ORIGINAL ARTICLE

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A new and alien species of "oyster leech" (Platyhelminthes, Polycladida, Stylochidae) from the brackish North Sea Canal, The Netherlands

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Abstract A new species of polyclad flatworm, *Imogine necopinata* Sluys, sp. nov., is described from a brackish habitat in The Netherlands. Taxonomic affinities with Asian species and the ecology of the animals suggest that the species is an introduced, exotic component of the Dutch fauna. The new species belongs to a group of worms with species that are known to predate on oysters.

Keywords Platyhelminthes · Polycladida · *Imogine necopinata* · Invasive species · The Netherlands

Introduction

The North Sea Canal or Noordzeekanaal is situated in the Province of North Holland and forms the connection between the port of Amsterdam and the North Sea. It is one of the last large brackish water gradients left in The Netherlands. The canal harbours a relatively rich assemblage of brackish water species, with both indigenous as well as exotic species. Because of intense shipping by cargo vessels from all over the world, new

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S. Rajagopal · G. van der Velde Department of Animal Ecology and Ecophysiology, University of Nijmegen, Toernooiveld, 6525 ED, Nijmegen, The Netherlands invasions of exotic animals and plants are to be expected in the North Sea Canal. Establishment of exotic species in the North Sea Canal mainly depends on their tolerance to existing variations in brackish water conditions and temperature regimes. The area along the North Sea Canal is highly industrialized and houses several power stations. For studying the biofouling in cooling water circuits, PVC panels were suspended in the inlet and outfall of cooling water conduits of the Velsen and Hemweg power stations from June 1994 to October 1994.

During this study period, numerous flatworms were observed on the PVC panels during the month of August. The objective of the present study is to describe this polyclad species and to present information on its habitat, ecology, and the type of potential ecologial impact of this animal.

Methods

Experimental PVC panels (200×100×5 mm) tied with mild steel rope were suspended at 1 m, 2 m, and 3 m as test coupons. For details of panel exposure procedures we refer to Rajagopal et al. (1996). The composition of the fouling community was determined from duplicate 25 cm^2 scrapings from the test panels. Each sample was studied for species composition. numerical abundance and dry biomass. For the present study, a single large polyclad specimen was collected from the panels for further description of the species. The specimen was killed in 4% formalin and subsequently transferred to 70% ethanol. Before further processing, the specimen was post-fixed in Steinmann's fluid. Sections were made at intervals of 6 µm, mounted on six glass slides, and stained in Mallory-Cason. This animal and other material examined are deposited in the Zoological Museum of the University of Amsterdam (ZMA).

Results

Polycladida Lang, 1884 Acotylea Lang, 1884 Stylochidae Stimpson, 1857 Genus *Imogine* Girard, 1853 *Imogine necopinata* Sluvs, sp. nov.

Material examined

Holotype: ZMA V.Pl. 978.1, North Sea Canal, Velsen Power Station, 19 October 1994, sagittal sections on six slides.

Other material examined: ZMA V.Pl. 979.1, North Sea Canal, km 2, 30 September 1993, sagittal sections on 2 slides; V.Pl. 980, IJmuiden, North Sea Canal, 10 September 2000, preserved specimen; V.Pl. 981, IJmuiden, Binnenspuikanaal, 30 October 2001, preserved specimens.

Diagnosis

Imogine necopinata is characterized by a tripartite, muscular seminal vesicle, a single, common gonopore, and marginal eyes that extend backwards up to the level of the nuchal tentacles.

Etymology

The specific epithet is from the Latin adjective "necopinatus", unexpected, and alludes to the fact that presumably the nearest relatives of the species live in Japanese waters.

Description

The preserved, oval-shaped specimen was white, with a length of 5.5 mm and a maximum width of about 4.5 mm. For the living specimens, it was reported that they were dorsally covered by brown pigment spots or maculae. Nuchal tentacles small, containing many eyes. Numerous eyes along the anterior body margin up to the level of the nuchal tentacles. Cerebral eyes present, varying between three and five eye spots; frontal eyes absent. Ruffled pharynx central; mouth opening central in the pharyngeal cavity (Fig. 1).

The epidermis is densely packed with short dermal rhabdites, especially the ventral and dorso-lateral sides of the body. The dorsal and ventral muscle layers differ in size and sequence. The dorsal one is formed inwards by smooth layers of circular, longitudinal, and diagonal layers. The musculature of the ventral body wall is stronger than the dorsal one, consisting inwards of circular, longitudinal, diagonal, and longitudinal muscle fibres.

Testes ventral, throughout the body length. Ovaries scattered dorsally throughout the body.

The uterine canals join at the distal end of the vagina, the latter receiving the openings of numerous shell glands. The vagina opens to the exterior through the common gonopore.

Each of the vasa deferentia opens into the lateral lobes of the large, tripartite seminal vesicle. This vesicle lies ventrally, partly underneath the prostatic organ. The muscular seminal vesicle tapers posteriorly to form the narrow ejaculatory duct, which opens into the narrow prostatic duct. The prostatic vesicle is large, with a thick coat of muscles, regularly traversed by narrow ducts.

Fig. 1 Imogine necopinata. Drawings combined from two specimens from samples V.Pl. 980 and V.Pl. 981. a Dorsal view, showing the arrangement of the eyes. b Ventral view. Scale bar: 1 mm. Abbreviations: br brain, ce cerebral eyes, cgo common gonopore, me marginal eyes, mo mouth opening, ph pharynx, pv prostatic vesicle, te tentacular eyes, vd vas deferens

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Fig. 2 Imogine necopinata. Holotype. ZMA V.Pl. 978.1. Sagittal reconstruction of the copulatory apparatus. Abbreviations: cgo common gonopore, ed ejaculatory duct, gd glandular duct, pd penial duct, pp penial papilla, pv prostatic vesicle, sv seminal vesicle, uc uterine canal, v vagina, vd vas deferens



These ducts lead the erythrophilic secretion, produced by extravesicular gland cells, to the lumen of the prostatic vesicle, which is lined with a folded and highly glandular epithelium (*Stylochus djiboutiensis* type) (Fig. 2).

The penial papilla is a well developed, stubby cone that completely fills the penial cavity. The latter opens to the exterior through the common gonopore.

Ecology

The holotype specimen was collected on 19 October 1994 from an experimental PVC panel suspended on 30 June 1994 at 1 m depth near the inlet area of the Velsen power station. The specimen was found on the panel surface along with other exotic species: the mussel *My*-tilopsis leucophaeata (Conrad, 1831), the barnacle Balanus improvisus Darwin, 1854 the calcareous tubeworm *Ficopomatus enigmaticus* (Fauvel, 1923), the hydroid *Cordylophora caspia* (Pallas, 1771), and the gammarid *Gammarus tigrinus* Sexton, 1939. During this period the water temperature varied from 23.9 to 26.1°C. The salinity values ranged from 4.8 to 5.3%. The chlorophyll-a values varied between 18.1 and 66.7 µg l⁻ 1. The pH values varied from 7.9 to 8.1.

Most specimens were collected from the Binnenspuikanaal to 2 km land inward along the North Sea Canal (Fig. 3). Here, they were collected from artificial substrates, the undersides of stones or from sandy bottom and occasionally from empty barnacles. They were found at a maximum depth of 9.6 m together with a large number of calcareous tubeworms *Ficopomatus enigmaticus*, overgrown by barnacles *Balanus improvisus* and the mussel *Mytilopsis leucophaeata* (Kaag 2002). The flatworms have been collected by A.G. Klink in the Noordzeekanaal as early as 30 September 1993 at 1 m depth at km 2 and km 13. In

October 1997 four polyclad flatworms were collected from the undersides of stones in the Noordzeekanaal near the recreation parc Spaarnwoude at depths of 0.2-0.5 m (Van Splunder 1998). On 17 June 2003 again, two specimens were collected at the same locality. In the Binnenspuikanaal at a depth of 9.6 m, densities of 6.7 specimens per square metre were recorded during October-November 2001 and densities of 2.9 specimens per square metre at a depth of 9.2 m during April-May 2002 (Kaag 2002). In the same area, some new specimens were collected from artificial substrate during 2002 (personal communication, Myra Swarte). Twentyone specimens were collected from the Afrika harbour during June, September, and October 2002 from stones at a depth of 0.5 m (Myra Swarte, personal communication). Salinity of the localities with the new species ranged from 3.8 to 14.9%; water temperatures ranged from 11.1 to 26.1°C.

Discussion

Although only one specimen was sufficiently well preserved for sectioning and even the preparations of this animal are not optimal, there are two distinct features enabling one to assign the animal to a particular genus. A single gonopore is not common among acotylean polyclads but has been described for Stylochus catus Marcus and Marcus, 1968, Stylochus uniporus Kato, 1944, and S. miyadii Kato, 1944. Each of the two Japanese species has a tripartite, muscular seminal vesicle, which is present also in S. catus. Marcus and Marcus (1968) proposed the new subgenus Stylochus (Imogine) Girard, 1853 for polyclads with such a tripartite seminal vesicle, a suggestion followed by Faubel (1983). In a recent study on the Australian representatives of this subgenus, Jennings and Newman (1996) re-elevated Imogine to genus level, with the tripartite, anchorFig. 3 Distribution records of *Imogine necopinata* in the North Sea Canal



shaped, muscular seminal vesicle as its diagnostic feature.

It is evident that because of the presence of such a tripartite seminal vesicle, the specimen described above belongs to the genus Imogine. Imogine necopinata is mainly characterized by a true common gonopore at the rear body end, marginal eyes along the anterior margin, nuchal tentacles containing numerous eyes, and frontal eyes being absent. Currently, this genus contains 33 species, of which only I. cata, I. unipora, and I. miyadii have a true common gonopore, suggesting a close relationship to I. necopinata. The difference between I. unipora and our specimen lies in the fact that the penial papilla in the Japanese species is very small and the marginal eyes extend to the anterior end of the last fourth of the body. In I. miyadii the penial papilla has about the same relative size as in our specimen, but I. miyadii differs from the Dutch animal in the much more posterior location of its nuchal tentacles and in the arrangement of marginal eyes, which extend up to the anterior level of mid-body. Apart from the absence of cerebral tentacles, I. necopinata resembles most closely I. cata with respect to the arrangement of marginal and tentacular eyes, location of pharynx, mouth, genital organs, and location of the gonopore. Differences exist in the succession of muscle layers of the body wall. Information on the sequence of the muscle layers in the body wall of *I. unipora* and *I. mivadii* is not readily available.

Consequently, the Dutch specimen represents a new, and hitherto unrecorded species. Geographically, the nearest representative of the genus *Imogine* is *I. mediterranea* (Galleni, 1976), described from Italian coasts; it is a species with separate male and female gonopores. Present knowledge does not allow a firm conclusion on the origin of the Dutch species; it may either be an introduced species with Australasian affinities, or an undescribed autochthonous species from the North Atlantic region.

In this context it is interesting to note that also *Stylochus flevensis*, described by Hofker (1930) from the former Dutch Zuiderzee (Southern Sea), which after closure in 1932 became the present freshwater Lake IJsselmeer, is a polyclad species presumed to have tropical affinities. According to Hofker (1931) *Stylochus flevensis* resembles *S. orientalis* Bock, 1913 from Formosa Strait (26°N 121°30'E, Gulf of Thailand), and Western Australia (Cape Jaubert), the latter species now considered to be a member of the genus *Imogine*. But Hofker (1930, 1931) considered *S. pusillus* Bock, 1913 from Hongkong to be the closest relative of *S. flevensis*; currently the former is considered to belong to another genus and is classified as *Distylochus pusillus* (Bock, 1913).

In our opinion, S. flevensis belongs to the species group characterized by a prostatic vesicle of the Stylochus neapolitanus type. However, S. flevensis is morphologically a relatively isolated species within this group because of its eye arrangement: presence of frontal eyes and marginal eyes extending up to mid-body. It is evident that in its gross morphology, notably body size, colour, arrangement of cerebral, marginal and tentacular eyes, S. flevensis very much resembles I. necopinata. However, a more detailed analysis of their anatomy reveals some distinct differences, albeit that we offer the caveat that in the absence of type specimens (we have been unable to trace the type material), we accept Hofker's (1930) account on S. flevensis as being an accurate representation of its anatomy. The marginal eyes of I. necopinata are developed only along the anterior margin of the body, up to the level of the tentacles, in contrast to S.flevensis in which the marginal eyes extend backwards well beyond the tentacles. The male and female gonoducts in *I.necopinata* unite to form a common opening to the exterior, whereas S.flevensis was described with separate male and female gonopores, even though these are located close together. However, all of these features may be subject to variation, either of ecophysiological nature or resulting from preservation artefacts. The most important difference between S. flevensis and I. necopinata lies in a feature that also forms the basis of the taxonomic difference between the genera Stylochus and Imogine. In the last-mentioned genus, the seminal vesicle is tripartite or anchor-shaped, receiving the separate openings of highly muscular sperm ducts, as is the case in I. necopinata. In contrast, S. flevensis was described by Hofker (1930) with a large muscular seminal vesicle receiving the openings of thin-walled, non-muscular or weakly muscular sperm ducts, which may be expanded to false seminal vesicles; this places S. flevensis squarely within the genus Stylochus.

Stylochus polyclads, and others as well, are wellknown and effective predators on cultured bivalves and on barnacles (cf. Skerman 1960; Branscomb 1976; Galleni et al. 1980; Chintala and Kennedy 1993; Jennings and Newman 1996; Newman et al. 1993, and references therein). From that standpoint, possible introductions of little known animals such as polyclad flatworms should not be taken lightly. Other flatworms introduced into Europe from Australasian regions, notably land planarians, have already demonstrated that they can become local pests when living outside of their native distributional areas and habitats (cf. Jones and Boag 2001, and references therein). Faasse (2003) recorded presumed Stylochus flevensis from the undersides of stones in the brackish Canal Through Walcheren. Examination of preserved specimens may reveal whether these animals concern S. flevensis or I. necopinata. It should be noted that the oyster cultures in this southern part of The Netherlands may form ample food supply for introduced stylochid flatworms, otherwise also known as oyster leeches (cf. Newman and Jennings 1997; O'Connor and Newman 2001).

It is interesting to note that during 2002 another alien polyclad species was discovered in the North Sea Canal, viz. *Euplana gracilis* (Girard, 1850), a species from the Atlantic coast of North America (Faasse and Ates 2003).

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