the localities cited by Oscoz et al. (2008a) and in the Jalon River, showing that Asian clam is spreading throughout the Ebro River basin (Figure 1).

Dreissena polymorpha (Pallas, 1771)

The zebra mussel (*D. polymorpha*) is native to the Ponto-Caspian region (Black, Caspian and Azov Seas) in Eurasia, but during the last two centuries it has invaded large regions of Europe through rivers and artificial channels and since the end of the 1980s also North America. This species has been nominated as among 100 of the "World's Worst" invaders (Lowe et al. 2000) because it causes severe economic and ecological impacts in the new colonized areas. Ecologically, and owing to its large filtration capacity and mass occurrence, this species competes with native mussel species for food and space, changes the phosphorus cycling, indirectly favours the blooms of blue green algae, increases water transparency, changes food-web dynamics, water chemistry, benthos and increases submerged macrophytes (reviewed in Minchin et al. 2002; Higgins et al. 2008). Moreover, limited availability of substrates in habitats with dense populations of zebra mussel leads to colonization of alternative substrates, including mobile hard-shelled animals (Duris et al. 2007). This species could also bioaccumulate contaminants and serve as the sole or intermediate host for different endosymbionts, including pathogenic helminth parasites of fish and waterfowl (Molloy et al. 1997; Karatayev et al. 2000). The sharp edges of the shells can also result in injuries to bathers. Economic impacts caused by zebra mussels include fouling of intake pipes, ship hulls, navigational structures or aquaculture cages. The main pathways of the expansion in the range of zebra mussel are through inland navigation, particularly since the opening of new waterways between eastern and central Europe at the beginning of the 1800s, the transfer of animals (including crayfish) for stocking in farms and the introduction into lakes of mussels attached to boat hulls. Larvae and adults may be distributed in ballast water or as fouling on ship and boat hulls, navigation buoys, fishing vessel wells, as well as by transport of timber or river gravel, fish stocking water and fishing equipment (Reynolds and Donohoe 2001).

The zebra mussel was first discovered in Spain in 2001 at Ribarroja Reservoir (lower Ebro River), and probably reached the Ebro River via sport fishing boats or by fish transport across the Pyrenees (Durán and Anadón 2008; Rajagopal et al. 2009). According to Rajagopal et al. (2009),

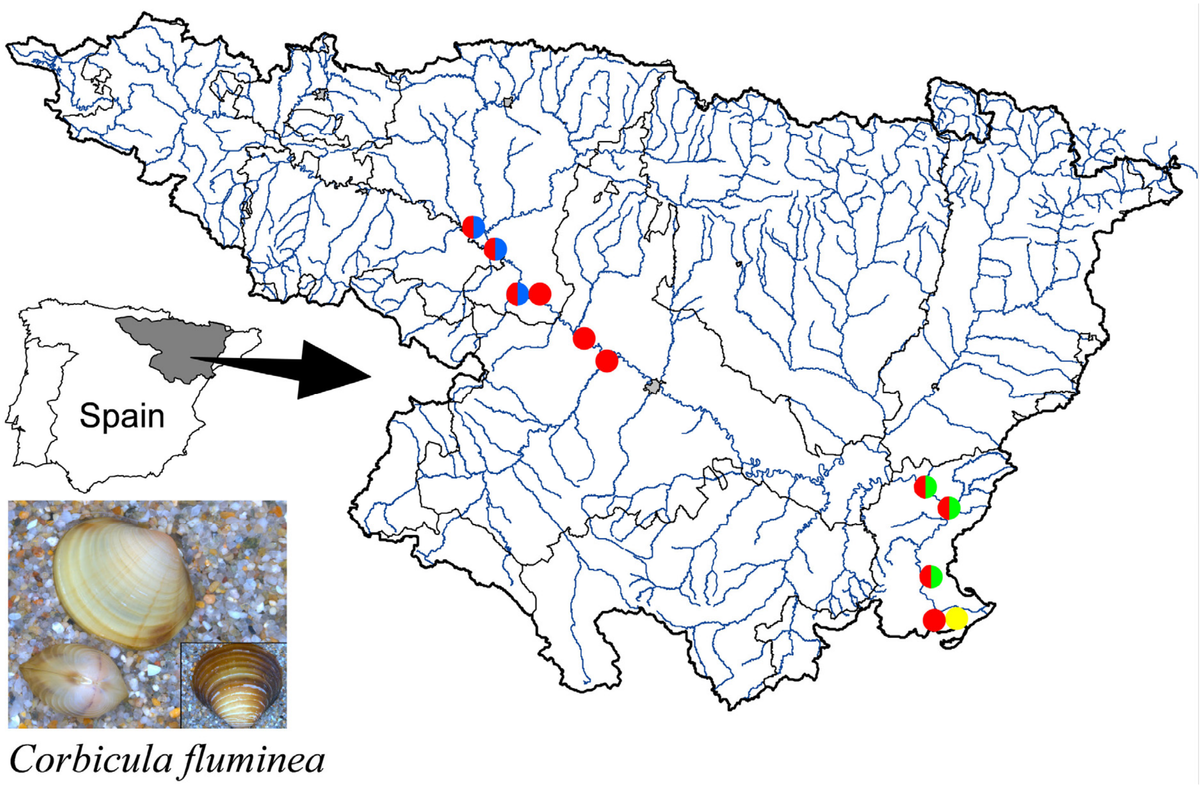

Figure 1. Presence of *Corbicula fluminea* in River Ebro basin. (Yellow circles: Lopez and Altaba (1997); Green circles: Oscoz et al. (2006); Blue circles: Oscoz et al. (2008a); Red circles: This study).

the recent invasion of zebra mussels in Spain is most likely from France. The relatively late invasion of Spain was most likely due to the presence of the Pyrenees, which isolate the Iberian Peninsula from the rest of the European continent, and act as a dispersal barrier for this species. However, since its introduction, the zebra mussel has invaded a great part of the Ebro river basin in the last years (for more details of invasion history and recent data in this basin see Durán and Anadón (2008) and Durán et al. (2010). The primary pathway for the spread of the zebra mussel in this basin is probably via transport on recreational boats and associated equipment, so in order to stop the invasion of new areas in this basin the Ebro Hydrographic Confederation has changed the navigation rules in the Ebro River basin. In addition, any equipment that has been in contact with this water must be disinfected, either by soaking, immersion or spraying with a disinfectant solution (Durán and Anadón 2008).

Xironogiton victoriensis Gelder and Hall, 1990

This crayfish worm or branchiobdellida (Annelida: Clitellata) is an obligate ecto-symbionts native from North America living primarily on freshwater astacoidean crayfishes. This species has been introduced with their host, the signal crayfish (*Pacifastacus leniusculus* (Dana, 1852)), in Japan, Sweden, Finland, Italy and Spain (Gelder 1999; Oberkofler et al. 2002; Ohtaka et al. 2005; Klobucar et al. 2006; Gherardi et al. 2008). The relationship between branchiobdellidans and their hosts is still poorly known. The crayfish worms have been variously described as diatom feeders, blood feeders or as parasites only in the adult stage, but according to Gelder (1999), branchiobdellidans are defined as ectosymbionts and not parasites. This species has not yet been found on European native crayfish, but has always been found on signal crayfish. No certain explanation can be given to this fact, but low density of signal crayfish in the studied localities, competition between the branchiobdellida species or the incompatibility to colonize other crayfish species has been hypothesized (Oberkofler et al 2002). Further research is needed to ensure that this exotic endosymbiont will not affect to European native crustaceans. Although this species has only been found in Spain in the Altube River (Nervión River basin) (Gelder 1999), in 2008 some branchiobdellidans specimens were found on the pincers of signal crayfish. Although they have not been yet classified, it is probably that these specimens belong to *X. victoriensis*. Further studies on identification and distribution in the Ebro River basin are needed, as more specimens of branchiobdellidans were found in more rivers during monitoring in 2009.

Artemia franciscana (Kellogg, 1906)

North American brine shrimp (*A. franciscana*) is endemic to hypersaline biotopes of North, Central and South America, but has been exported worldwide since the 1950's for use in the aquarium trade and in fish farming. Amat et al. (2005) suggest that exotic *A. franciscana* populations originate as intentional or non-intentional inoculations through aquacultural (hatchery) effluents or via pet market activities, whereas Gherardi et al. (2008) suggest that its introduction in Italy was intentional. However this species can also disperse readily via gut passage through migratory shorebirds (Green et al. 2005). In Europe *A. franciscana* was first detected in 1981 in the Portuguese Algarve (Amat et al. 2005) and has since spread to different important Iberian sites for shorebirds along the East Atlantic Flyway, as well as to France and Italy (Amat and Green 2005; Green et al. 2005; Mura et al. 2006; Amat et al. 2007). Native populations of *Artemia* in the Mediterranean region are under severe threat from competition with this expanding species (Amat et al. 2005). In the Ebro River basin this exotic species has been found in Ebro delta salterns (Alfaques Bay, Tarragona) (Amat et al. 2007).

Dolerocypris sinensis Sars, 1903

According to McKenzie (1986), Rossi et al. (2003) and to García-Berthou et al. (2007) this freshwater ostracod is native to Asia, although it is frequently encountered in the circum-Mediterranean region (Meisch 2000) and Baltanás et al. (1996) cited it as a Paleartic species. It is supposed to have been introduced in Europe along with such useful plants as rice and hemp (McKenzie and Moroni 1986). This species has been cited in rice fields of the Ebro River delta (Forés et al. 1986).

Cypris subglobosa Sowerby, 1840

This non-marine ostracod is native to tropical and subtropical areas from America, Africa and Asia and, as in other ostracods found in ricefields, it has been introduced unintentionally by humans (Baltanás et al 1996). This species was recorded in rice fields of Ebro River delta (Forés et al. 1986).

Isocypris beauchampi (Paris, 1920)

According to Rossi et al. (2003) and García-Berthou et al. (2007) this freshwater ostracod is native to Africa, although Baltanás et al. (1996) point out that it is widely distributed in Western Europe but with a low number of records. It has been reported in Spain in five localities (Baltanás et al. 1996) and it seems to tolerate warmer waters (Rieradevall and Roca 1995). In the Ebro River basin it has been reported from the province of Alava (Baltanás et al. 1996).

Stenocypris major (Baird, 1859)

This ostracod has a circumtropical distribution, but it has been also cited in Spain, southern France, Italy, the Balkans and Turkey (Meisch et al. 2007). It is supposed that this species was introduced in Europe along with some tropical plants (Baltanás et al. 1996; Rossi et al. 2003). In the Ebro River basin this ostracod species has been cited in rice fields of the Ebro River delta (Forés et al. 1986).

Strandesia vinciguerrae (Masi, 1905)

This freshwater ostracod seems to be a native of Africa (Baltanás et al. 1996; Rossi et al. 2003; García-Berthou et al. 2007), but it has been reported from southern Europe (Italy, Balkans, and Spain), Canary Islands, Pacific Islands and India (Beyer et al. 1997; Meisch et al. 2007). However different authors suggest that it is a non indigenous ostracod in Europe (Baltanás et al. 1996; García-Berthou et al. 2007; Gherardi et al. 2008). In the Ebro River basin this species has been cited in rice fields of Ebro River delta (Forés et al. 1986).

Tanycypris sp.

This is a genera of Ostracoda distributed in tropical and sub-tropical regions from Asia, but one species has been cited in some regions of Europe (Rossi et al. 2003; Karan-Znidarsic and Petrov 2007; Gherardi et al. 2008), specially in rice fields (McKenzie and Moroni 1986). In the Ebro River basin, Forés et al. (1986) report this genus from rice fields of the Ebro River delta. Probably this may be the same species of this genus already reported in Europe (*Tanycypris pellucida* (Klie, 1932)), but in any case it must be considered as a non-indigenous taxon (Baltanas et al. 1996).

Lernaea cyprinacea Linnaeus, 1758

The anchorworm (*L. cyprinacea*) is a copepod parasite (only female) of freshwater and marine fish, although it can also use amphibians as hosts (Wellborn and Lindsey 1970; Kupferberg et al. 2009). It is native of Asia, but nowadays it is widely distributed in the world. Its spread is mainly attributed to international trade of tropical fishes (Robinson and Avenant-Oldewage 1996). It is usually reported as having a wide range of host susceptibility.

This parasite can have serious deleterious effects on their freshwater fish hosts, and death can occur as a result of hemorrhage and secondary infections. It is a harmful parasite of cultured and aquarium fishes. This species has been reported from different fish species and areas of the Iberian Peninsula (Moreno et al. 1986; Pérez-Bote 2000; Pérez-Bote 2005; Gutiérrez-Galindo and Lacasa-Millán 2005). In the Ebro River basin this species was reported from Ebro nase (*Parachondrostoma miegii* (Steindachner, 1866)) specimens in a tributary of the Ebro River (Sterling et al. 1995).

Pacifastacus leniusculus (Dana, 1852)

The signal crayfish (*P. leniusculus*) is endemic to northwestern USA and southwestern Canada where it occupies a wide range of habitats from small streams to large rivers and lakes (Souty-Grosset et al. 2006), but it was introduced into more southerly states, as well as into Europe and Japan. Signal crayfish is an aggressive competitor and it has been responsible for displacing indigenous crayfish species wherever it has been introduced. In addition, it is a carrier and it acts as a vector for the crayfish plague oomycete pseudofungus (*Aphanomyces astaci* (Schikora, 1906)). It can also have a considerable impact on populations of macroinvertebrates, fish, and aquatic plants (Nyström and Strand 1996; Guan and Wiles 1997; Nyström 1999; Crawford et al. 2006; Gherardi 2007b) and their burrowing activities can have a serious impact on bank morphology, causing considerable damage to river and lake margins (Souty-Grosset et al. 2006). This species also has introduced branchiobdellidans in several countries (see *X. victoriensis* section). Due to the fact that the introduction of signal crayfish has a negative impact on the native species and the ecosystem, it is important to emphasize the necessity of preventing its spread. The management of the exotic crayfish populations should inevitably be focused on controlling their means of dispersal, which usually occur by human activities. It has been proposed the use of fishing pressure as a way to control introduced crayfish populations (both signal and red swamp crayfish), but it should be carefully addressed. Fishermen could act as agents for the dispersal of exotic crayfish, especially in areas where there is a high demand for fishery development (Alonso et al. 2000). Once an introduced species is established in a new area, managers face the dilemma of whether it is better to allow or forbid fishing. Opening the fishery will result frequently in the spread of the species to other places, whereas on the other hand it is difficult to explain to fishermen and the local public why they can not exploit these exotic crayfish populations. It would be important to develop and apply methods for eradicating populations as an alternative to this dilemma, even if they were not very efficient (Alonso et al. 2000).

In Europe, signal crayfish was first released into Swedish waters in 1960, in order to replenish stocks of crayfish with an ecological and gastronomic homologue replacing noble crayfish (*Astacus astacus* (Dana, 1852)), which had been affected by crayfish plague (Holdich and Pöckl 2007). It became a popular species for stocking and culture, and as a result of secondary introductions (both from Sweden and North America) it was established in several European countries, making it the most widespread non-indigenous crayfish (Souty-Grosset et al. 2006). In most of the countries into which it has been introduced, it has become established in the wild either as a result of escapes or deliberate seeding of waters.

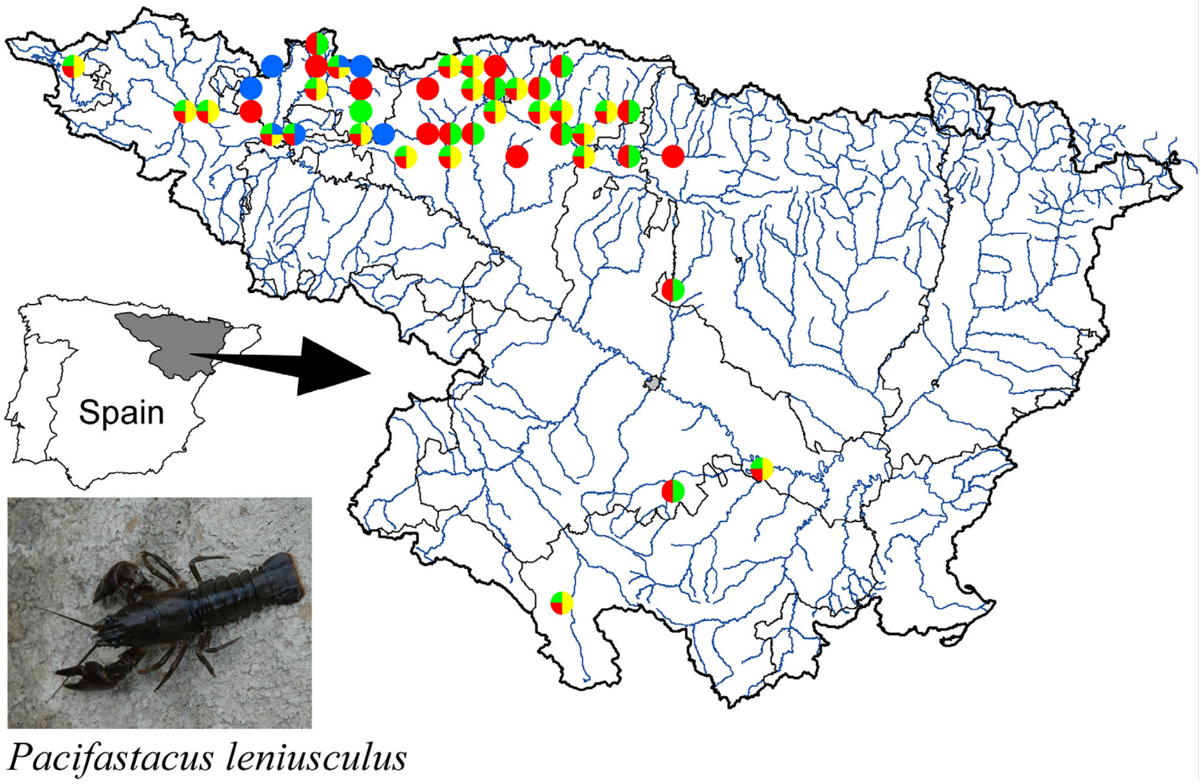

Figure 2. Presence of *Pacifastacus leniusculus* in River Ebro basin (Yellow circles: Oscoz and Durán (2005); Green circles: Oscoz et al. (2008b); Blue circles: Desma Estudios Ambientales (2009); Red circles: this study).

It was introduced in Spain in 1974-1975 in Tajo and Duero river basins (Habsburgo-Lorena 1979). Later different local administrations, specially Castilla-Leon, Pais-Vasco and Navarra (Carral et al 1993), carried out more stocking-programs in order to replenish stocks of crayfish with an ecological homologue replacing white-clawed crayfish, as well as to act as an "ecological barrier" to prevent red swamp crayfish (*Procambarus clarkii* (Girard 1852) colonization of new areas (Reynolds et al. 1992). However non scientific data has been found about habitat segregation between both exotic crayfish (Alonso et al. 2000), moreover it has been observed that both species can be present and coexist in the same river stretch (Oscoz et al. 2008b). On the other hand, signal crayfish inevitably escape from crayfish farms, and new populations can be detected in the vicinity of crayfish farms (Alonso et al. 2000).

In the Ebro River basin the signal crayfish is present mainly in the north-west region, although some specimens has been recorded in Aragón (Oscoz and Durán 2005; Oscoz et al. 2008b). This north-west area belongs to Castilla-Leon, Pais Vasco and Navarra territories, areas where stocking-progams were carried out by their local administrations. Figure 2 shows the signal crayfish records in the Ebro River basin according to our data.

Cherax destructor (Clark, 1936)

The yabby (*Ch. destructor*) is a crayfish native to eastern Australia, where it it is one of the principal cultured freshwater crayfish and is farmed commercially in different regions of Australia and North America (Souty-Grosset et al. 2006). This species is considered a delicacy, being sold live for restaurants in several European countries, such as Switzerland, Germany, and England (Souty-Grosset et al. 2006). Yabbies show strongly aggressive, competitive and predatory behaviour towards other animals, and burrowing by yabby can be a problem by the damage caused to crops, lawns, golf courses, dams walls, dykes, irrigation canals, rivers and lakes banks (Gherardi 2007b; Lynas et al. 2007).

The first European introduction of this species was carried out in a pond in Girona in 1983 for commercial farming, but fortunately no yabby population was established. However, in 1984-1985 another introduction was carried out into the province of Zaragoza and a population became established (Bolea 1996). Later, some specimens were found in ponds in Navarra. Recently a second European population of this species has been reported in Italy (Scalici et al. 2009). In the Ebro River basin there are currently four populations of yabby, three in Navarra and one in Zaragoza (Souty-Grosset et al. 2006). As this species is susceptible to the crayfish plague, it has been proposed to eradicate yabby populations using crayfish plague, either directly or with infected signal crayfish (Scalici et al. 2009). Nevertheless, the eradication of alien crayfish by spreading of crayfish plague could represent a serious risk of infection and disease for native populations of white-clawed crayfish (*Austropotamobius pallipes* (Lereboullet 1858)).

Procambarus clarkii (Girard, 1852)

The red swamp crayfish is native to the southcentral United States and north-eastern Mexico, but it has been transplanted world-wide (Souty-Grosset et al. 2006). It is a prolific and aggressive species, it tolerates bad environmental conditions (low oxygen concentrations, drought periods and a wide range of water salinity and acidity) and it is well adapted to life in areas with drastic, seasonal fluctuations in water levels, where it survives by digging deep burrows (Barbaresi and Gherardi 2000). As the preceding species, red swamp crayfish has negative environmental and economic impacts, and in some of the continents where it has been introduced it is often an invasive pest. Their burrowing activities can lead to damage to water courses, recreational areas and crops (particularly rice crops) and indirectly crayfish activity could increase water turbidity, thus reducing light penetration and thus plant production (Barbaresi and Gherardi 2000). Their feeding habits could lead to changes in food webs and even disappearance of some species, and it could have a potential impact on macroinvertebrates, macrophytes or amphibians (Cruz et al. 2006; Souty-Grosset et al. 2006; Gherardi 2007b; Dana and Ortega 2010). This species has also a negative impact to native fauna as it contributes to the spread of numerous parasitic helminths of vertebrates (Sogandares-Bernal 1965), as well as the crayfish plague fungus (it may harbour the fungus within its body in a chronic or latent infection and thus may function as a vector for the disease into Europe (Huner and Lindqvist 1995)). A high risk of diffusion of this fungus is also represented by the movement of fishermens' equipment, carrying spores, through different streams (Barbaresi and Gherardi 2000). For exotic crayfish, special attention should be given to live trade, as limiting their live commercialization is perhaps the most repeated advice issued by the scientific community for the control of introduced crayfish populations (Alonso et al. 2000). On the other hand, red swamp crayfish represents a valuable resource and could bring undoubted benefits to a local economy through its harvesting or fishery; according to Cano et al. (2003), in the Guadalquivir marshes the annual production is more than 3,000 Tm. This species has also been considered responsible for the increase of some carnivorous avian species and of the otter (*Lutra lutra* (Linnaeus, 1758)) (Beja 1996; Barbaresi and Gherardi 2000; Elvira et al. 2007; Gherardi 2007b).

In Europe it was introduced to southern Spain in 1973-1974 (Habsburgo-Lorena 1979) for aquacultural purposes, but soon became widely established in the wild and is now present in 13 European countries, including Sardinia, Sicily and the Azores, Balearic and Canary islands (Souty-Grosset et al. 2006). In Spain, after their introduction into the Guadalquivir marshes, red swamp crayfish dispersed very quickly by human means, and in a few years appeared in distant areas (for example the Albufera of Valencia, the Ebro delta, Zamora in Castilla-Leon, Lugo in Galicia). The absence of adequate legislation for preventing the introduction of non-native species and the trade of live crayfish were factors that contributed to the rapid spread of this species in Spain (Alonso et al. 2000). Today red swamp crayfish is the most abundant species in the Iberian Peninsula (Alonso et al. 2000; Souty-Grosset et al. 2006) and dense populations are found in most Iberian regions, specially in lower and medium reaches of main rivers (Alonso et al. 2000; Oscoz and Durán 2005).

In the Ebro River basin this species is widely distributed, specially in lower and medium reaches of the Ebro River and its tributaries,

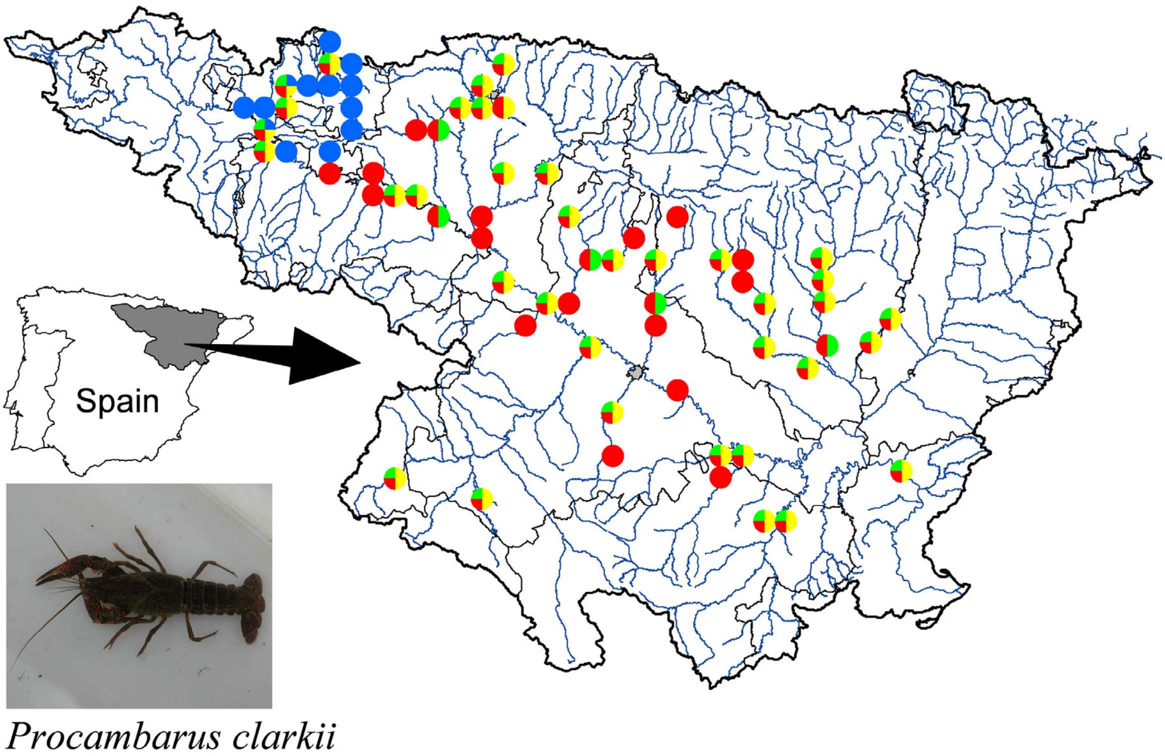

Figure 3. Presence of *Procambarus clarkii* in River Ebro basin. (Yellow circles: Oscoz and Durán (2005); Green circles: Oscoz et al. (2008b); Blue circles: Desma Estudios Ambientales (2009); Red circles: This study).

although some populations has been located in upper reaches near urban areas, dams or recreational ponds (Oscoz and Durán 2005; Oscoz et al. 2008b). Figure 3 shows the red swamp crayfish records in the Ebro River Basin according to our data.

Conclusions

According to these data, 23 exotic freshwater invertebrate species have been introduced into the Ebro River basin, and one more (the branchiobdellida *X. victoriensis*) could be also present in this area. The total number of introduced species we recorded is probably an underestimate of the real number of alien species inhabiting the Ebro River basin. As it noted by Gherardi et al. (2008), that notwithstanding the increased scientific interest for biological invasions during the past decade, there is still a gap of knowledge about some invertebrate taxa and functional groups.

Most of these exotic invertebrates probably arrived to Ebro River basin unintentionally, and were already found in other European countries (Gherardi et al. 2008), where accidental transport, in association with both fish (for aquaculture or stock enhancement) and crops, especially rice, have been the main vector of invertebrate introductions in aquatic ecosystems. Although, the ranking of likely species introduction vectors varies throughout Europe shipping and species movements for aquaculture or stocking purposes are considered the dominant invasion vectors in freshwater invaders (Gollasch 2006; 2007). An assessment of the pathways followed to alien species should be made on a case-by-case basis.

Biological invasions in freshwater ecosystems are a major threat to the biological diversity, economy, and human health and wellbeing. Aquatic non indigenous species are difficult to detect, tend to disperse rapidly and, by the time they are detected, they are already extremely difficult to eradicate and control. It is important to emphasize the necessity to study introduction pathways of exotic species in order to control their means of introduction, and to prevent the spread of already introduced species. The understanding of these introduction mechanisms in previous invaders could help to protect aquatic ecosystems from the impacts of future invasions. To address this problem it is essential to focus on prevention, by improving the capacity of countries/states and institutions to exchange information, address pathways of introduction, regulate importation and introduction of species, and mitigate the impacts once prevention has failed (Genovesi 2007). In order to reach these objectives each state should develop a national action strategy on exotic invasive species, and the different states and institutions should work jointly toward a consistent global policy on invasive non indigenous species.

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Annex 1. Records of freshwater invertebrate alien species in the Ebro River basin (Spain) according to published bibliography.

Species	Locality – River	Reference
Dugesia tigrina	Santa Ana Reservoir - Noguera Ribagorzana River	Baguña et al. 1982
	Near Xerta - Ebro River	Ribas et al. 1989
	Móra d'Ebre - Ebro River	Vila-Farré et al. 2008
	Deltebre - Ebro River	
	Ullals de Baltasar - Ebro River	
Pseudodactylogyrus anguillae	Ebro Delta	Maillo et al. 2005
Phyllodistomum folium	Ebro River	Peribañez et al. 2006
Anguillicola crassus	Ebro Delta	Maillo et al. 2005
Gyraulus chinensis	Ebro Delta	Welter-Schultes 2009
Melanoides tuberculata	Alhama de Aragón (Zaragoza)	Álvarez Halcón 1995
Physella acuta	Segre River Basin (Lleida)	Bofill and Haas 1920
	Zaragoza	Haas 1924
	Logroño (La Rioja)	Haas 1929
	Huesca	
	Zaragoza	
	Zaragoza	Navas 1932
	Huesca	Altimira and Balcells 1972
	Alamus (Lleida)	Bech 1974
	Navarra	Larraz and Equisoain 1993
	Corella - Alhama River	Larraz et al. 2007
	Villafranca - Aragón River	
	Yesa - Aragón River	
Pomacea canaliculata	Ebro Delta	GEIB 2009
Potamopyrgus antipodarum	Alfes - Set River (Lleida)	Vilella 1967
	Huesca	Altimira and Balcells 1972
	Zaragoza	
	Unzué - Zidacos River	Larraz 1986
	Yesa - Aragón River	
	Navarra	Larraz and Equisoain 1993
	Álava, Basque country	Desma Estudios Ambientales 2009
	Teruel	Álvarez-Halcon, pers. com.
Corbicula fluminea	Ebro Delta	López and Altaba 1997
	Peralta (Navarra)	Araujo 2004
	Lower Ebro River	Oscoz et al. 2006
	Peralta - Arga River	Oscoz et al. 2008a
	Funes - Arga River	
	Milagro - Aragón River	
	Tudela - Ebro River	
Dreissena polymorpha	Lower Ebro River	Oscoz et al. 2006
	Bubal Reservoir - Gállego River	Durán and Anadón 2008
	Calanda Reservoir - Guadalope River	
	Flix Reservoir - Ebro River	
	Lanuza Reservoir - Gállego River	
	Mequinenza Reservoir - Ebro River	
	Rialb Reservoir - Segre River	
	Ribarroja Reservoir - Ebro River	
	Sabiñánigo Reservoir - Gállego River	
	S Lorenzo de Mongay Reservoir - Segre River	
	Sobrón Reservoir - Ebro River	

Annex 1 (continued).

Species	Locality – River	Reference
Dreissena polymorpha	Sobrón Reservoir - Ebro River	Oscoz et al. 2006
	Talarn Reservoir – Noguera Pallaresa River	Durán and Anadón 2008
	La Tranquera Reservoir - Piedra River	
	Ullivarri-Gamboa Reservoir - Zadorra River	
Artemia franciscana	Alfaques Bay, Tarragona	Amat et al. 2007
Dolerocypris sinensis	Ebro Delta	Forés et al. 1986
Cypris subglobosa	Ebro Delta	Forés et al. 1986
Isocypris beauchampi	Álava	Baltanás et al. 1996
Stenocypris major	Ebro Delta	Forés et al. 1986
Strandesia vinciguerrae	Ebro Delta	Forés et al. 1986
Tanycypris sp.	Ebro Delta	Forés et al. 1986
Lernaea cyprinacea	Ebro River basin	Sterling et al. 1995
Cherax destructor	Zaragoza	Bolea 1996
	Navarra	Souty-Grosset et al. 2006
Pacifastacus leniusculus	Arga River (Navarra)	Muez and Muez 1988
	Aragón River (Navarra)	Oscoz and Durán 2005
	Arakil River (Navarra)	
	Arga River (Navarra)	
	Arlabán River (Álava)	
	Ayuda River (Álava)	
	Bayas River (Burgos)	
	Ebro River (Cantabria)	
	Ebro River (Burgos)	
	Ega River (Navarra)	
	Erro River (Navarra)	
	Izki River (Álava)	
	Jerea River (Burgos)	
	Jiloca River (Teruel)	
	Larraun River (Navarra)	
	Linares River (Navarra)	
	Martín River (Zaragoza)	
	Oca River (Burgos)	
	Onsella River (Navarra)	
	Oroncillo River (Burgos)	
	Salazar River (Navarra)	
	Ulzama River (Navarra)	
	Zadorra River (Álava)	
	Huesca	Torralba-Burrial 2008
	Aragón River (Navarra)	Oscoz et al. 2008b
	Arakil River (Navarra)	
	Arga River (Navarra)	
	Biniés River (Navarra)	
	Ega River (Navarra)	
	Erro River (Navarra)	
	Iranzu River (Navarra)	
	Irati River (Navarra)	
	Juslapeña River (Navarra)	
	Larraun River (Navarra)	
	Linares River (Navarra)	
	Onsella River (Navarra)	
	Robo River (Navarra)	
	Salazar River (Navarra)	
	Ulzama River (Navarra)	
	Oroncillo River (Burgos)	
	Álava, Basque country	Desma Estudios Ambientales 2009
Procambarus clarkii	Headwaters of the Ebro catchment	Alonso et al. 2000
	Aguasvivas River (Teruel)	Oscoz and Durán 2005
	Alcanadre River (Huesca)	
	Aragón River (Navarra)	
	Arba de Biel River (Zaragoza)	
	Arba de Riguel River (Zaragoza)	
	Arlabán River (Álava)	
	Cinca River (Huesca)	
	Clamor Amarga River (Huesca)	
	Clamor Amarga River (Lleida)	
	Ebro River (Navarra)	
	Ebro River (Zaragoza)	
	Ebro River (Tarragona)	
	Elorz River (Navarra)	
	Flumen River (Huesca)	
	Gállego River (Huesca)	
	Guadalope River (Teruel)	

Annex 1 (continued).

Species	Locality – River	Reference
Procambarus clarkii	Guatizalema River (Huesca)	
	Huecha River (Zaragoza)	
	Huerva River (Zaragoza)	
	Isuela River (Huesca)	
	Linares River (Navarra)	
	Oroncillo River (Burgos)	
	Piedra River (Zaragoza)	
	Sadar River (Navarra)	
	Tirón River (Logroño)	
	Vero River (Huesca)	
	Zadorra River (Álava)	
	Zadorra River (Burgos)	
	Zidacos River (Navarra)	
	Beraiz Pond (Navarra)	
	Aragón River (Navarra)	Oscoz et al. 2008b
	Arga River (Navarra)	
	Beraiz pond (Navarra)	
	Elorz River (Navarra)	
	Iranzu River (Navarra)	
	Linares River (Navarra)	
	Sadar River (Navarra)	
	Zidacos River (Navarra)	
	Álava, Basque country	Desma Estudios Ambientales 2009
	Ebro Delta	Querol, pers. Com.

Annex 2. Records of the different non-indigenous invertebrate species found in the Ebro River basin in different monitoring studies carried out in the last years.

Species	Locality - River	Record coordinates	
		Latitude	Longitude
Craspedacusta sowerbii	Montcortes Lake (Lleida)	42º19'N	0º59'E
Corbicula fluminea	Milagro - Aragón River	42º14'N	1º45'W
	Peralta - Arga River	42º20'N	1º47'W
	Funes - Arga River	42º18'N	1º47'W
	Castejón - Ebro River	42º10'N	1º41'W
	Tudela - Ebro River	42º04'N	1º35'W
	Ribaforada - Ebro River	42º00'N	1º30'W
	Gallur - Ebro River	41º52'N	1º18'W
	Alagón - Ebro River	41º47'N	1º08'W
	Flix - Ebro River	41º14'N	0º33'E
	Móra d'Ebre - Ebro River	41º05'N	0º39'E
	Tortosa - Ebro River	40º48'N	0º31'E
	Amposta - Ebro River	40º42'N	0º35'E
	Alagón - Jalón River	41º44'N	1º08'W
Dreissena polymorpha	Downstream Sobrón Reservoir - Ebro River	42º45'N	3º05'W
	Miranda de Ebro - Ebro River	42º41'N	2º57'W
	Downstream Miranda de Ebro - Ebro River	42º40'N	2º54'W
	Ircio - Ebro River	42º39'N	2º53'W
	Upstream Salinillas de Buradón - Ebro River	42º38'N	2º52'W
	El Ciego - Ebro River	42º29'N	2º37'W
	Varea - Ebro River	42º28'N	2º22'W
	Lodosa - Ebro River	42º25'N	2º04'W
	Tudela - Ebro River	42º04'N	1º35'W
	Ribaforada - Ebro River	42º00'N	1º30'W
	Flix - Ebro River	41º14'N	0º33'E
	Móra d'Ebre - Ebro River	41º05'N	0º39'E
	Jaca - Gas River (?)	42º33'N	0º36'W
Pacifastacus leniusculus	Lagata - Aguasvivas River	41º14'N	0º48'W
	Puentelarreina de Jaca - Aragón River	42º33'N	0º47'W
	Downstream Berdún - Aragón River	42º36'N	0º55'W
	Yesa - Aragón River	42º37'N	1º12'W
	Sangüesa - Aragón River	42º34'N	1º16'W
	Iturmendi - Arakil River	42º53'N	2º07'W
	Irañeta - Arakil River	42º55'N	1º56'W
	Izurdiaga - Arakil River	42º54'N	1º49'W
	Errotz - Arakil River	42º53'N	1º49'W
	Asiain - Arakil River	42º49'N	1º47'W

Annex 2 (continued).

Species	Locality - River	Record coordinates	
		Latitude	Longitude
Pacifastacus leniusculus	Huarte - Arga River	42º50'N	1º35'W
	Pamplona - Arga River	42º48'N	1º42'W
	Landa - Arlabán River	42º57'N	2º35'W
	Mijancas - Ayuda River	42º42'N	2º49'W
	Miranda de Ebro - Ayuda River	42º40'N	2º53'W
	Miranda de Ebro - Bayas River	42º41'N	2º55'W
	Burgui - Biniés River	42º43'N	0º59'W
	Aldea de Ebro - Ebro River	42º54'N	4º01'W
	Trespaderne - Ebro River	42º47'N	3º23'W
	Zubielki - Ega River	42º40'N	2º03'W
	Señorío Arínzano - Ega River	42º38'N	1º59'W
	Aberin - Ega River	42º36'N	1º59'W
	Allo - Ega River	42º33'N	1º58'W
	Lizoain - Erro River	42º48'N	1º27'W
	Urroz - Erro River	42º47'N	1º27'W
	Downstream Urroz - Erro River	42º46'N	1º26'W
	Liberri - Erro River	42º46'N	1º25'W
	Upstream Villaveta - Erro River	42º46'N	1º24'W
	Villaveta - Erro River	42º46'N	1º23'W
	Burgui - Esca River	42º43'N	1º00'W
	Berganzo - Inglares River	42º39'N	2º47'W
	Estella - Iranzu River	42º38'N	2º00'W
	Olaldea - Irati River	42º55'N	1º17'W
	Upstream Oroz Betelu - Irati River	42º54'N	1º17'W
	Aos - Irati River	42º45'N	1º23'W
	Upstream Lumbier - Irati River	42º40'N	1º19'W
	Upstream Liédena - Irati River	42º37'N	1º17'W
	Liédena - Irati River	42º36'N	1º16'W
	Antoñana - Izki River	42º40'N	2º23'W
	Palazuelos de Cuesta Urria - Jerea River	42º47'N	3º21'W
	Ojos de Monreal - Jiloca River	40º46'N	1º21'W
	Arazuri - Juslapeña River	42º49'N	1º43'W
	Downstream Muguiro - Larraun River	42º58'N	1º50'W
	Urritza - Larraun River	42º57'N	1º50'W
	Ventas de Urritza - Larraun River	42º57'N	1º49'W
	Latasa - Larraun River	42º57'N	1º49'W
	Downstream Latasa - Larraun River	42º56'N	1º49'W
	La Ferrería - Larraun River	42º56'N	1º49'W
	Irurtzun - Larraun River	42º55'N	1º50'W
	Izurdiaga - Larraun River	42º54'N	1º50'W
	Torres del Río - Linares River	42º33'N	2º16'W
	Escatrón - Martín River	41º17'N	0º18'W
	Oña - Oca River	42º44'N	3º24'W
	Espejo - Omecillo River	42º48'N	3º02'W
	Sangüesa - Onsella River	42º33'N	1º16'W
	Orón - Oroncillo River	42º40'N	2º58'W
	Obanos - Robo River	42º40'N	1º47'W
	Aspurz - Salazar River	42º43'N	1º09'W
	Ollerías - Santa Engracia River	43º00'N	2º40'W
	Gurrea de Gállego - Sotón River	42º01'N	0º45'W
	Upstream Murua - Subialde-Zayas River	42º59'N	2º43'W
	Orgui - Ulzama River	42º57'N	1º40'W
	Downstream Orgui - Ulzama River	42º57'N	1º40'W
	Upstream Latasa - Ulzama River	42º57'N	1º39'W
	Olave - Ulzama River	42º53'N	1º36'W
	Otxandio - Urkiola River	43º02'N	2º39'W
	Zuazu - Zadorra River	42º52'N	2º24'W
	Heredia - Zadorra River	42º52'N	2º26'W
	Mendibil - Zadorra River	42º54'N	2º38'W
	Durana - Zadorra River	42º53'N	2º38'W
	Miranda de Ebro - Zadorra River	42º40'N	2º53'W
	Barasoain - Zidacos River	42º36'N	1º38'W
	Downstream Barasoain - Zidacos River	42º36'N	1º38'W
Procambarus clarkii	Azaila - Aguasvivas River	41º17'N	0º29'W
	Ontiñena - Alcanadre River	41º40'N	0º05'E
	Alfaro - Alhama River	42º10'N	1º45'W
	Cáseda - Aragón River	41º31'N	1º21'W
	Marcilla - Aragón River	41º19'N	1º43'W
	Luna - Arba de Biel River	42º11'N	0º55'W
	Erla - Arba de Biel River	42º07'N	0º56'W
	Ejea de los Caballeros - Arba de Luesia River	42º07'N	1º09'W
	Tauste - Arba de Luesia River	41º54'N	1º16'W

Annex 2 (continued).

Species	Locality - River	Record coordinates	
		Latitude	Longitude
Procambarus clarkii	Sádaba - Arba de Riguel River	42º17'N	1º15'W
	Pamplona - Arga River	42º48'N	1º42'W
	Ororbia - Arga River	42º48'N	1º44'W
	Upstream Zabala - Arga River	42º43'N	1º50'W
	Señorio Sarria - Arga River	42º41'N	1º49'W
	Ozaeta - Arlabán River	42º57'N	2º35'W
	Señorío Beraiz - Beraiz Pond	42º54'N	1º35'W
	Las Pilas Bridge - Cinca River	42º04'N	0º12'E
	Upstream Monzón - Cinca River	41º57'N	0º10'E
	Conchel - Cinca River	41º53'N	0º09'E
	Santa Lecina - Cinca River	42º46'N	0º07'E
	Altorricón - Clamor Amarga River	41º48'N	0º29'E
	Almacelles - Clamor Amarga River	41º45'N	0º24'E
	Miranda de Ebro - Ebro River	42º41'N	2º57'W
	Downstream Miranda de Ebro - Ebro River	42º40'N	2º54'W
	Ircio - Ebro River	42º39'N	2º53'W
	El Ciego - Ebro River	42º29'N	2º37'W
	Varea - Ebro River	42º28'N	2º22'W
	Lodosa - Ebro River	42º25'N	2º04'W
	Azagra - Ebro River	42º19'N	1º55'W
	Tudela - Ebro River	42º04'N	1º35'W
	Gallur - Ebro River	41º52'N	1º18'W
	Alagón - Ebro River	41º47'N	1º08'W
	Presa Pina - Ebro River	41º33'N	0º40'W
	Azud de Rueda - Ebro River	41º17'N	0º19'W
	Flix - Ebro River	41º14'N	0º33'E
	Zubielki - Ega River	42º40'N	2º03'W
	Barañain - Elorz River	42º47'N	1º41'W
	Quicena - Flumen River	42º08'N	0º20'W
	Tierz - Flumen River	42º08'N	0º21'W
	Downstream Sariñena - Flumen River	41º45'N	0º10'W
	Ayerbe - Fontobal River	42º17'N	0º40'W
	Marracos - Gállego River	42º06'N	0º45'W
	Gurrea de Gállego - Gállego River	41º49'N	0º47'W
	Upstream Alcañiz - Guadalope River	41º01'N	0º08'W
	Alcañiz - Guadalope River	41º03'N	0º08'W
	Siétamo - Guatizalema River	42º07'N	0º16'W
	Sesa - Guatizalema River	42º00'N	0º14'W
	Huerto - Guatizalema River	41º54'N	0º07'W
	Magallón - Huecha River	41º50'N	1º27'W
	Mallen - Huecha River	41º54'N	1º25'W
	Villanueva de Huerva - Huerva River	41º21'N	1º01'W
	Botorrita – Huerva River	41º30'N	1º01'W
	Estella - Iranzu River	42º38'N	2º00'W
	Pompenillo - Isuela River	42º05'N	0º23'W
	Santa María de Huerta - Jalón River	41º15'N	2º10'W
	Alagón - Jalón River	41º44'N	1º08'W
	Arazuri - Juslapeña River	42º49'N	1º43'W
	Upstream Zuera - La Violada River	41º54'N	0º46'W
	Agoncillo - Leza River	42º26'N	2º18'W
	Mendavia - Linares River	42º26'N	2º11'W
	Hijar - Martin River	41º10'N	0º27'W
	Orón - Oroncillo River	42º40'N	2º58'W
	Cimballa - Piedra River	41º06'N	1º46'W
	Murchante - Queiles River	42º01'N	1º39'W
	Pamplona - Sadar River	42º48'N	1º39'W
	Upstream Monzón - Sosa River	41º54'N	0º12'E
	Tirgo - Tirón River	42º32'N	2º56'W
	Barbastro - Vero River	42º00'N	0º09'E
	Villodas - Zadorra River	42º50'N	2º46'W
	Nanclares de la Oca - Zadorra River	42º48'N	2º48'W
	La Puebla de Arganzón - Zadorra River	42º46'N	2º49'W
	Olite - Zidacos River	42º29'N	1º38'W