

***Proceraea exoryxae* sp. nov. (Annelida, Syllidae, Autolytinae), the first known polychaete miner tunnelling into the tunic of an ascidian**

Comment [cg1]:

Comment [cg2]: sp. nov. Check for consistency throughout - I saw at least one sp. n. on line 216

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**ABSTRACT**

While studying organisms living in association with the solitary tunicate *Phallusia nigra* (Asciacea, Ascidiidae) from a shallow fringing reef at Zeytouna Beach (Egyptian Red Sea), one of the collected ascidians showed peculiar perforations on its tunic. Once dissected, the perforations revealed to be the openings of a network of galleries excavated in the inner tunic (atrium) by at least six individuals of a polychaetous annelid. The worms belonged to the Autolytinae (Syllidae), a subfamily that is well known to include specialized predators and/or symbionts, mostly associated with cnidarians. The Red Sea worms are here described as *Proceraea exoryxae* sp. nov., which are anatomically distinguished by the combination of simple chaetae only in anterior chaetigers, and a unique trepan with 33 teeth in one outer ring where one large tooth alternates with one medium-sized tricuspid tooth, and one inner ring with small teeth located just behind the large teeth. Male and female epitokes were found together with atokous individuals within galleries. *Proceraea exoryxae* sp. nov. constitutes the first known miner in the Autolytinae and the second species in this taxon known to live symbiotically with ascidians. The implications of finding this specialised parasite are discussed considering that *P. nigra* has been introduced worldwide, in tropical and sub-tropical ecosystems, where it has the potential of becoming invasive.

## INTRODUCTION

There are approximately 11,840 polychaete annelids known, spanning a remarkable array of habitats, ecological niches, and trophic modes (Read & Fauchald 2016). Among these, symbiotic species (sensu Castro 2015) span at least 28 different families (Martin & Britayev 1998). These symbiotic interactions, in general, are poorly understood, but cases of inquilinism, commensalism, mutualism and parasitism have been documented. Interestingly, parasitism seems to be among the least common modes of life for polychaetes (< 0.5% of known species, spread among 13 families), most of them being found within the Spionidae and most often being shell borers (Martin & Britayev 1998). Several reports of associations with tunicates (Phylum Chordata) are available, but the polychaetes have not been identified in some of these instances (Fiore & Jutte 2010; Illg 1958; Monniot 1990; Okada 1935; Spooner et al. 1957). There are few details known for these associations although consumption of the ascidian host has been reported in one case (Spooner et al. 1957).

*Phallusia nigra* Savigny, 1816, is a solitary ascidian that has been introduced into tropical and subtropical ecosystems worldwide since it was originally discovered in the Red Sea (Shenkar 2012; Vandepas et al. 2015; Zhan et al. 2015). The ascidian hosts a remarkable array of crustacean symbionts, including amphipods and at least eight confirmed copepod species (Kim et al. 2016). During studies on the ecology of *P. nigra* and its associated fauna in the Egyptian coast of the Red Sea, one of the collected specimens showed various perforations on its tunic. Upon dissection, we discovered a network of excavated galleries resembling the habit of some leaf-mining herbivores in terrestrial and marine habitats (Brearley & Walker 1995; Connor & Taverner 1997; Mejaes et al. 2015; Sinclair & Hughes 2010). The galleries were inhabited by several specimens of a small polychaete species belonging to the subfamily Autolytinae (Annelida, Syllidae). Although some bivalves and crustaceans have been reported to live within ascidian tunics (Cañete & Rocha 2013; Lambert 2005; McClintock et al. 2009; Morton & Dinesen 2011), no previous reports of annelids exhibiting a similar habit are known (Lambert 2005; Monniot 1990).

The Autolytinae are small free-living polychaetes, ranging from 1–60 mm long and from 0.1–1.2 mm wide. They are distributed worldwide and inhabit shallow waters, mostly restricted to the continental shelf. They often live in a more or less intimate association with sedentary

**Comment [cg3]:** I believe that Spooner et al. actually identified the polychaete to species- and because it was an autolytine, it is therefore relevant background information. Ok I see you mention it later - could also refer to Discussion

125 Subclass ERRANTIA Audouin & Milne-Edwards, 1832

126 Order PHYLLODOCIDA Dales, 1962

127 Suborder NEREIDIFORMIA

128 Family SYLLIDAE Grube, 1850

129 Subfamily AUTOLYTINAE Langerhans, 1879

130 Tribe PROCERINI Nygren, 2004

131 Genus *Proceraea* Ehlers, 1864

132

133 *Proceraea exoryxae* sp. nov.

134 LSID. urn:lsid:zoobank.org:act:34373CE6-A0D4-488D-B4A5-12CF4E103504

135 (Figures 2–7)

136

137 **Type material.** Holotype. MNCN16.01/17717: atokous anterior fragment, Zeytouna Beach,  
138 Egyptian Red Sea, 27°24'09.2"N 33°41'08.5"E, October 7 2010, 3–7 m depth, E. Cruz-Rivera  
139 coll.; fixed in 4% formalin seawater, preserved in 70% ethanol. Paratypes. MNCN16.01/17718:  
140 atokous anterior fragment, pharynx dissected, same as holotype; MNCN16.01/17719: atokous  
141 anterior fragment, prepared for SEM, same as holotype; MNCN16.01/17720: atokous anterior  
142 fragment, pharynx dissected, same as holotype; MNCN16.01/17721: male stolon, anterior  
143 fragment, same as holotype; MNCN16.01/17722: female stolon, anterior fragment, same as  
144 holotype; MNCN16.01/17723: atokous mid-body fragments, same as holotype.  
145 MNCN16.01/17724: atokous posterior fragments, same as holotype.

146

147 **Diagnosis.** *Proceraea* with simple chaetae in anterior chaetigers, and a trepan with 33 teeth with  
148 one outer ring where one large tooth alternates with one medium-sized tricuspid tooth, and one inner ring  
149 with small teeth located just behind the large teeth.

150

151 **Description.** All observations are from preserved specimens if not otherwise stated. Length 3–  
152 10.5 mm for 10–68 chaetigers in four anterior fragments, 3–14.5 mm for 19–90 chaetigers in  
153 nine median fragments, and 6.5–22 mm for 50–125 chaetigers in three posterior fragments.  
154 Width of anterior fragments, excluding parapodial lobes, c. 0.4 mm. Live individuals dorsally  
155 with light brown transverse stripes, one per segment, not known whether these are inter- or

**Comment [cg4]:** Institutional  
acronym needs to be spelled out  
earlier

intrasegmental, or if there is any other additional coloration (Fig. 2B-E); **proventricle** white (Fig. 2B, C). Formalin preserved specimens without any sign of coloration.

Body shape, excluding parapodial lobes, cylindrical in transection, ventrally flattened. Body long and slender, with slowly tapering end. Nuchal epaulettes ciliated, otherwise ciliation not assessed. Prostomium rounded rectangular (Fig. 3A, C). Four eyes with lenses, anterior pair larger, confluent in dorsal view, eye spots absent (Fig 3C). Palps in dorsal view projecting c. half of prostomial length, fused (Fig. 3A, B).

Nuchal epaulettes extending to median part of chaetiger 1 (Fig. 3A). Prostomium with three antennae, median antenna inserted medially on prostomium, lateral antennae on anterior margin. Median antenna reaching chaetiger 8–10, lateral antennae about half as long as median antenna. Tentacular cirri two pairs. Dorsal tentacular cirri about two third as long as median antenna, ventral tentacular cirri about half as long as dorsal tentacular cirri. Alternation in direction of cirri not assessed. First dorsal cirri about as long as median antenna, second dorsal cirri as long as ventral tentacular cirri. From chaetiger 3 to chaetiger 20–25, cirri alternate indistinctly in length, shorter cirri slightly shorter and longer cirri equal or slightly longer than body width excluding parapodial lobes (Fig. 3A, B), dorsal cirri in more posterior chaetigers more or less equal in length, c. half of body width excluding parapodial lobes; anal cirri as long as half body width, excluding parapodial lobes at level of proventricle.

Cirrophores on tentacular cirri, first and second dorsal cirri (Fig. 3A), otherwise absent. Antennae, tentacular cirri, dorsal cirri, and anal cirri cylindrical. Parapodial lobes rounded. Aciculae 2–3 in anterior chaetigers, 1–2 in median and posterior chaetigers.

Chaetal fascicle with 9–12 chaetae in anterior chaetigers (Fig. 4A), 4–10 in median and posterior chaetigers. Chaetiger 1–5 with simple chaetae only (Fig. 4B, 5A–D), chaetiger 6 with simple chaetae only (n=3), or with single compound chaeta in addition to the simple chaetae (n=1). From chaetiger 7 to between chaetiger 10–13 with an increasing proportion of compound chaetae (Fig. 4A). Except for the single, thick, distally denticulated bayonet chaeta (Fig. 5E), starting at the earliest in chaetiger 9, more posterior chaetigers with compound chaetae only. Simple chaetae unidentate with rows of spines subdistally (Fig. 4B, 5A–D, F). In anterior 4–5 chaetigers most simple chaetae with a proportionally long region distal to the swollen neck (Fig. 4B, 5B–D), one or two of the inferior-most chaetae with a shorter region distal to the swollen neck (Fig. 4B, 5A, C, D), similar in appearance to the shafts of the compound chaetae found in

**Comment [cg5]:** Yes, this is the preferred term, but 'proventriculum' used in figure caption. Check for other instances.

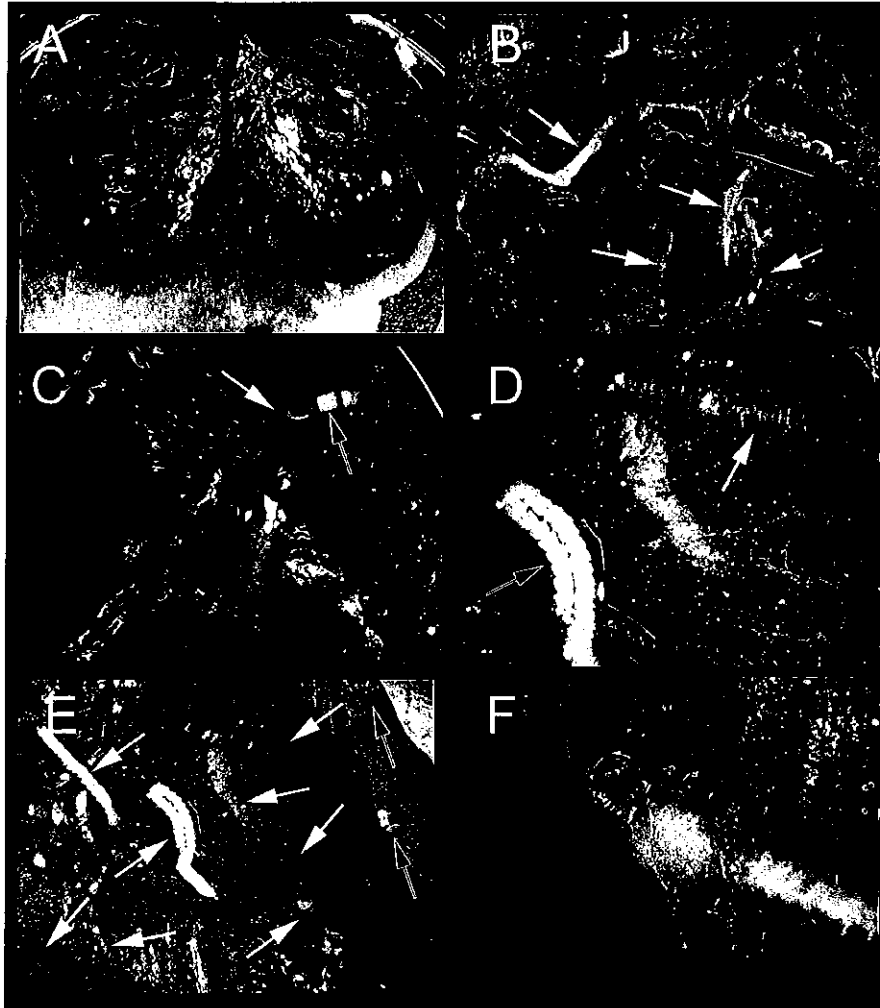
**Morphology of the epitokous female.** Length 5 mm for 6+27+9 chaetigers in region a, b and c (see Nygren 2004), incomplete; width in region a and c 0.6 mm excluding parapodial lobes, in region b 1 mm including parapodial lobes. Colour of living individuals unknown. Preserved specimen yellowish, with body filled by eggs (Fig. 7D); colour markings absent. Ciliation not assessed. Prostomium rounded rectangular, wider than long, anterior margin straight. Four eyes with lenses, situated ventro-lateral and dorsal on prostomium, ventro-lateral pair larger (Fig. 7C, D). Palps absent. Nuchal organs reaching beginning of chaetiger 1 (Fig. 7D). Median antenna inserted medially on prostomium, reaching c. chaetiger 5; lateral antennae inserted on anterior margin, about two third in length of median antenna. Tentacular cirri 2 pairs (Fig. 7C), dorsal tentacular cirri, as long as prostomial width, ventral tentacular cirri about 1/2 as long as dorsal pair. First dorsal cirri (Fig. 7D), equal in length to lateral antennae. Achaetous knobs absent. Cirri in region a reciprocally equal, slightly shorter than first dorsal cirri, equal in length to body width excluding parapodial lobes, cirri in region b reciprocally equal, slightly longer than cirri in region a, cirri in region c reciprocally equal, slightly shorter than cirri in region a. Ceratophores on median and lateral antennae, cirrophores present on all dorsal cirri, but tentacular cirri without cirrophores. Antennae, tentacular cirri, and dorsal cirri cylindrical. Parapodia in region a uniramous, neuropodial lobes rounded, parapodia in region b with additional notopodial lobes. Single neuropodial acicula in all chaetigers; 2–3 anterodorsal and 6–7 posteroventral notopodial aciculae in region b. Neuropodial fascicle with 7–8 compound chaetae and a single bayonet chaeta of the same types described for the atokous form. Notopodial chaetal fascicle with 20–25 long and thin swimming chaetae.

**Comment [cg6]:** Can these be illustrated?, especially because syllid swimming chaetae are not often illustrated.

## DISCUSSION

### Taxonomic remarks

The combination of morphological features in *Proceraea exoryxae* sp. nov. makes it difficult to place it in any specific genus within the Autolytinae. The thick type of bayonet chaeta, distally denticulated, and the presence of cirrophores only on anterior-most cirri indicate, however, that



752  
 753 Figure 2. A. An uninfected *Phallusia nigra* dissected to show normal atrial surface (left) and  
 754 internal organs. B. Inner atrial surface showing the presence of several atokous forms inside their  
 755 galleries (white arrows). C. Detail of the colour of the anterior region of an atokous form; white  
 756 | arrow: position of pharynx; black arrow: position of proventriculus. D. Detail of colour of the  
 757 mid-body of an atokous form (white arrow) and the posterior region of a male epitokous form  
 758 (black arrow). E. Inner atrial surface showing the presence of atokous (white arrows) and male  
 759 epitokous (yellow arrows) forms inside their galleries, as well as part of the banded body of an  
 760 atokous form protruding from an external tunic opening and other empty tunic openings (black  
 761 arrows). F. Close-up view of the head of a male epitoke in its gallery (specimen not preserved).

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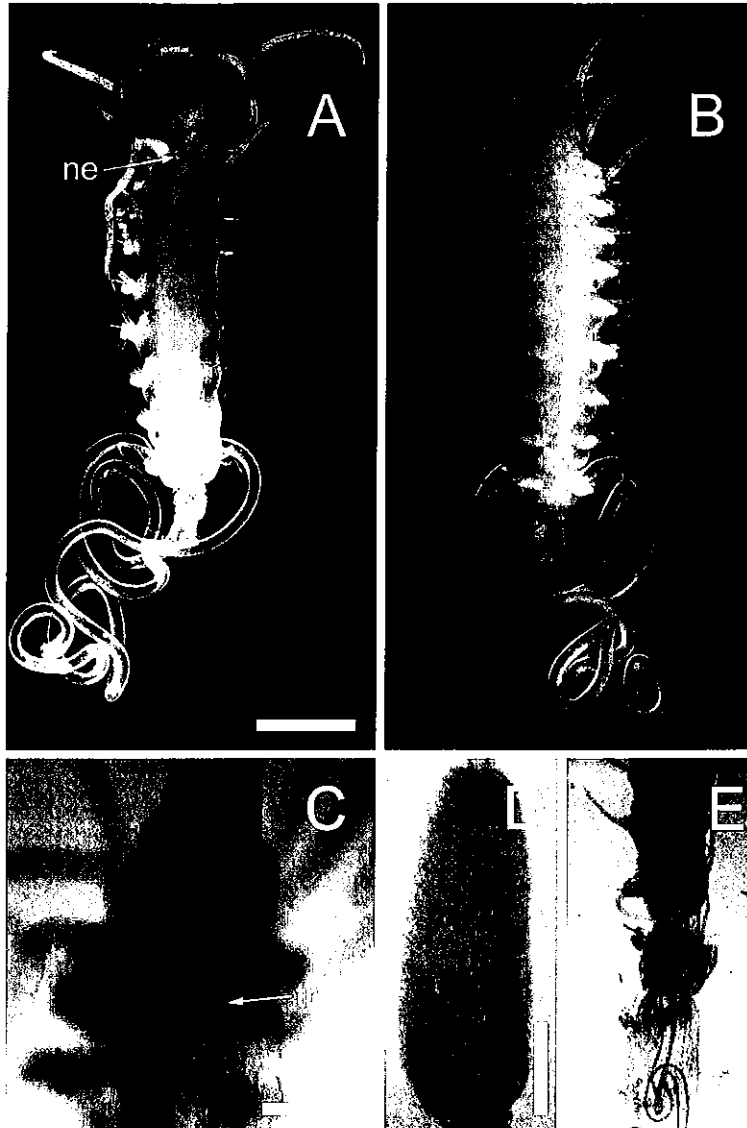


Figure 3. *Proceraea exoryxae* sp. nov. A. Anterior fragment, dorsal view [MNCN16.01/17719]. B. Anterior fragment, ventral view [MNCN16.01/17719]. C. Anterior end, dorsal view [MNCN16.01/17719]. D. Proventricle [MNCN16.01/17719]. E. Pharynx situation in chaetiger 9–14, dorsal view [MNCN16.01/17720]. Abbreviations as follows: ne = nuchal epaulettes, tr = trepan. Scale bars A, B, E = 0.5 mm, C, D = 0.2 mm.

**Comment [cg7]:** Mention that the body is broken after chaetiger 10 exposing the pharynx

**Comment [cg8]:** Hmmm, picture quality is marginal; even with a polychaete imagination, I struggle to see the trepan.

**Comment [cg9]:** The image is not sharp - can it be enhanced?

**Comment [cg10]:** Is this figure necessary considering that the pharynx is shown in A, B.

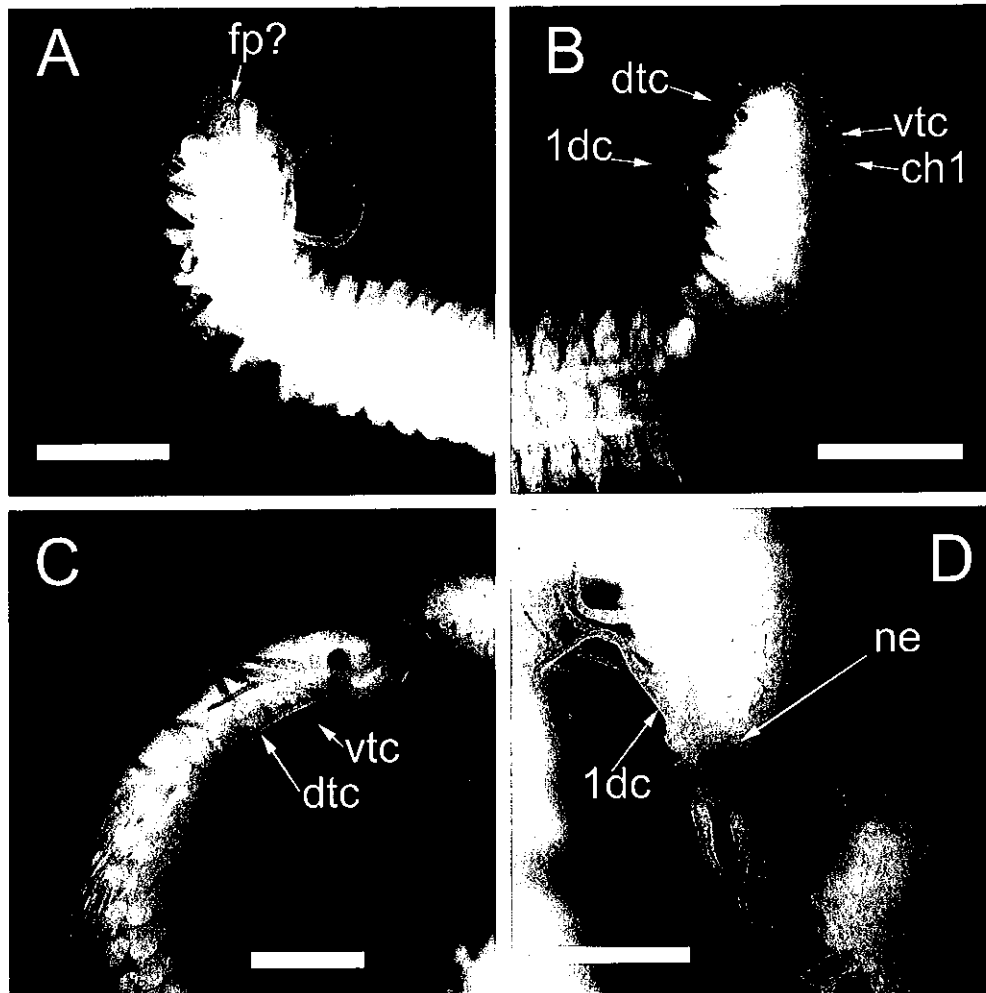


Figure 7. *Proceraea exoryxae* sp. nov. A. Male stolon, anterior end, dorsal view [MNCN16.01/17721]. B. Male stolon, anterior end, ventral view [MNCN16.01/17721]. C. Female stolon, anterior end, anteroventral view [MNCN16.01/17722]. D. Female stolon, anterior end, dorsal view [MNCN16.01/17722]. Abbreviations as follows: dtc = dorsal tentacular cirri, vtc = ventral tentacular cirri, 1dc = first dorsal cirri, ch1 = chaetiger 1, fr = frontal process, ne = nuchal epaulettes. Scale bars A–D = 0.5 mm.

**Comment [cg11]:** Consider omitting reference to ne - they are not clear on this copy