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# Redescription and biology of *Diopatra neapolitana* (Annelida: Onuphidae), a protandric hermaphrodite with external spermaducal papillae

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ABSTRACT

A one-year study of the reproductive biology of a population of *Diopatra neapolitana* at Villaviciosa estuary, northern Spain, was undertaken. Field observations together with a histological study of monthly collected individuals revealed that the population was iteroparous, had a discontinuous reproductive season with a resting period during August and September and a spawning season from March to July. The study showed that *D. neapolitana* was not dioecious as previously suggested but consisted of protandric sequential hermaphrodites, pure males and pure females with a male biased sex ratio of 3:1. During the peak reproductive period from May to August we observed simultaneous hermaphrodites with two dorsal papillae per segment in the branchial region. Histological studies demonstrated that the papillae were acting as seminal vesicles, storing own sperm, and also as sperm ducts, providing an exit route; hence we termed them 'spermaducal papillae'. The papillae are not the only sperm repositories as the coelom of males and simultaneous hermaphrodites in smaller size classes is also filled with sperm. The worms are broadcast spawners with a brief pelagic larval stage as previously reported but the finer points of this unusual fertilisation system need still to be elaborated. *Diopatra cryptornata* was recently described as a new species, supposedly differing from *D. neapolitana* in chaetal detail and the possession of the papillae. We have shown conclusively with morphological and genetical studies that the former species is a junior synonym of the latter. In the absence of type material we are here designating a neotype from recently collected material from Naples.



## 1. Introduction

The tube-building polychaete *Diopatra neapolitana* Delle Chiaje, 1841 is one of the earliest known species of the genus, described in the third volume of "Animali senza Vertebre del Regno di Napoli". Although the original description was brief, the diagnostic characters generic rather than specific and the exact locality was not specified, the report mentioned already that this large littoral species was used as fish bait (Delle Chiaje, 1841). From the initial harvest of *D. neapolitana* by local fishermen, the activity has grown into a considerable industry with live bait being shipped throughout the Mediterranean and Iberia (Dagli et al., 2005; Pires et al., 2012a). *Diopatra neapolitana* is distributed throughout the Mediterranean and along the Atlantic Iberian and French coast in intertidal and shallow subtidal depths, often reaching high densities (Berke et al., 2010). This species plays important ecological roles: i) by constituting a food source for populations of marine biota such as some species of birds and fishes (Rangel and Santos, 2009); ii) as an ecosystem engineer by stabilising the sediment with its tubes, and thus increasing the structural complexity and biodiversity of its habitat (Bailey-Brock, 1984; Thomsen et al., 2011); iii) by providing refugia from disturbance and predation (Bailey-Brock, 1984) and iv) facilitating the settlement and the attachment of some algal species (Thomsen and McGlathery, 2005). Also, recently it was demonstrated that *D. neapolitana* is an

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excellent bioindicator of metal contamination, organic matter enrichment and pharmaceuticals in coastal environments (Freitas et al., 2012, 2015; Carregosa et al., 2014). Furthermore, its regenerative capacity was shown to be affected by abiotic factors (i.e. seawater pH, temperature and salinity) and that it can be used as a sensitive marker to assess the metabolic effects of the imminent climate change on marine invertebrates (Pires et al., 2015).

Despite the long history, wide distribution and great ecological and economic importance of *D. neapolitana*, our knowledge of its reproductive biology is still incomplete. The earliest mention of its reproduction was by Lo Bianco (1899) in his paper describing sexually mature animals of the Gulf of Naples. He stated that many small yellowish/brown eggs were attached to the tube of *D. neapolitana* near its distal opening in a gelatinous brown egg sac of about one square centimetre. However, other studies contradict this type of development. Cazaux (1970) artificially fertilised *D. neapolitana* eggs from Arcachon Bay (France) on the Atlantic Ocean that were green in colour and found that the lecithotrophic larvae had a brief pelagic phase before they settled, indicating that they are broadcast spawners. These findings were confirmed with studies by Conti et al. (2005) who described the sperm of *D. neapolitana* as a typical ect-aquasperm as defined by Jamieson and Rouse (1989).

Dagli et al. (2005) carried out a one-year study of a population in Izmir Bay (Aegean Sea, eastern Mediterranean). They reported coelomic gametes in all months of the year except January, with a peak reproductive period from April to August. The overall male:female ratio was 3:4, but interestingly seemed to change by months. Pires et al. (2012b) studied a population from Ria de Aveiro (Atlantic coast of northwestern Portugal) where they found coelomic gametes all year round with a peak period from May to August and an overall sex ratio of 1:1. Although not explicitly stated, both studies implied that *D. neapolitana* is dioecious.

*Diopatra cryptornata* Fauchald et al., 2012 was recently described from the eastern Atlantic coast at Obidos, Portugal and Huelva, Spain. Two features were described as unique for the new species: paired dorsal papillae and parapodia with falcate hooks on anterior segments. We have studied the reproductive biology of a population of *D. neapolitana* from Villaviciosa estuary, northern Spain, Bay of Biscay. During the course of our investigations we discovered that some members of the population, as well as some mature specimens from Vigo estuary (northwestern Spain, Atlantic Ocean) and Cádiz Bay (southwestern Spain, Atlantic Ocean), had dorsal papillae of the same kind and distribution as described for *D. cryptornata*.

The aim of the present paper is to report the findings of the reproductive biology of *D. neapolitana* from the study site and to determine whether *D. cryptornata* is a valid species. We propose to examine the morphology of *D. neapolitana* from the type locality and other localities as well as the types of *D. cryptornata*, utilise a molecular approach to establish whether there is any distinction between the *D. neapolitana* specimens with and without dorsal papillae by characterising a mitochondrial and a nuclear DNA gene, to designate a neotype, formally redescribe *D. neapolitana* and to discuss the fate of *D. cryptornata*.

## 2. Material and methods

### 2.1. Study area and sampling

Samples of *Diopatra neapolitana* were collected with a shovel to a depth of 30 cm from the intertidal sandy flats of Villaviciosa estuary, Asturias, northern Spain, Bay of Biscay, 43° 18′–43° 32′N, 5° 29′–5° 32′W at monthly intervals from September 2010 to August 2011. At this site *D. neapolitana* is periodically exposed at low tides, like almost the entire estuary. Additional samples of *D. neapolitana* were collected from April to September 2013 in, Eo estuary (Asturias, Spain), Vigo estuary (Galicia, Spain), Pontevedra estuary (Galicia, Spain), Santander Bay (Cantabria, Spain), Cádiz Bay (Andalucía, Spain) and Arcachon Bay (France). Population densities of *D. neapolitana* from the study site were estimated at low tide from counts of 50 × 50 cm quadrats taken every 5 m along a 50 m transect line parallel to the axis of the estuary in its outer basin within the *D. neapolitana* zone.

### 2.2. Treatment of live specimens

Collected specimens from Villaviciosa estuary were brought alive to the laboratory and anaesthetised in a 7.5% $MgCl_2$ solution isotonic with seawater. Subsequently the width of the 10th chaetiger (without parapodia) of all specimens (N = 162) was measured as size reference and the specimens were classified by size classes. Also, when possible the total length of complete specimens was measured and correlated with the width of the 10th chaetigerous segment. Then, a sample of the coelomic fluid (~1 ml) of about 12–16 worms per month was extracted with a Pasteur pipette after making a short incision in their body wall at about the sixtieth chaetigerous segment. The extracted coelomic fluid was then mounted fresh on a cavity slide with seawater and examined under a compound microscope. We also estimated the mean number of coelomic eggs in mature ovigerous complete specimens. For each individual, the mean oocyte size from forty oocytes was calculated using the average value of the longest and shortest oocyte diameter as estimate of oocyte size. All measurements were done monthly on freshly mounted oocytes using a calibrated eyepiece graticule. The oocyte diameter was used as an index of the stage of maturation.

### 2.3. Preservation and histological analyses

Eight to ten tubes with worms were sampled during the annual breeding period and fixed in 10% buffered formalin to assess the presence/absence of brooding eggs, embryos or attached egg masses. In order to study gametogenesis and sexual strategy, serial histological sections were examined after treating individuals as follows. Specimens were fixed in Bouin's solution for 24–48 h, washed thereafter in 70% ethanol for two days prior to dehydration, dehydrated following standard methods, transferred to xylene and embedded in paraffin wax. Serial, 6 μm thin sections were cut with a Leica 2045 microtome and double stained with haematoxylin and eosin. A total of 50 individuals (without evident signs of regeneration) collected from all sampling months and distributed among all size classes established were histologically examined.

Selected specimens were prepared for scanning electron microscopy (SEM) for the study of their general morphology. Specimens were dehydrated in an ascending series of ethanol, critical point dried using acetone as transition liquid and sputter coated with gold. Samples were then imaged using a JEOL 6610 LV scanning electron microscope. To study the spermatozoa ultrastructure, mature sperm were extracted from the coelomic cavity of recently fixed worms. The extracted sperm was prepared for SEM examination as described above.

### 2.4. Deposition of specimens and taxonomic procedures

Voucher specimens from the study sites (fixed in 10% buffered formalin, preserved in 70% ethanol) have been deposited at the Museo Nacional de Ciencias Naturales, Madrid (MNCN) and the Australian Museum, Sydney (AM). Voucher specimens used for genetic study (fixed in 100% ethanol) have been deposited at the University Museum of Bergen (ZMB).