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Short communication

Occurrence of the first non-indigenous ascidian *Phallusia nigra* Savigny, 1816 (Tunicata: Ascidiacea) in Greek waters

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Abstract

The ascidian *Phallusia nigra* is listed among the non-indigenous species of the Mediterranean Sea and its first occurrence in Hellenic waters is noted here. The westward expansion of the black sea squirt from the north Levantine coast, up to the Aegean Sea has been revealed by scuba diving, as established populations were recorded offshore and in one of the harbours of Rhodes Island. Maintenance conditions of the species in captivity are briefly discussed.

Key words: Tunicata, Ascidiidae, Phallusia nigra, non-indigenous species, Aegean Sea, Mediterranean Sea

Alien species continuously increase in numbers in the Mediterranean waters (Zenetos 2010). Among the Hellenic seas, where a recent update lists 193 alien species introductions (Zenetos et al. 2009), the south-eastern Aegean region is considered the main cross roads for the northward and westward dispersion of aliens from the warm waters of the Levant (Pancucci-Papadopoulou et al. 2005). There are also indications that the increased rate of alien introductions observed in this area during the last fifteen years is strongly associated with the warming of marine waters (Pancucci-Papadopoulou et al. 2009).

Populations of *Phallusia nigra* Savigny, 1816 (Ascidiidae) have been revealed for the first time on natural and artificial substrates of Rhodes Island, increasing the number of alien species established in the region. Furthermore, this is the first non-indigenous ascidian reported in Hellenic waters.

Ten specimens of *Phallusia nigra* were collected offshore of the beach "Stegna" (geographic coordinates: 36°11'03"N, 28°08'07"E), off the eastern coast of Rhodes Island, at a depth of 12-14 meters, during a multipurpose field trip (Figure 1). Two groups of 16 and 10 individuals, approximately 100 meters apart, were observed on the morning of the 24th of November, 2009. The rocky substrate was dominated by holothurians and serpulids, while young individuals of *Oblada melanura* (Linnaeus, 1758) dwelled nearby. At the surface, salinity was 39.3 psu and temperature 20.5°C, while the concentration of dissolved oxygen was 5.13 mL/L. Nine specimens were introduced into exhibition tanks of the Hydrobiological Station of Rhodes (HSR), whilst the tenth was photo-graphed (Figure 2), measured (69.4 mm), fixed in a 4% formaldehyde solution for the HSR collection (catalogue number: HSR49) and re-measured the next day (59.4 mm). On the 10th of December 2009, many specimens (150-200) were observed on the hull of an old, anchored metallic recreational boat, in the new shipyard of Rhodes (geographic coordinates: 36°25'03"N, 28°14'01"E) (Figure 1). Of the three collected (65-74 mm) all were mature females (Figure 3).

The largest specimen measured 86 mm in its natural position, while the length of the remaining live specimens ranged between 60.3 and 77.1 mm.

Up until the time of writing this paper, the ascidians have become accustomed to the HSR facilities in 50-100 L aquaria, illuminated by fluorescent lamps under a 12 h light cycle (Figure 4). The seawater is collected directly from the sea, for the closed recirculation system (water temperature of 22.5°C, salinity 39 PSU), whereas for the open water system the water is pumped from a well (water temperature of 20.0°C, salinity 35 PSU). Up to now, a supply of

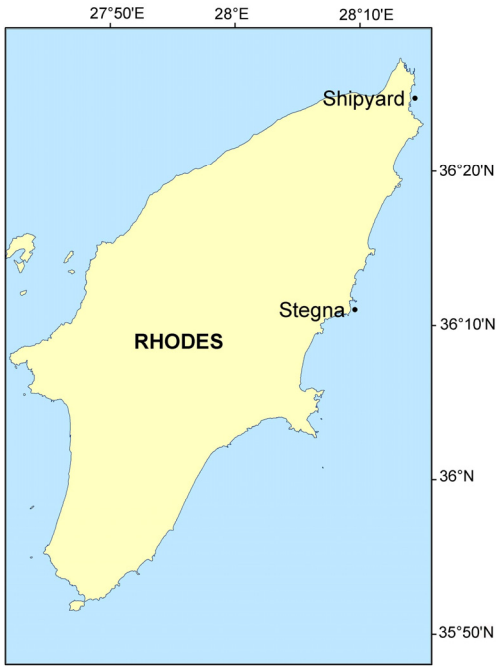

Figure 1. Sampling locations.

phytoplankton and defrosted, ground mussels and shrimps, has appeared nutritionally sufficient, as no alteration has been observed in the appearance or the overall health status of the animals. The use of submerged water circulators for the simulation of continuous currents, resembling the natural environment, is considered essential. All tanks are exhibited to the public. With reference to other ascidians, adult specimens of *Phallusia mammillata* (Cuvier, 1815), were maintained in the aquarium at 16°C with a 12 h light cycle (Zega et al. 2005), whereas *Halocynthia papillosa* (Linnaeus, 1767) and *Microcosmus sulcatus* (Coquebert, 1797) are kept at 17-19°C in curatorial and exhibition tanks of the CRETAquarium under a 12 h light cycle (Doxa Ch., personal communication).

Among the eleven species of the family Ascidiidae occurring in the Mediterranean Sea (Monniot and Monniot 1987; Koukouras et al. 1995), two are considered non-indigenous, namely *Ascidia cannelata* Oken, 1820 and *Phallusia nigra* (Zenetos et al. 2005; Izquierdo-Muñoz et al. 2009; Shenkar and Loya 2009). *A. cannelata* is a species of Indo-Pacific origin, also known from the Gulf of Suez, the Suez Canal and the lake Timsah (Por 1978) with a distribution restricted, up to date, on artificial and natural substrate of the Mediterranean coasts of Israel (Shenkar and Loya 2009). Already

Figure 2. A *Phallusia nigra* specimen freshly collected at Rhodes Island (69.4 mm). Photo by G. Kondilatos.

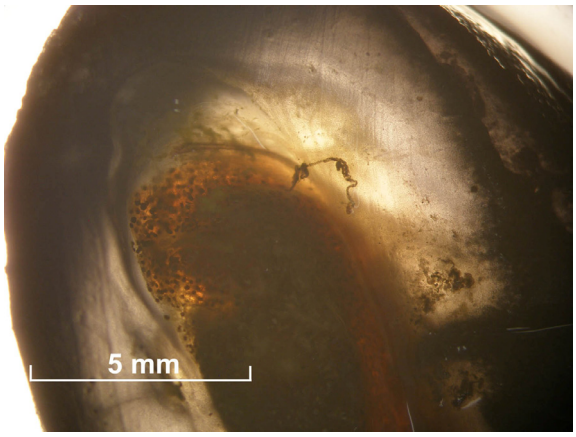

Figure 3. Gonads from a mature female. Photo by G. Kondilatos.

Figure 4. Alive *Phallusia nigra* in aquarium. Photo by G. Kondilatos.

listed among the Lessepsian migrants by Por (1978), it is considered a true Lessepsian migrant by Koukouras et al. (1995), while, following Izquierdo-Muñoz et al. (2009), the species has been introduced into the Mediterranean as fouling on ship hulls through the Suez Canal.

According to De Felice et al. (2001), the origin of *Phallusia nigra* is disputed; it may be native to the Red Sea and the Indian Ocean area, or to the tropical western Atlantic Ocean. The western Atlantic (Galil 2007) and the tropical western Atlantic Ocean (Galil et al. 2008) are reported as the native ranges of the species. *Phallusia nigra* is today classified as a tropical cosmopolitan or pantropical species (Koukouras et al. 1995; Izquierdo-Muñoz et al. 2009), due to its current wide distribution, comprising the Mediterranean, Suez Canal, Gulf of Suez, Red Sea, Gulf of Aden, Indo-Pacific Ocean and the western Atlantic ocean from Florida and Bermudas to southern Brazil (Por 1978; Lambert 2002; Koukouras et al. 1995; Subba Rao 2005; Palomares and Pauly 2009; Shenkar and Loya 2009). It is considered a non-indigenous species introduced by vessels to Hawaiian Islands (De Felice et al. 2001) and to Indian seas (Subba Rao 2005). In terms of the Mediterranean Sea, the oldest record of the solitary black tunicate is from Israeli waters by Pérès (1958). More than forty years later, Zibrowius and Bitar (2003) reported it from Lebanon where it occurs frequently and can be easily collected. Very recently, Çinar et al. (2006) documented its abundant occurrence along the Turkish Mediterranean coasts, up to Kekova, while Shenkar and Loya (2009) ascertained the wide distribution of the species on artificial and natural substrate off the Israeli coasts. Therefore, *Phallusia nigra* is known to occur today along the Levantine coasts. It is obvious that this ascidian has been a resident species in the Mediterranean waters for a long time before it begun to spread further north. However, it seems that the observed warming of the Eastern Mediterranean Sea, which showed an abrupt shift by the end of 1990's, results to more favourable conditions for the establishment of alien species (Raitsos et al. 2010). A strong correlation between alien species entry and climate warming has been detected in the area (Rhodes Island, Dodecanese) during the last decades (Pancucci-Papadopoulou et al. 2009), favouring the colonization of shallow coastal waters by tropical alien species such as *Phallusia nigra*. Due to its worldwide distribution, the species is not considered a Lessepsian migrant by Koukouras et al. (1995), while Zibrowius and Bitar (2003) underline that transoceanic navigation has largely contributed to ascidians dispersion. Galil et al. (2008) and Izquierdo-Muñoz et al. (2009) agree that the black sea squirt is an alien species introduced to the Mediterranean via fouling of vessels. Izquierdo-Muñoz et al. (2009) argue furthermore that *P. nigra* has been introduced from the Red Sea as part of the fouling fauna of the ships' hulls that enter the Mediterranean Sea through the Suez Canal. Nevertheless, whatever the origin, the spread out of the black tunicate within the Mediterranean waters follows the same pathways and patterns of most of the lessepsian species.

Due to its distinct, uniform dark colour, the black tunicate is easily distinguished from other ascidians. The openings of the two siphons are round with fringed edges. Its right region is characterized by a thick cartilaginous and smooth to the touch tunic with protrusive blood vessels. The quite sudden appearance of the species and the extended distribution that it demonstrated within a short time along the eastern coasts of Rhodes, both in offshore waters and in the harbour, support the invasive character of the species, as already pointed out by Izquierdo-Muñoz et al. (2009). Furthermore, the record of *P. nigra* from Rhodes indicates a westwards expansion of about 150 Km from its distribution off the north Levantine coasts of Turkey (Kekova, cfr. Çinar et al. 2006) up to the Aegean Sea.

The expansion of *P. nigra* should be further investigated with regard to possible lack of predation, as it has been reported that the species has no epibionts and there is a possibility that its tunic acid could be an important mean for protection against predation and bio-fouling (Stoecker 1980; Hirose et al. 2001). Concerns may involve a possible risk to human health because of its strongly acidic tunic (Hirose et al. 2001) and the occurrence of paralytic toxins, such as the paralytic shellfish toxins (PSTs) (Freitas et al. 1996). Furthermore, because this ascidian is a fouling organism and can live attached to any hard substrate (De Felice et al. 2001), it could apparently compete with other fouling organisms, both indigenous or non-indigenous, and have a negative effect on the local fauna, hence biodiversity, as has been observed for other ascidians (Lambert 2002; Lambert and Lambert 2003; Lambert 2007; Mastrototaro and Dappiano 2005).

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