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Rapid Communication

On the presence of the Ponto-Caspian hydrozoan *Cordylophora caspia* (Pallas, 1771) in an Iberian estuary: highlights on the introduction vectors and invasion routes

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Abstract

Several non-native invertebrate and vertebrate species have been detected in the Guadiana Estuary (SW-Iberian Peninsula, Europe) during the 21st century. In June 2015, the non-native hydroid *Cordylophora caspia* (Pallas, 1771) was detected for the first time in this estuary, which motivated an assessment of its distribution during late Spring and Summer 2016. The main goals of this paper were to: i) report the presence of *Cordylophora caspia* and its distribution in the Guadiana Estuary, ii) record the substrates colonized, salinity, and water temperatures at locations where the species was detected, iii) evaluate possible introduction vectors and invasion routes; and iv) discuss the potential impacts and management options. *Cordylophora caspia* occupied a 25-km stretch of the estuary with salinities between 0.2 and 13.8 and occupied a variety of human-made substrates. Shipping was the most likely introduction vector of *C. caspia*, which might have originated from populations in the Atlantic Ocean or the Mediterranean Sea. Currently, the potential ecological impacts are likely low since the population size is small due to an apparent shortage of suitable habitat. Economic effects are minimal at present because there are no major industries along the basin extracting water from the estuary. An integrated ecohydrological approach—i.e. freshets released from dams to control the populations of Cnidaria—was proposed to minimize or mitigate the potential negative effects of this species in the Guadiana Estuary.

Key words: non-native species, Cnidaria, Ponto-Caspian species, Guadiana Estuary, Portugal, Spain, Iberian Peninsula

Introduction

Detecting non-native species and assigning the time of introduction is particularly difficult in locations without a comprehensive species list. This is the case of the Guadiana Estuary (SW-Iberian Peninsula, Europe—Figure 1) where some non-native aquatic species have been found in recent years in the few studies that have been carried. So far, six non-native species are known: *Corbicula fluminea* (Müller, 1774) (Mollusca: Bivalvia) (Morais et al. 2009); *Palaemon macrodactylus* Rathbun, 1902 (Crustacea: Decapoda), *Blackfordia virginica* Mayer, 1910 (Cnidaria: Hydrozoa) (Chícharo et al. 2009), *Cynoscion regalis* (Bloch and Schneider, 1801) (Pisces: Sciaenidae) (Morais and Teodósio 2016), *Acartia tonsa* (Dana, 1849 (Crustacea: Copepoda) (Cruz et al. 2017), and *Fundulus heteroclitus* (Linnaeus, 1766) (Pisces: Fundulidae) (R. Gonçalves, CCMAR, unpublished data).

The freshwater hydroid *Cordylophora caspia* (Pallas, 1771) (Cnidaria: Hydrozoa) (Figure 2), a non-native invertebrate species, was initially detected in the Guadiana Estuary in June 2015 during a study aimed at detecting polyps of *B. virginica*. *Cordylophora caspia* is a hydrozoan native from the Ponto-Caspian region inhabiting brackish and freshwater environments (Fulton 1960). *C. caspia* has a panmictic but discontinuous distribution (e.g. Roch 1924; Rzóska 1949;

Conde et al. 2013; Deserti et al. 2015). This species is present in six continents: Africa—Egypt, Sudan (Boulenger 1908; Rzóska 1949); Asia—China (Roch 1924); Europe—France, Germany, Hungary, Italy, Luxembourg, Netherlands, Poland, Portugal, Spain (Marchand 1972; Roos 1979; Ambrogi et al. 1983; Massard and Geimer 1987; Gruszka 1999; Escot et al. 2003; Musko et al. 2008; Correia et al. 2012; Conde et al. 2013; Chainho et al. 2015); North and South America—Argentina, Chile, Panama, USA (Jones and Rützler 1975; Smith et al. 2002; Galea et al. 2007; Wintzer et al. 2011; Deserti et al. 2015; Richardson and Hammond 2016); and Oceania—Australia, New Zealand, Tasmania (Roch 1924). However, it is important to highlight that the genus *Cordylophora* includes an unknown number of cryptic species, so its biogeographic distribution might change as new taxonomic studies are completed (Folino-Rorem et al. 2009), which is likely common for cryptic invasive species (Morais and Reichard 2018).

Cordylophora caspia reproduces asexually through fragmentation and sexually, in which the two sexes occur in separate colonies (Fulton 1960). Fertilized eggs develop in the gonophores (Figure 2A), which release a planula larvae that swim or crawl until finding a suitable substrate; and then evolve into a new colony (Fulton 1960). This species forms branching colonies composed of three types of chitin tubes: stolons, uprights, and branches (Fulton 1960). Colonies withstand adverse conditions (e.g. cold temperatures) by producing spheres of coenosarc tissue and remaining in the perisarc of the hydrocauli, called menonts. The menonts regenerate under favorable conditions and represent a crucial survival mechanism (Roos 1979; Folino-Rorem and Indelicato 2005). The optimal temperature for growth of colonies is between 18 and 26 °C, but polyps survive temperatures ranging from 8 to 30 °C (Fulton 1962). *Cordylophora caspia* tolerates salinities ranging from 0 to 40; however, the optimum is between 15 and 17 (Folino-Rorem et al. 2009). *Cordylophora caspia* also tolerates a broad range of pH (> 50% survival: $5.0 < pH < 8.5$; 100% mortality: $pH < 4$ and $pH > 10$) (Gutierre 2012) and some hypoxia (>2 mg O_2 L^{-1}) (Fulton 1962).

To date, few studies document the ecological impacts of *Cordylophora caspia*. Nevertheless, *C. caspia* is included in Europe's 100 worst invasive species (DAISIE 2017), as it facilitates the settlement of invasive dreissenid mussels (Moreteau and Khalanski 1994; Pucherelli et al. 2016), and it competes for food with larval, juvenile, and benthivorous fish (Berg and Folino-Rorem 2009). It was also argued that a slight increase in water temperature induced by climate change might lead to the spread of non-indigenous populations of *C. caspia* within estuarine ecosystems if the current water temperature is below optimal conditions during the growing season (Meek et al. 2012). Regarding economic consequences, *C. caspia* can produce deleterious impacts on industrial infrastructure such as water treatment plants (Mant et al. 2011), coal power plants (Folino-Rorem and Indelicato 2005), and nuclear power plants (Moreteau and Khalanski 1994). Other impacts include the fouling of aquatic infrastructure (e.g. docks, piers) and the hulls of recreational and commercial vessels.

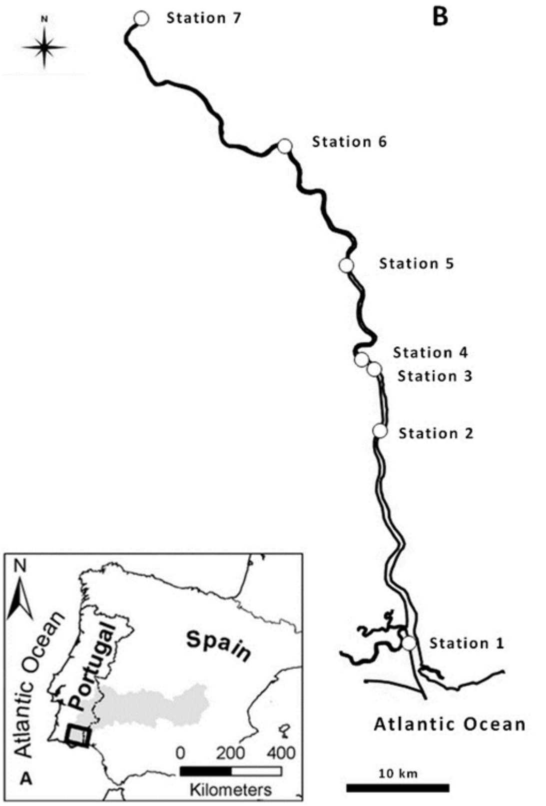

Figure 1. Location of the Guadiana River basin in the Iberian Peninsula (A) and of the sampling stations surveyed along the estuary on July 26th, 2016 (B).

In this study, we aimed to (1) document the presence of *Cordylophora caspia* in the Guadiana Estuary, (2) determine the distribution of this species in the estuary and record the substrates colonized and the salinity of locations sampled, (3) evaluate possible introduction vectors and invasion routes, and (4) discuss the potential ecological and economic impacts of this species within the estuary.

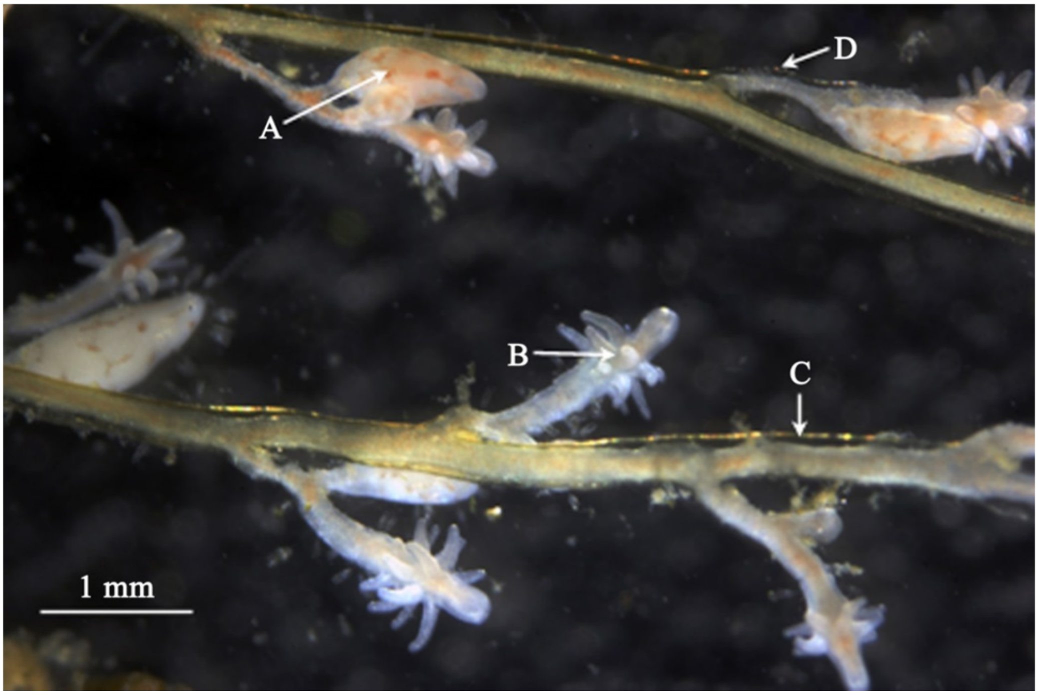

Figure 2. Photograph of *Cordylophora caspia* sampled at Station 2 showing the gonophores (A), hydranth (B), chitin layer (C), and the ring-shaped structure (D). Photo credits: Thomas Seyer.

Material and methods

Study area

The Guadiana River is 810 km long, and the last 50 km serves as the southern border between Portugal and Spain (Iberian Peninsula, Europe) (Figure 1), and it drains into the Gulf of Cadiz (Atlantic Ocean). The estuary occupies an area of 22 km^2. The tidal amplitude varies between 1.3 and 3.5 m, and tidal effects extend inland for 70 km. River flow varies substantially within and among years because of variations in rainfall. However, the river discharge has usually been lower than 20 m^3 s^{-1} since the completion of the Alqueva dam in February 2002 (Garel and Ferreira 2011) despite episodic flooding events (e.g. March – April 2013).

Field sampling and laboratory analyses

In May 2016, clean artificial structures—roof tiles and PVC plates (Figure 3)—were deployed in stations 1 and 2, which were sampled every month, and removed in August 2016. The rapid colonization of these structures in station 2, led us to assess the distribution of *C. caspia* along the Guadiana Estuary. Thus, a comprehensive spatial assessment was done on July 26th, 2016 through visual inspection of piers and other hard structures present along the estuary. These structures were visually inspected by snorkeling in seven sites—sites 1 and 7 were located at 4 km and 70 km from the river mouth, respectively (Figure 1).

In each station, the water temperature and salinity were recorded with a YSI Professional Plus Multi-parameter Meter (Yellow Spring Instruments, Yellow Springs, Ohio, USA). Also, the individuals present in an area of 10 cm^2 were collected and placed in a container with water from that site and transported alive for examination under a stereo-microscope. In the laboratory, the presence or absence of gonophores (Figure 2A), the shape of hydranths (Figure 2B), and the maximum length of branches was registered.

Results

A small ring-shaped structure (Figure 2D) in the chitin layer (Figure 2C), and at the base of each division, is a diagnostic trait of *C. caspia*. The presence of gonophores (Figure 2A) attested to the occurrence of sexual reproduction in the Guadiana Estuary.

Polyps of *C. caspia* were preferentially present on surfaces protected from exposure to direct sunlight and were found on all types of artificial substrates (e.g., metallic barrels, PVC buoys, and tires). *Cordylophora caspia* was collected within a 25 km stretch of the estuary, between stations 2 (20 km from the mouth) and 6 (45 km from the mouth)

(Supplementary material Table S1). Here, salinity varied between 0.2 (station 6) and 13.8 (station 2), while water temperature varied between 27.1 (station 2) and 28.8 °C (station 6).

In station 2, we observed a nudibranch and amphipods feeding on *C. caspia* (Table S1). In station 6, polyps had gonophores but no hydranths, however structures suspected to be decaying hydranths were observed. The maximum stem length was up to 5.7 times smaller in station 6 (maximum length: 1.0 cm) than in other sites (maximum length: 3.2–5.7 cm).

Discussion

Cordylophora caspia in the Guadiana Estuary

A patch of *Cordylophora caspia* was identified for the first time in the Guadiana Estuary in June 2015. However, this observation does not indicate a recent colonization because *C. caspia* was detected in a stretch of 25 km of the estuary a year later, indicating it had already established a reproducing viable population as confirmed by the speed with which the organism colonized our settlement plates. This species is easily overlooked, likely due to its resemblance with epiphytic algae, and was not detected (or not recognized) in the few previous surveys targeting benthic and planktonic taxa (Morais et al. 2009; Chícharo et al. 2009).

The optimal salinity conditions of *C. caspia* range between 15 and 17, despite tolerating high (up to 40) and low salinity conditions (down to 0) (Smith et al. 2002; Folino-Rorem et al. 2009). However, *C. caspia* is not present in stations 1 and 7 where salinity was 34.0 and 0.1, respectively. At least three non-exclusive hypotheses might explain this limited distribution. The first hypothesis is that not enough time has elapsed since the introduction of *C. caspia* to allow the colonization of less-than-optimal upstream and downstream areas. The second hypothesis is that the Guadiana population prefers intermediate abiotic conditions of salinity/conductivity and temperature. The third hypothesis is that other abiotic and biotic factors are responsible for the absence of *C. caspia* from those areas. On the one hand, the high water temperature (31.7 °C) registered in the uppermost station (Table S1) might be fatal to *C. caspia*, because its maximum tolerable temperature is 30 °C (Fulton 1962). In the upper Guadiana, salinity does not seem to have been a factor because the range was only 0.1 to 0.3 (Table S1).

Predation by marine/brackish invertebrates might have prevented *C. caspia* from colonizing the lower estuary. Indeed, a nudibranch and several amphipods were observed feeding on *C. caspia* at station 2. Interestingly, predation by nudibranchs has been reported in the San Francisco Bay (USA) (Mills and Sommer 1995) and Baltic Sea (Evertsen et al. 2004). Further work is needed to assess the predator-prey interactions between *C. caspia* and the potential predators in the Guadiana Estuary.

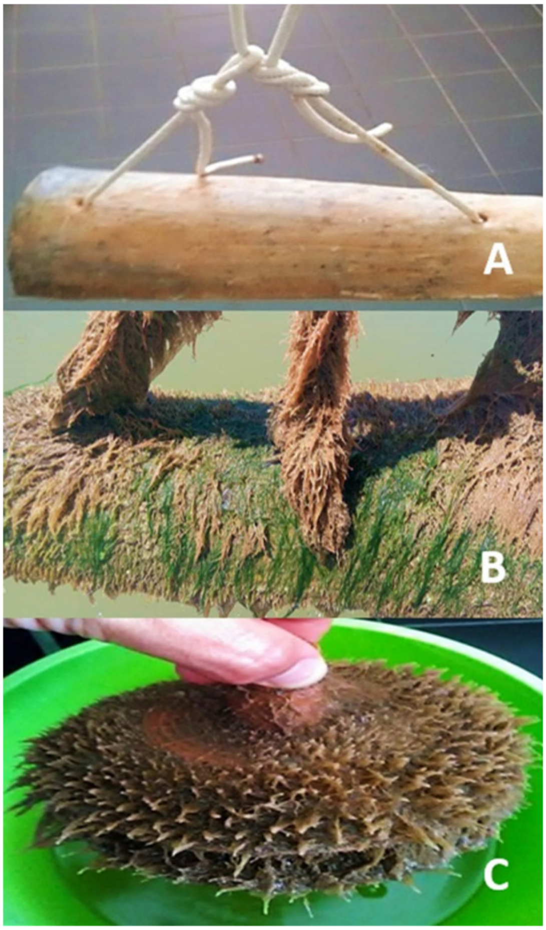

Figure 3. Photographs of a roof tile (30 cm long) before (A) and after (B) being deployed at the Guadiana Estuary to be used as an attachment structure for *Cordylophora caspia*. The figure also shows PVC plates (10 cm in diameter) colonized by *C. caspia* (C). Photo credits: Thomas Seyer.

A literature search on the optimal abiotic conditions and tolerance limits is insufficient to explain the distribution of a population in a given river, due to multiple interacting biological and ecological factors (e.g. availability of substratum, the cost of sexual reproduction, predation) (Roos 1979; Jormalainen et al. 1994). Therefore, monitoring this non-indigenous population is essential to validate or reject the first hypothesis, while the second hypothesis can only be properly evaluated in laboratory trials aiming to test the response of Guadiana's population to key abiotic (e.g. temperature, salinity, turbidity) and biotic factors (e.g. predation, competition).

Introduction vectors and invasion routes

Cordylophora caspia is an invasive species with a panmictic but discontinuous distribution (e.g. Roch 1924; Rzóska 1949; Conde et al. 2013; Deserti et al. 2015). *Cordylophora caspia* has four biological traits conducive to invasiveness: their broad physiological tolerances (Fulton 1962), the capability to produce the menonts, the short planktonic duration of the planula larvae (Fulton 1960), and the capacity to reproduce asexually through fragmentation (Kinzelbach 1995). For example, in the Laurentian Great Lakes and nearby lakes in North America, the dispersal of planula larvae only enables local expansion, while regional expansion is achieved through anthropogenic dispersal vectors (Darling and Folino-Rorem 2009).

The presence of *C. caspia* in Western Europe (Iberian Peninsula) dates back to at least to the early 1980's when it was first found in Lagoa de Santo André (Portugal), an intermittent land-locked coastal lagoon (Correia et al. 2012). Four other records were made in different basins since then, namely in the estuaries of the Minho (in 2006; Rivas-Rodríguez et al. 2010), Tagus (in 2011; Conde et al. 2013), and Guadalquivir Rivers (in 2003; Escot et al. 2003); and in Albufera de València (Conde et al. 2013). Thus the colonization of the Guadiana Estuary may have been inevitable given the boat traffic patterns.

Shipping is the most likely introduction vector of *C. caspia* into the Guadiana Estuary, either by transport in ballast water or ship fouling (or both), as is proposed for colonization of other Iberian estuaries (Escot et al. 2003; Rivas-Rodríguez et al. 2010; Conde et al. 2013). In central and eastern Europe, the introduction is promoted by ships, but dispersion is also facilitated by the numerous artificial canals connecting distinct hydrographic basins, and that has resulted in three major invasion corridors (Bij de Vaate et al. 2002).

There are two possible non-exclusive introduction routes into the Guadiana Estuary, one that originates in the French Atlantic coast (route α) and the other on the French Mediterranean coast (route β). Route α implies the arrival of *C. caspia* on the French Atlantic coast via the Loire River (established there at least since 1901, Marchand 1972) and subsequent southward transport. Route β involves the arrival of *C. caspia* into the French Mediterranean coast via the Rhone River followed by a westward transport through the Mediterranean Sea. A definitive answer to these hypotheses could be obtained through genetic comparisons of the Iberian and central and eastern European populations. Nonetheless, it is possible to eliminate some source populations because there is a relationship between *C. caspia* population's salinity tolerance and its genetic lineage (Folino-Rorem et al. 2009).

Cordylophora populations are divided into two main lineages, lineages 1 (subdivided in lineages 1A and 1B) and 2 (subdivided in lineages 2A and 2B) (Folino-Rorem et al. 2009). Lineage 1A populations are present in freshwater sites (salinity < 0.5), lineage 1B populations are found in both fresh and brackish (salinity > 0.5) water sites, while both lineage 2 populations are only present in brackish water sites (Folino-Rorem et al. 2009; Pucherelli et al. 2016). Lineage 2B was only detected on the Pacific coast of North America (Folino-Rorem et al. 2009). While the necessary genetic analyses have yet to be done, it seems more likely that Guadiana's population originated from a lineage 1B or 2A population, or both as observed in the Squamscott River (New Hampshire, USA) (Folino-Rorem et al. 2009). Only six European populations have been studied to date: a) three population belong to lineage 2A (France—Canet St. Nazaire Lake in the Mediterranean coast, Germany—Ryck River, Ireland—Shannon River), b) one population to lineage 1A (Grafham Water, England), c) another to lineage 1B (Lake Balaton, Hungary), d) and one population that has individuals from lineages 1A and 1B (Waal River, Netherlands) (Folino-Rorem et al. 2009). Although it seems more likely that Guadiana's population has originated from a 2A lineage, due to proximity to lineage 2A sites, currently available data advice caution and a lineage 1B source population is also possible.

Impacts and management

The ecological impacts associated with *C. caspia* establishment are yet to be determined in the Guadiana Estuary. Nonetheless, and contrary to what is predicted to occur in the San Francisco Estuary (Meek et al. 2012), we hypothesize that climate change (i.e. increased water temperature) will shorten the upstream distribution limit of *C. caspia* in the Guadiana because the species upper thermal limit was exceeded in the upper estuary (site 7) during summer. A future impact assessment could investigate how community structure and trophic relationships shift when *C. caspia* increases fractal complexity to uncolonized habitats. The competition for settlement space may be relevant with other sessile invertebrates, like mussels, oysters, and bryozoans. However, the potential for competition for food, with planktivorous fish species and larval phases, is probably minimal since the population standing stock is low owing to the small amount of settlement habitat available (e.g. piers, harbors, boulders, bivalve shells) (Berg and Folino-Rorem 2009), despite the species broad distribution along the estuary.

Currently, the economic impacts are minimal in the Guadiana Estuary because there are no major industries using water from the estuary for their operations (Morais 2008). Salt pans and aquaculture facilities have not been affected by *C. caspia* because they are located in uncolonized areas of the lower estuary. Also, there are still no reports of irrigation channels in agricultural areas being colonized by this species. Thus, impacts are restricted at present to biofouling of boat hulls and piers, which are also fouled by other species (e.g. algae, bivalves). Nonetheless, the impacts might increase if the abundance of *C. caspia* increases and if their distribution expands.

Management practices aiming to confine the distribution or reduce the standing stock of the *C. caspia* population ought to access basic ecological traits (e.g. optimum environmental conditions, type and period of reproduction, interspecific relationships) to propose the most effective measures. For example, in the northern Baltic Sea, *C. caspia* allocates energy in colonial growth and reproduction during early summer (Jormalainen et al. 1994), so it is likely that the investment in reproduction start earlier in southern latitudes. A possible management action would be to increase hydrodynamic disturbance, through an increase of river discharge, during key life-history periods thereby disrupting reproduction. Ideally, this would occur during late winter or early spring to coincide with a management proposal aiming to reduce the standing stocks of a bloom-forming jellyfish in the Guadiana Estuary (K. Amorim, CCMAR, unpublished data). This solution highlights the need for integrated management solutions to manage biological invasions and other nuisance blooms in estuarine environments (Wolanski et al. 2004; Chícharo and Chícharo 2006; Chícharo et al. 2006; Morais et al. 2012).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Temperature and salinity records registered at the seven sampling stations surveyed along the Guadiana Estuary on July 26th, 2016.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2017/Supplements/BIR_2017_Seyer_etal_Table_S1.xlsx