



Communication

First Record of the Nudibranch *Tenellia adspersa* (Nordmann, 1845) in Portugal, Associated with the Invasive Hydrozoan *Cordylophora caspia* (Pallas, 1771)

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Abstract: The estuarine nudibranch *Tenellia adspersa* (Nordmann, 1845) was recorded for the first time in Portugal, while sampling for fouling fauna of artificial structures along the salinity gradient of the Guadiana estuary (SW Iberian Peninsula). Two specimens were found in association with the invasive hydrozoan *Cordylophora caspia* (Pallas, 1771) and kept in the laboratory for taxonomy purposes. After two days, batches of eggs were seen in *C. caspia* branches, while the nudibranchs were also actively feeding on the hydrozoan polyyps. The fast generation times of *T. adspersa*, along with its food preference for *C. caspia*, might suggest a positive role of the nudibranch on controlling this invasive hydrozoan in the Guadiana estuary. Introduction routes and facilitation interactions are discussed.

Keywords: Heterobranchia; nudibranch; sea slug; *Tenellia*; Portugal; estuarine; facilitation; invasive species; biological control

1. Introduction

Nudibranchs, the most diverse group of sea slugs (Gastropoda: Heterobranchia), are a widely distributed and diverse group of molluscs, found at their highest diversity in marine shallow coastal areas, with fewer associated with estuarine and brackish habitats [1,2]. The cosmopolitan aeolid *Tenellia adspersa* (Nordmann, 1845), along with the recently described *Bohuslania matsmichaeli* (Korshunova, Lundin, Malmberg, Picton and Martynov, 2018), are widely recognized as the only nudibranchs associated with brackish-water habitats [2–4].

The study species, *T. adspersa*, although considered a cosmopolitan species nowadays, the historical records point out to an European origin, with the first description of the species being made in the Black Sea, followed by other records in the Baltic Sea, North-West Iberian Peninsula and the British Isles [5–7]. Past misidentifications and nomenclature changes undermine present-day access to information on the distribution of *T. adspersa*, as several synonyms existed, namely *Embletonia grayi* Kent, 1869, *Embletonia pallida* Alder and Hancock, 1854, *Eolis armoricana* Hesse, 1872, *Eolis ventilabrum* Dalyell, 1853, *Tenellia mediterranea* Costa A., 1866, *Tenellia pallida* (Alder and Hancock, 1845) or *Tergipes adspersus* Nordmann, 1845. The available literature using some of these synonyms, confirms its cosmopolitan distribution across the North-East Atlantic Ocean and Mediterranean Sea [8,9]. Still, only a few recent records across Europe have been published, namely in the Baltic Sea [4] or the Mediterranean Sea [10]. Available records in online databases such as GBIF [11] or OBIS [12] show scattered records across the coasts of Finland, Sweden, Germany, Netherlands, the United Kingdom and Spain. In other parts of the world, such as North America [13–15] or India [16], it is considered a non-indigenous species.

In the Iberian Peninsula, according to the latest checklist on ‘opisthobranchs’ made in 2004 for Spain and Portugal, including islands and archipelagos, only three records were identified for *T. adspersa*, all in mainland Spain [17]. The oldest record is on the Atlantic coast in Galicia, namely inside ‘Ría do Ferrol’, found on the hydrozoan *Ectopleura larynx*, on the hull of a shipwreck [6]. A second record of eight individuals was made in the Mediterranean coast of Spain inside ‘Mar Menor’, feeding on the hydrozoans *Obelia dichotoma* and *Pachycordyle pusilla* [18]. These specimens were initially identified as *T. pallida*, a synonym of *T. adspersa*. A more recent record dates from 2001 on the Atlantic coast, in Sancti-Petri, close to the city of Cadiz (SW Iberian Peninsula) (unpublished record, see Cervera et al. 2004; GBIF occurrence ID 31777043). The present study aims at describing the first record of *T. adspersa* in Portugal, including additional information on development stages and remarks on its association with the invasive hydrozoan *Cordylophora caspia* (Pallas, 1771).

2. Materials and Methods

2.1. Study Area

Sampling took place during the summer of 2016 in the Guadiana estuary, located in SE Portugal/SW Spain (Figure 1). The tidal influence on this mesotidal estuary extends to Mértola (Station 7, Figure 1), 70 km away from the coast, while the river flow varies substantially among and within years, as is characteristic of regions with a Mediterranean climate, with the additional regularization by the Alqueva dam, located 180 km from the river mouth [19]. As in other regions with Mediterranean climate, the rainfall regime in the area is very irregular between years, with 80% concentrated in autumn and winter, while summers are very dry [19]. The estuary can be divided into three areas (Figure 1): the upper estuary, with salinity close to zero (<0.5 PSU), the middle estuary, characterized as the mixing zone of brackish water (0.5–25 PSU), and the lower estuary, with salinity closer to seawater (>25 PSU) [20]. The mixing zone of the middle estuary, where sampling took place (Figure 1), is also one of the zones with higher fluctuations in environmental variables. At Station 2, annual values of surface water temperature can change between 9 and 27 °C, while salinity ranges between 0 and 15 PSU, all changing according to tidal influence and seasonal river flow [21].

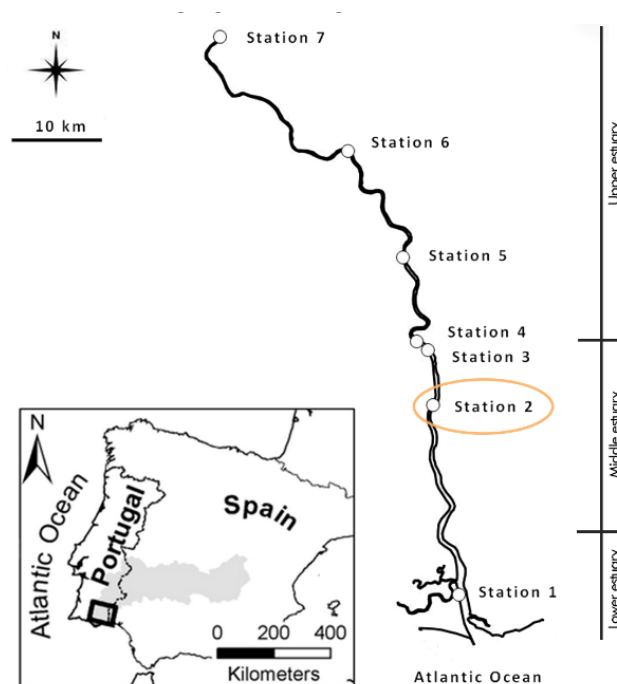


Figure 1. Location of the Guadiana estuary in SW Iberian Peninsula and all the seven sampling stations. Station 2, where *Tenellia adspersa* was found, is highlighted. Adapted from Seyer et al. (2017).

2.2. Sampling Methods

Sampling took place along a salinity gradient that was intended to characterize fouling fauna of artificial structures, namely hydrozoans [22]. Visual inspection and sampling of floating artificial structures, such as pontoons, in each sampling station resulted in many samples being retrieved to the laboratory. For detailed sampling methods, see Seyer et al., 2017. During live sorting, two nudibranchs from a sample collected on 26 July 2016 at Station 2 (GPS: 37.353544; -7.440794) were separated in an individual container for further identification, as they constituted unusual fauna for the study area. Specimens were kept in saltwater with some branches of the hydrozoan *C. caspia*, as they were originally found in the Guadiana estuary. On subsequent days of observation, the specimens laid several batches of eggs, which led us to document the process over the following weeks. Nevertheless, the experimental conditions were not entirely controlled, as the initial purpose was purely species identification.

3. Results

The two specimens retrieved at Station 2 were identified as *Tenellia adspersa* (Nordmann, 1845) (Figure 2) according to several diagnostic features, such as the round oral veil and the arrangement of the cerata in four to six groups, each with one to three elongated, cylindrical and semi-transparent cerata [5,7,8,23]. The sampling station number 2, located in the middle estuary (Figure 1), was characterized by a salinity value of 13.8 and a water temperature of 27.1 °C, measured at the surface in the day of sampling (Table 1).



Figure 2. One of the adults of *Tenellia adspersa* collected in the Guadiana estuary on 26 July 2016.

Table 1. Salinity and temperature measurements along the seven sampling stations in the Guadiana estuary on 26 July 2016.

Station	1	2 *	3	4	5	6	7
Salinity	34.0	13.8	4.9	3.3	0.3	0.2	0.1
Temperature (°C)	25.1	27.1	28.0	27.9	28.4	28.8	31.7

* Indicates the sampling station where *Tenellia adspersa* was found.

After 2 days at the laboratory, several batches of eggs were found in *C. caspia* branches (Figure 3A). Eggs developed (Figure 3B) and metamorphic individuals were observed inside egg masses after 5–7 days (Figure 3C). Hatching occurred after 9–12 days from collection in the Guadiana estuary (Figure 3D) and juveniles were observed in subsequent days (Figure 3E). After 2–3 weeks, the number of egg masses was increasingly higher, mostly due to the new generation reaching maturity and starting to spawn.

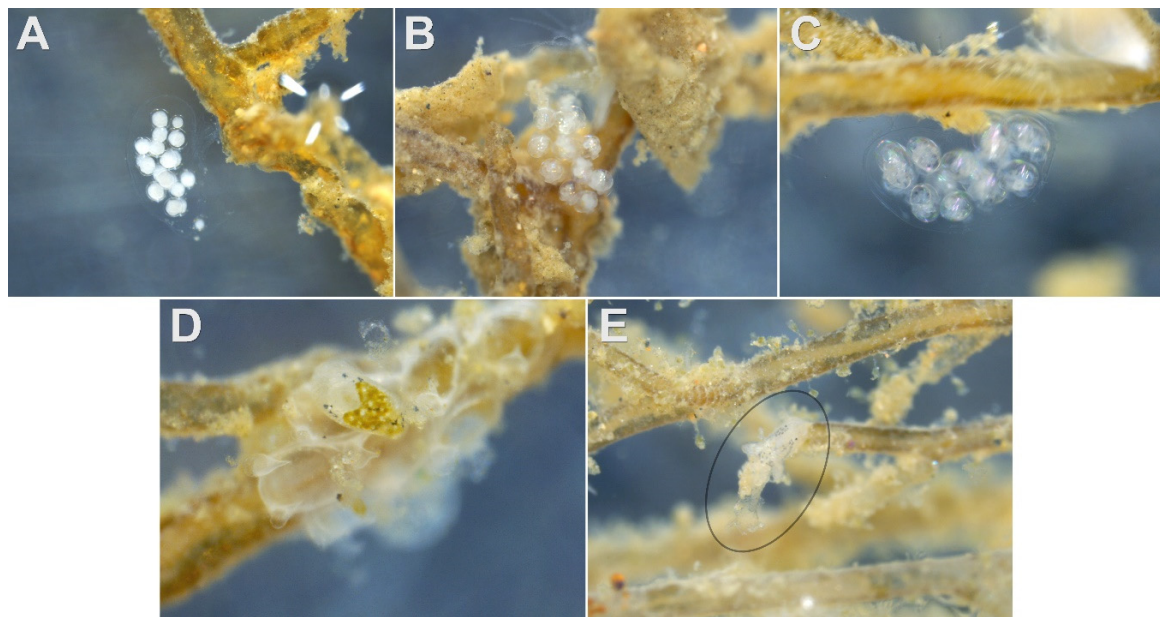


Figure 3. The several stages of development of *Tellenia adspersa* documented in laboratory. (A) Batch of eggs after 2 days at laboratory; (B) intermediate phase of development; (C) metamorphic individuals inside eggs; (D) juveniles after hatching; (E) a juvenile days after hatching.

4. Discussion

Considering the available literature, this record of *T. adspersa* in the Guadiana estuary is the first known occurrence of this species in Portugal. The habitat where it was found, the brackish-water zone of an estuary, coincides with other literature describing it as a tolerant species in terms of salinity, with an apparent optimum in the range between 12 and 25 [3,24], although it is found in the Baltic Sea at salinities of 6 PSU [4]. The reproductive biology of *T. adspersa* also makes it a very adaptable species, namely its fast generation times and reaching sexual maturity within 2 to 3 weeks after hatching [3,23]. Two different reproductive modes may also be used by the same population, the first where batches of small eggs produce pelagic free-swimming veliger larvae, and a second mode where batches of fewer, larger eggs produce capsular metamorphic individuals [23]. This capacity may also increase the survival and adaptability of *T. adspersa*, as the pelagic free-swimming larvae disperse over larger areas, while the metamorphic larvae will hatch as well-developed juveniles, increasing the chances of survival [23].

The origin of this species is difficult to pin-point, although several hypotheses can be explored. Shipping and ballast-water have always been major drivers of species dissemination across distant geographical areas, as also suggested for the presence of *T. adspersa* in North America [14] or India [16]. This vector of introduction can either bring a species from thousands of kilometres across an entire ocean, or from a neighbouring country just a few hundreds of kilometres away. Looking at the past known distribution of *T. adspersa*, the closest known record was in Cadiz, just ca. 150 km from the Guadiana estuary [17], or in the NW Iberian Peninsula in Galicia, ca. 690 km away [6]. Therefore, individuals from the Gulf of Cadiz may have naturally reached the Guadiana estuary, as there are some coastal lagoon systems in between, or aided by human-mediated vectors of introduction.

The habitat where the specimens were found also suggests possible explanations on the presence of the species in the Guadiana estuary, or rather why it was recorded now and not before on other recurrent sampling campaigns. The specimens were found on *C. caspia* hydrozoans and in laboratory were observed feeding actively on their polyps. The presence of the invasive hydrozoan *C. caspia* in the Guadiana estuary at least since 2015 [22] may have a facilitative effect on the native nudibranch. Facilitation processes by invasive species on native species have already been documented worldwide, and may range from habitat modification or creation, trophic subsidy, or food source diversification

and nutrient enrichment [25]. Studies made on predation by *T. adspersa* on *Cordylophora lacustris*, nowadays considered a synonym of *C. caspia*, indicated a preference for this prey upon other hydroids such as *Obelia commiserialis* or *Hydractinia echinata* [3,24]. Several other authors also recorded this food preference, namely in Finland [4] or North America [15].

Fouling organisms such as *C. caspia* can create a variety of economic problems related to water intakes, namely by blocking tubes, filters, irrigation systems or reducing flow in water cooling systems [26–28]. Although thermal and chemical treatments with chloride have been shown to effectively control *C. caspia*, these pose several economic and environmental challenges [26,29]. Biological control of invasive species as long been a subject of intense debate, namely on the unpredictable effects on non-target species [30]. Nevertheless, this control does not have to be through the deliberate introduction of new species. Control of invasive species by predation of native species may go unnoticed, and is generally neglected in the literature, but several examples have been documented. In the British Isles, the sacoglossan nudibranchs *Placida dendritica* and *Elysia viridis* showed, in some cases, a preference for an invasive *Codium* macroalgae over other native congeners [31]. In North America, in Chesapeake Bay, a natural control by the native blue crab *Callinectes sapidus* on young invasive rapa whelk *Rapana venosa* was documented [32]. In Europe, the use of the European eel *Anguilla anguilla* as an indigenous predator was proposed and tested towards the invasive red swamp crayfish *Procambarus clarkii*, while also contributing to the conservation efforts of the already threatened native European eel [33].

Our observations are in fact the first documentation of a potential predator for the invasive *C. caspia* in the Guadiana estuary, already impacted by several other invasive species [22,34–37]. Regarding the presence of *C. caspia* in the Guadiana estuary, it was first detected in June 2015, and in 2016 was recorded in five sampling stations across a 25 km stretch along the Guadiana estuary, on salinities between 0.2 and 13.8 [22]. The availability of hard substrate along this brackish area of the estuary, represented by artificial structures or mooring pontoons, also created a suitable habitat for *C. caspia*, which then created a suitable habitat for the nudibranch *T. adspersa*. Therefore, the discovery of the *T. adspersa* nudibranch in the Guadiana estuary in June 2016 is most likely the result of a facilitation process that may have occurred not only regarding food availability but also by an increase in habitat availability. The fast generation times of *T. adspersa* of about 17 days from egg to egg reported by Chester [3], which was consistent with what we observed at the laboratory, and food preference for *C. caspia*, suggest that it might play an important role in controlling the dispersal of the invasive hydrozoan, which must also be further investigated in future sampling campaigns and prey selection experiments.

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Conflicts of Interest: The authors declare no conflict of interest.

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