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Moonshine worms (*Diopatra aciculata*: Onuphidae, Annelida) in the Knysna Estuary, South Africa; taxonomy and distribution

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Abstract

Moonshine worms are a popular bait species used for fishing. The taxon was not detected during surveys of the macrobenthos conducted in Knysna in the 1940s and 1990s, and was first reported as a harvested bait species in the mid-2000s, suggesting that it appeared for the first time in the estuary in the last three decades. A previous molecular analysis identified the worms as *Diopatra aciculata*, a species first described from Australia. This study provides an updated detailed morphological description of *D. aciculata* in South Africa to facilitate future identifications and also investigates the species' distribution and population size in the Knysna Estuary. Specimens were examined by scanning electron, stereo- and compound microscopes. *Diopatra aciculata* has tubes that protrude from the sediment in sandy areas, often decorated with algae and shell fragments; a large body size, up to 600 mm long and 11.5 mm wide. It has 10–18 rings on ceratophores; 5–10 teeth on pectinate chaetae; uni- and bidentate pseudo-compound falcigers and dorsal cirri approximately as long as branchiae. *Diopatra aciculata* was detected up to 12 km from the mouth of the Knysna Estuary with densities measured at 18 sampled sites. Statistical analysis retrieved high and low density groups that were significantly different from one another (Kruskal-Wallis $H_{(14,\ 800)} = 376.55$; $P = 0.01$), but distribution of high density sites was patchy. We estimate that the population comprises 20–24 million individuals. Given the size of individual worms and the population estimate, this species can be expected to have significant ecological impacts in the estuary.

Introduction

Knysna Estuary is the largest clear-water estuary along the coast of South Africa (Allanson *et al.*, 2000*a*), and is ranked the most important estuary in the country based on size and the high level of biodiversity it supports (Turpie & Clark, 2007). The estuary is incorporated into the Garden Route National Park and as such is managed and protected by South African National Parks. Knysna Estuary is also a popular tourist destination and fishing spot (Hodgson *et al.*, 2000). Consequently, behaviour of fishermen and bait collectors and their impacts on fish and bait stocks have been investigated multiple times (Hodgson *et al.*, 2000; Napier *et al.*, 2009; Simon *et al.*, 2019). These studies show a gradual change in the use of polychaetes as bait in the region. Hodgson *et al.* (2000) found that only few recreational fishermen harvested polychaetes, and that the only species used was the bloodworm *Arenicola loveni* Kinberg, 1866. A decade later, Napier *et al.* (2009) found that while *A. loveni* was still harvested, more fishermen, including subsistence fishermen, were also collecting other polychaete species, with moonshine worms (identified as *Diopatra* sp.) being the third most frequently collected species. After another decade, Simon *et al.* (2019) found that moonshine worm was the most preferred (albeit not the most collected) bait polychaete among subsistence and recreational fishermen in Knysna Estuary.

Despite being harvested in Knysna Estuary for more than a decade, moonshine worms from this and Swartkops (240 km to the east) estuaries were only recently identified as *Diopatra aciculata* Knox & Cameron, 1971 after intensive molecular and morphological analyses (van Rensburg, 2019; Elgetany *et al.*, 2020). These studies showed that despite high morphological similarities and low interspecific genetic distances, *D. aciculata* should be considered separate from *Diopatra neapolitana* Delle Chiaje, 1841, a species originally described from the Mediterranean, but also recorded in South Africa (Macnae, 1957; Day, 1967; Branch *et al.*, 2016). This distinction is extremely important, since *D. neapolitana* had previously been reported in Swartkops Estuary where it was used as bait (van Der Westhuizen & Marais, 1977) and in the nearby Sundays River Estuary (McLachlan *et al.*, 1984), another popular fishing site (Cowley *et al.*, 2013). Additionally, Branch *et al.* (2016) reports *D. neapolitana* from Namibia to southern Mozambique. It is probable that some, if not all these records are incorrect identifications. Thus, it is important that an updated description of *D. aciculata* from Knysna and Swartkops estuaries, the only sites where identification has been confirmed, is generated to mitigate future identification errors.

The increased harvesting of *D. aciculata* in Knysna Estuary probably reflects, in part, an increase in density over the last few decades. *Diopatra aciculata* is harvested at many popular

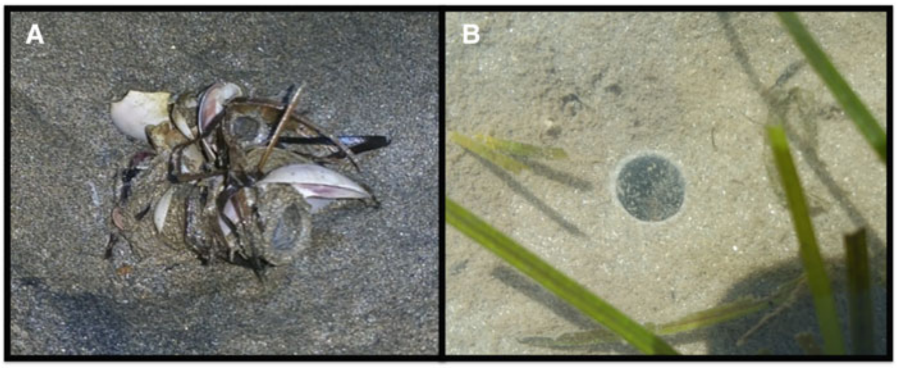

Fig. 1. Two types of *Diopatra aciculata* tubes. (A) In more sandy areas tubes protrude from substrate and are often bent in the direction of water flow with shell and plant fragments attached. (B) In areas with more muddy/silty substrates, often amongst seagrasses, tubes are flush with substrate but can be differentiated from other infauna by the presence of the off-white inner lining of the tube.

bait collecting sites within the estuary (Simon *et al.*, 2019), suggesting that the species is now widespread in the region. Since *Diopatra* species, which build conspicuous tubes (Figure 1A), are known ecosystem engineers (Berke *et al.*, 2010; Arias *et al.*, 2016), high density and wide distribution of *D. aciculata* may exert important ecological impacts in the estuary, especially because the species is so large (up to 60 cm, van Rensburg, 2019). It is therefore important that density and distribution of *D. aciculata* be determined to facilitate improved management of the species in Knysna Estuary.

The aims of this study are therefore to provide (1) a detailed morphological description of *Diopatra aciculata* from South Africa and (2) an assessment of its distribution and density in Knysna Estuary as a first step towards understanding the ecological impact that this species may have in this important estuary.

Materials and methods

Study area

Most of Knysna Estuary is entirely marine dominated and is therefore more accurately described as a marine or estuarine embayment (Allanson *et al.*, 2000*a*). Tidal and saline influence extends from the mouth (known as 'The Heads') for 19 km along the main winding channel (Allanson *et al.*, 2000*a*; Largier *et al.*, 2000). The estuary has two inhabited islands, Leisure Island and Thesen Island, and is bordered to the east by the Knysna central business district (Figure 2).

Morphology

Samples were collected over 4 hours around low tide in February and March 2017. Specimens for morphological analysis were collected from Bollard Bay (34°04′13.5″S 23°03′24.7″E; Site B, Figure 2) in Knysna Estuary and Swartkops Estuary (33°52′00.7″S 25°36′42.5″E) in Port Elizabeth and deposited at the Iziko Museum of South Africa (MB-A090394–MB-A090408). Worms were collected by inserting a thin wire with a hooked tip into the tube, turning it a few times to hook the worm and extracting by slowly pulling out the wire (Napier *et al.*, 2009; van Rensburg, 2019; Supplementary video S1). After collection, specimens were relaxed with 7% magnesium chloride solution in tap water and photographed live (using a Samsung Galaxy S6 smartphone). Specimens were then fixed in 4% formalin in seawater and stored in 70% ethanol.

Specimens were identified according to published identification keys (Day, 1967; Paxton, 1993; Arias *et al.*, 2016). Preserved specimens and sections of chaetigers were examined on dissecting (Leica MZ 7.5) and light (Leica DM1000) microscopes, respectively, and images captured using a Leica EC3 microscope camera and processed with the Leica Application Suite EZ (LAS EZ) software.

For scanning electron microscopy, specimens were dehydrated according to a protocol developed by L.-M. Joubert (Central Analytic Facility, Stellenbosch University); two washes in 100% ethanol of 10 min each, one wash in a 1:1 mixture of 100% ethanol and hexamethyldisilazane (HMDS) for 15 min, and finally two washes in HMDS for 30 min each. The HMDS was discarded and the specimens left overnight for residual HMDS to evaporate. Specimens were sputter-coated with gold palladium and viewed on a Zeiss Merlin scanning electron microscope at the Stellenbosch University Central Analytical Facility.

Distribution, density and population estimate

We sampled 18 sites (Figure 2) from the mouth of the estuary to about 14 km upstream, covering most of the estuary. The sampled sites included those surveyed by Day *et al.* (1951) and Allanson *et al.* (2000*b*) and popular bait collecting sites (Hodgson *et al.*, 2000; Napier *et al.*, 2009; Simon *et al.*, 2019). Thirteen sites were in the low intertidal zone (i.e. at the spring low-water mark ± 0.5 m) and five were in the subtidal zone (i.e. below the intertidal zone).

For density measurements, a 1 m^2 steel quadrat was used to sample a minimum of 20 m^2 per site. In each quadrat the number of visible *Diopatra aciculata* in tubes were recorded. Worms were detected by luring them to their tube openings using bait bags (frozen sardines (Clupeidae) in nylon stockings) that were squeezed near the tube entrances. Subtidal sampling was conducted by two free-divers following the same protocol.

To calculate a population estimate for the estuary we used the following equations (Wheater *et al.*, 2011):

$$\text{Population estimate } (\hat{P}) = \frac{\bar{x} \times n}{\text{SF}} = \frac{\text{mean number of worms per sample} \times \text{number of samples}}{\text{Sampling Factor}} \quad (1)$$

$$\text{Sampling Factor (SF)} = \frac{\text{Area sampled}}{\text{Total area}} \quad (2)$$

$$95\%\text{ Confidence Interval} = \frac{2 \times \text{Standard Error} \times n}{\text{SF}} \quad (3)$$

The mean number of worms per sample (for equation (1)) was calculated using data from all sites where *D. aciculata* was present. Similarly, area sampled (equation (2)) included only sites where *D. aciculata* occurred. The total area (equation (2)) was calculated using a conservative estimate of the area likely occupied by moonshine worms based on the area of the estuary covered by water

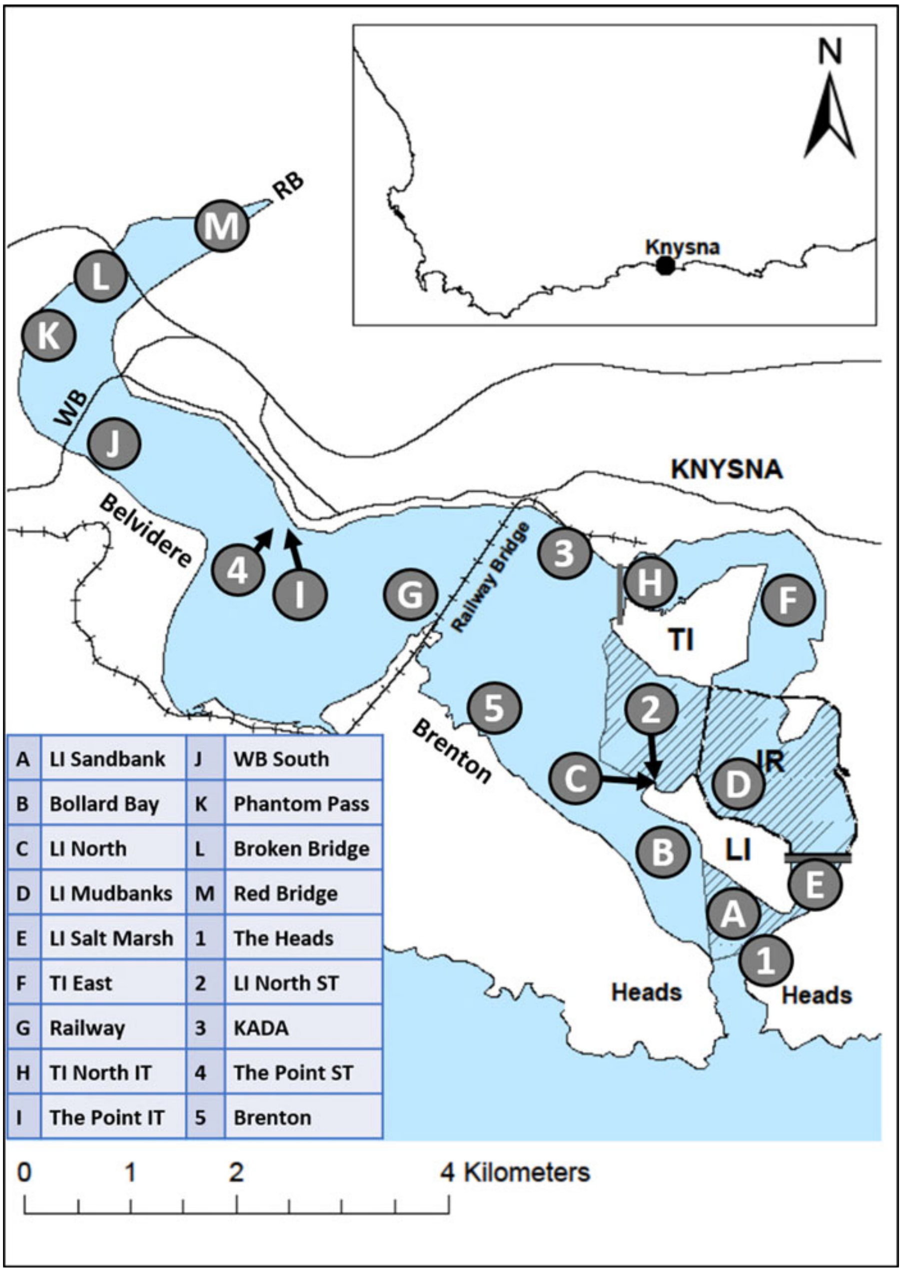

Fig. 2. Map of Knysna Estuary showing the sampling sites of *Diopatra aciculata*. Intertidal sites are given as letters A–M and subtidal sites are denoted 1–5. The invertebrate reserve is shown as the shaded area. WB = White Bridge, TI = Thesen Island, LI = Leisure Island, IR = Invertebrate reserve, RB = Red Bridge, IT = Intertidal, ST = Subtidal.

during neap low tide (Largier *et al*., 2000) and the depths at which this species is known to occur (cf. Knox & Cameron, 1971; Paxton, 1993).

Statistical analysis

All statistical analyses were performed in R-STUDIO and run in the R v.1.0.153 environment. In all instances, data were tested for normality using Shapiro–Wilks tests. Differences in density between sampling sites were calculated using Kruskal–Wallis rank sum test followed by Dunn's post hoc test for multiple comparisons using rank sums with Bonferroni correction.

Results

Taxonomy

SYSTEMATICS
Order EUNICIDA
Family ONUPHIDAE Kinberg, 1865
Subfamily ONUPHINAE Kinberg, 1865
Genus *Diopatra* Audouin & Milne Edwards, 1833
Diopatra aciculata Knox & Cameron, 1971
(Figures 1, 3–7)

D. aciculata: Knox & Cameron, 1971; Day & Hutchings, 1979; Paxton, 1986; Paxton, 1993; Elgetany *et al*., 2020

? *D. neapolitana*: Macnae, 1956; Macnae, 1957; Day, 1960; Day, 1967; van der Westhuizen & Marais, 1977; McLachlan *et al*., 1984; Branch *et al*., 2016

Diopatra sp.: Napier *et al*., 2009; Allanson *et al*., 2016; Simon *et al*., 2019

Material examined

16 specimens (MB-A090376–MB-A090391), incomplete, Knysna Estuary (34°04′17.6″S 23°03′30.5″E), Knysna, Western Cape, South Africa, coll. H. van Rensburg, 20 February 2017; 15 specimens (MB-A090394–MB-A090408), incomplete, Swartkops Estuary (33°52′00.7″S 25°36′42.5″E), Port Elizabeth, Western Cape, South Africa, coll. H. van Rensburg, 30 March 2017.

Diagnosis

Large body size, up to 600 mm long and 11.5 mm wide with 10–18 rings on ceratophores; 5–10 teeth on pectinate chaetae; uni-

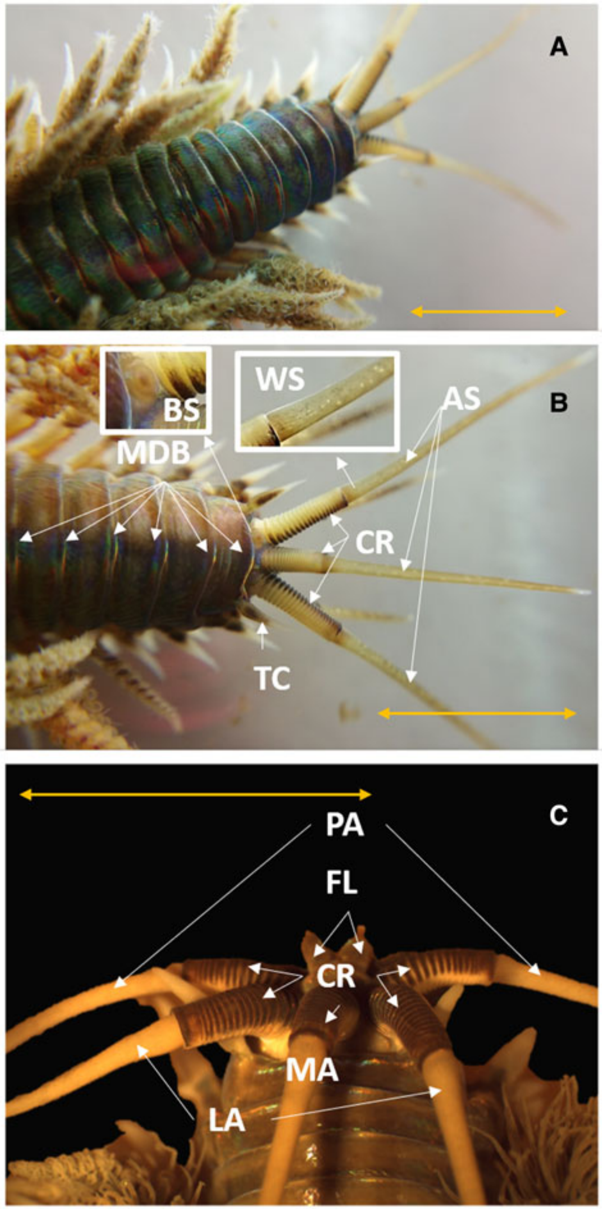

Fig. 3. Anterior regions of *Diopatra aciculata*, (A) a darker and (B) a lighter live specimen, and (C) a preserved specimen. Mid-dorsal bars (MDB) very difficult to see in live individuals, especially darker specimens. AS = Antennae styles; BS = Brown spot in center of nuchal organ; CR = Ceratophore rings; LA = Lateral antennae; MA = Median antenna; MDB = Mid-dorsal bar; PA = Palps; PC = Peristomal cirri; FL = Frontal lips; WS = White spots. Scale bars denote 10 mm.

and bi-dentate pseudo-compound falciger and dorsal cirri approximately as long as branchiae which distinguishes it from *D. neapolitana*.

Description

All specimens comprised large adults, maximum live incomplete length at least 60 cm. Preserved width excluding parapodia up to 11.4 mm at chaetiger 10, length of anterior fragment up to 158 mm for 131 chaetigers. Preserved body colour pale to dark brown, anterior regions sometimes darker. Single short black mid-dorsal bar on anterior margin of each chaetiger in branchial region (Figures 3A, B, 7A). Inner surface of ceratophore rings dark brown (Figure 3B, C). Median and posterior segments pale brown to cream. In live specimens, anterior often iridescent dark blue-green (Figure 3A) or darker brown (Figure 3B), mid-dorsal bar difficult to see on specimens with darker anterior regions. Inside of peristomal cirri brown (Figure 3A, B). Live specimens with small white spots irregularly spaced on antennae styles (Figure 3B).

Prostomium extended anteriorly, two smooth subulate frontal lips. Upper ventral lips have distal lobes (Figure 3C). Nuchal grooves almost completely circular, horseshoe-shaped (Figure 4A), some live specimens with small brown dot in centre of nuchal organ (Figure 3B). Three occipital antennae and two ventro-lateral palps mounted on 10–18 ceratophore rings, rings equally sized proximally, distal one longer (Figure 3C). Antennae styles smooth, long, slender, tapering to blunt end, reaching to chaetiger 9–15 (median) or 13–17 (lateral). Rows of interrupted sensory buds on antennae (Figure 4B, C), buds flattened, circular and irregularly spaced (Figure 4C, D), serous gland pores gradually disappearing distally.

Peristomium as long as succeeding chaetiger, two widely spaced peristomal cirri 1.5–2 times length of peristomium mounted on anterior margin, laterally to posterior occipital antennae (Figures 3A, B, 4A).

Three or four anterior abranchiate chaetigers; parapodia larger than on branchiate chaetigers, directed antero-ventrally; dorsal cirri elongated, slender, tapering, longer than ventral cirri (Figure 5A). Pre-chaetal lobes rounded, post-chaetal lobes long and subulate (Figure 5A). Pseudo-compound falciger distally uni- or bi-dentate, covered by pointed hood.

Unmodified parapodia usually from the fifth chaetiger. Dorsal cirri slender, elongated, longest in branchial region, similar in length to branchiae. Ventral cirri pad-like (Figure 6A). Post-chaetal lobes elongated, triangular (Figure 6B, C). Pre- and post-chaetal lobes gradually become smaller toward posterior. Pre-chaetal lobes disappear but post-chaetal lobes remain distinct. Limbate and pectinate chaetae present, pectinate chaetae having 5–10 teeth (Figure 5B, C), one lateral tooth often thicker than rest. Two bi-dentate subacicular hooks from chaetiger 19–23 onwards (Figure 6B, C).

Spiralled branchiae from fourth or fifth chaetiger, up to 20 branchial whorls arranged close together, brush-like or bushy appearance tapering towards tips (Figure 7A). After 20–40 segments, branchiae gradually shorten and whorls reduce until a single filament remains, terminate shortly thereafter (Figure 7B).

Taxonomic remarks

The specimens from South Africa match descriptions of *Diopatra aciculata* (Paxton, 1993) and *Diopatra neapolitana* (Arias *et al.*, 2016) with regards to number of chaetigers with subulate ventral cirri (four), branchiae starting on chaetiger 4 or 5, circular sensory buds, horseshoe-shaped to almost complete circular nuchal grooves, number of rings on ceratophores (10–18), maximum number of branchial whorls (20), number of teeth on pectinate chaetae (5–10) and presence of uni- and bi-dentate pseudo-compound falciger.

Compared with the original description of *D. aciculata* (Knox & Cameron, 1971), specimens examined here were wider (11 mm *vs* 5 mm), had longer antennae (reaching chaetiger 18 *vs* 12) with more ceratophores (18 *vs* 15) and no knob-like structures on pseudo-compound falcigers. However, barring the absence of the knob on the pseudo-compound falcigers, these differences can probably be attributed to the smaller size of the single specimen examined by Knox & Cameron (1971). Our specimens conformed best with descriptions of *D. aciculata* by Paxton (1993, 2016). Similar features included width at tenth chaetiger (11.5 mm) and lengths of palps, median and lateral antennae reaching chaetigers 2–5, 8–15 and 8–15 respectively. However, the adult specimens from South Africa differed from *D. aciculata* in Australia in that they never possessed tridentate falcigers (Paxton, 1993, p. 146, Figure 34), and although no complete specimens were collected in our study, these fragments were often

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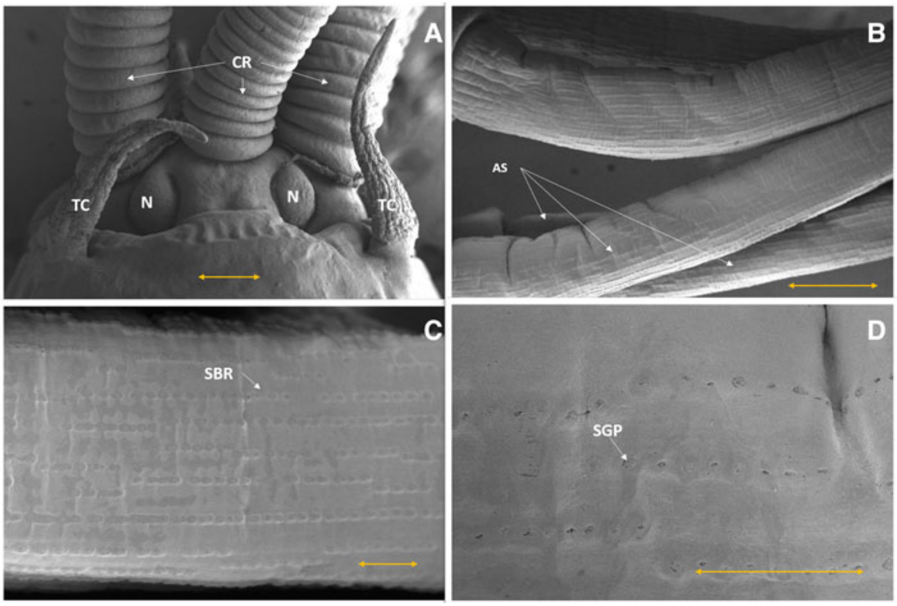

Fig. 4. Scanning electron micrographs of *Diopatra aciculata* showing (A) Nuchal grooves and peristomal cirri; (B) Irregular rows of sensory buds on antenna styles; (C) Mid antenna area with fewer serous gland pores in sensory buds; (D) Closer view of serous gland pores in sensory buds. AS = Antennae styles; CR = Ceratophore rings; N = Nuchal groove; SBR = Sensory bud rows; SGP = Serous gland pores; PC = Peristomal cirri. Scale bars denote: (A) 500 µm; (B) 500 µm; (C) 100 µm and (D) 100 µm.

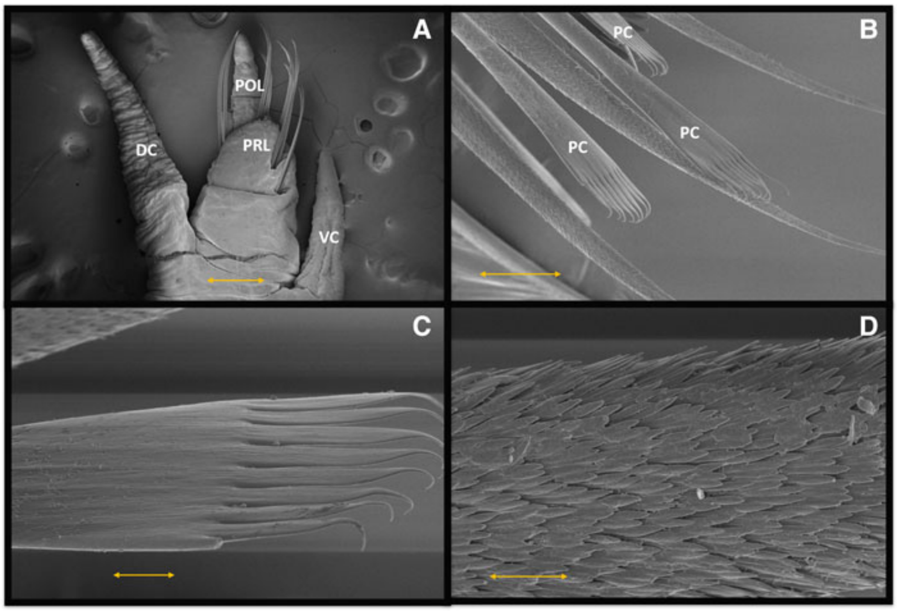

Fig. 5. Scanning electron micrograph of *Diopatra aciculata* showing (A) Modified parapodium; and (B–D) chaetae. (A) Ventral cirri elongated and subulate; (B) pectinate cheatae with 5–10 teeth; (C) Close up of pectinate cheatae; (D) Serrated surface of mid regions of limbate cheata. DC = dorsal cirrus; PC = pectinate chaetae; POL = post-chaetal lobe; PRL = pre-chaetal lobe; VC = ventral cirrus. Scale bars denote: (A) 400 µm; (B) 50 µm; (C) 10 µm and (D) 5 µm.

twice as long as the 340 mm length reported by Paxton (1993, 2016). Peristomal cirri of specimens from South Africa were 1.5–2 times longer than the peristomium, in accordance with Paxton (2016).

The South African specimens of *D. aciculata* differ from *D. neapolitana* in the following characteristics: they were wider at 10th chaetiger (7–11.5 mm *vs* 4–9 mm) and had longer palps (reaching chaetiger 2–5 *vs* 1–3), longer antennae (reaching chaetiger 8–15 *vs* 4–10) and longer dorsal cirri (reaching same length as branchiae in *D. aciculata* but less than half the length of branchiae in *D. neapolitana*) (Arias *et al.*, 2016). Furthermore, the branchial region was slightly shorter in *D. aciculata* than in *D. neapolitana* (up to chaetiger 61 *vs* 70). The observed length of live specimens collected here agreed best with the re-description of *D. neapolitana* (Arias *et al.*, 2016).

Distribution

Diopatra aciculata is known to occur on the southern coast of Australia from Perth in the west to Newcastle in the east (Paxton, 1993) and has also been reported from the Suez Canal in Egypt (Elgetany *et al.*, 2020). Confirmed South African range includes Knysna and Swartkops estuaries. Presence in estuaries where *D. neapolitana* was reported (Day, 1967; McLachlan *et al.*, 1984; Branch *et al.*, 2016) needs to be confirmed.

Ecology

Tubes lined with white parchment-like material. In sandy environments, tubes have protruding tube-caps that are often bent horizontally (Figure 1A). Tube-caps are made of sand with a smooth texture and plant material and shell fragments usually embedded into the tube with larger shell pieces often found distally (Branch *et al.*, 2016, p. 67, figure 26.4; this study, Figure 1A). Some tube-caps had no plant or shell attachments and appeared smooth. In muddy or silty environments, openings of tubes are flush with sediment surface and the off-white parchment-like lining is clearly visible (Figure 1B).

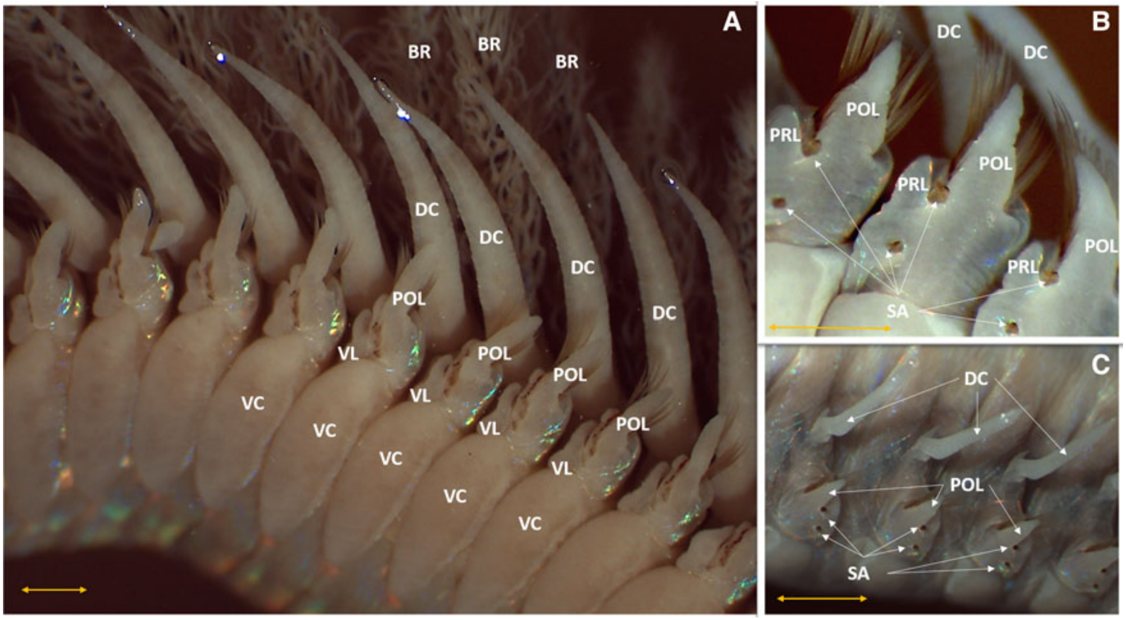

Fig. 6. Progression of parapodia of *Diopatra aciculata* showing (A) Latero-ventral view of branchial region with very long dorsal cirri, pad-like ventral cirri and presence of a ventral lobe on parapodia and lack of subacicular hooks. (B) Ventral view towards end of branchial region, longer dorsal cirri visible in background, appearance of subacicular hooks. (C) Lateral view past branchial region, dorsal cirri become reduced, ventral lobes and pre-chaetal lobes disappear, subacicular hooks remain. BR = branchia; DC = Dorsal cirrus; POL = post-chaetal lobe; PRL = Pre-chaetal lobe; SA = subacicular hook; VC = Ventral cirrus; VL = ventral lobe. Scale bars denote 10 mm.

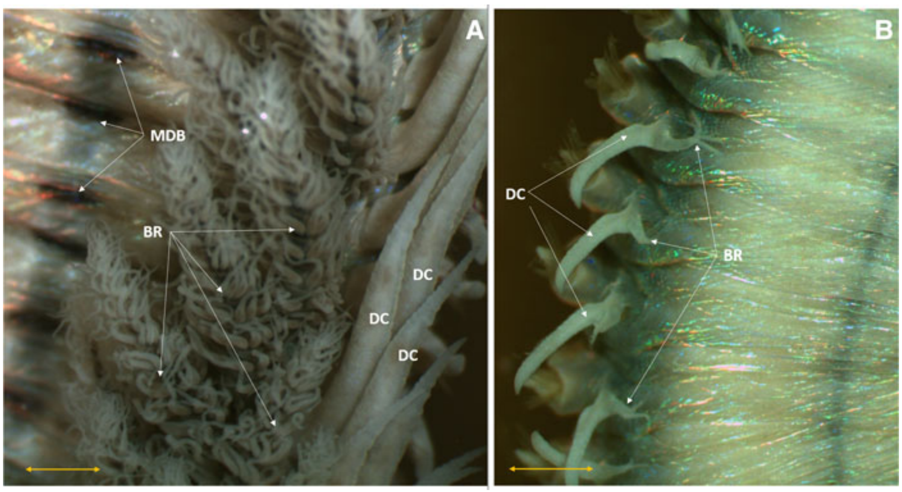

Fig. 7. Progression of branchiae from dorsal view of *Diopatra aciculata* showing (A) Main branchial region where branchiae have several whorls, large and bushy in appearance, dorsal cirri here characteristically long, mid-dorsal bar clearly present in preserved specimens. (B) Shows branchiae reducing, branchiae eventually disappear, absence of mid-dorsal bar. BR = Branchia; DC = Dorsal cirrus; MDB = Mid-dorsal bar. Scale bars denote 10 mm.

Distribution, densities and population estimate

A total of 860 m^2 were sampled at the 18 sites, with a mean of 47.8 m^2 covered per site. In total, 458 m^2 were sampled in the intertidal zone and 402 m^2 in the subtidal zone. No worms or holes were found in the three sites north of the White Bridge (Sites K, L & M, Figure 2), and these sites (60 m^2) were therefore excluded from further analyses. Of the 800 quadrats sampled from the remaining 15 sites, no worms were observed in 443 quadrats.

Densities varied significantly by site (Kruskal–Wallis $H_{(14,\ 800)} = 376.55$; $P = 0.01$, Figure 8). Post-hoc analysis revealed six overlapping homogeneous groups (I–VI, Figure 8). However, all the data could be divided into two exclusive groups, one with high and one with low densities (I and II, respectively, Figure 8). The high-density group (I) contained seven sites with median densities of 3–8 worms m^{-2}: Bollard Bay, Railway Bridge, Thesen Island east, Leisure Island mudbanks, Leisure Island north, White Bridge and Knysna Angling and Diving Association (KADA). Median and maximum densities were highest at Bollard Bay and Railway Bridge (median = 8 worms m^{-2} at each, maximum densities = 52 worms m^{-2} and 26 worms m^{-2} at the two sites, respectively). All but one site (KADA) from the high-density group (I) were from the intertidal zone. The low-density group (II) contained the remaining eight sites; all with median densities of 0 worms m^{-2} and a maximum density of 14 worms m^{-2} at The Heads. Despite overlap in homogeneous groups, two sites in groups I and III (Bollard Bay & Railway, Figure 2) never overlapped with sites in groups II and VI (The Heads, Leisure Island North ST, The Point ST & IT, Brenton, Leisure Island Sandbank, Leisure Island Salt Marsh, Thesen Island North, Figure 2). Sites with high densities were patchily distributed throughout the estuary (Figure 9).

The mean density per sample was 3.47 worms m^{-2} for 800 samples, covering a total area of 800 m^2. A conservative estimate of the total area that could be occupied by *D. aciculata* was determined as the area submerged during neap low tide south of the White Bridge. This amounted to 6,487,600 m^2 of the

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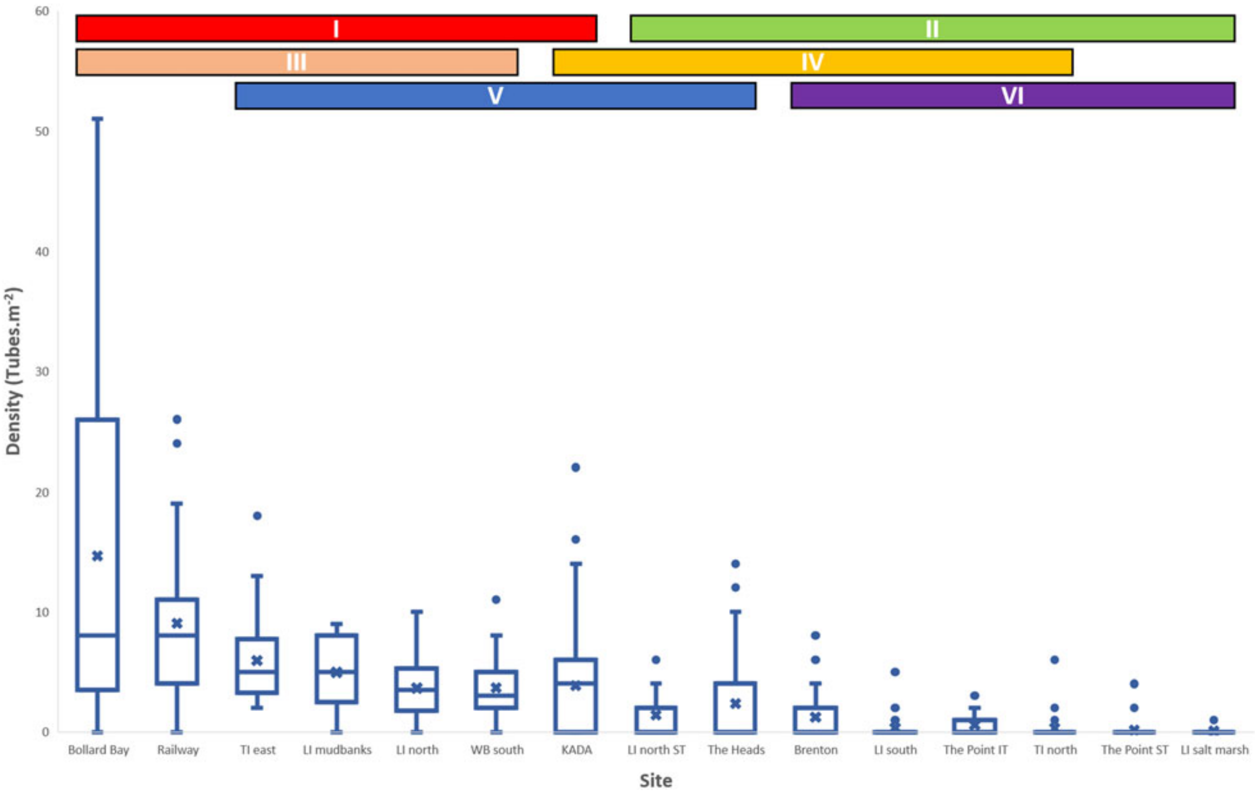

Fig. 8. Boxplots showing densities of *Diopatra aciculata* in Knysna Estuary at all sampled sites where worms were found. Results of post hoc Dunn's test showing homogeneous groups (I – VI) are shown visually as bars above boxplots. Crosses (X) denote means while centre bars show medians (indistinguishable in groups II & VI). Box and whiskers shows quartiles with minimum and maximum values. Dots represent outliers.

18,270,000 m^2 area of the Knysna Estuary. The sampling factor (SF) was therefore 1.233×10^{-4}, and the population size estimate was 22,514,193 worms with a 95% confidence interval of 2,338,229 worms. Thus, the estimated population size of *D. aciculata* in the Knysna Estuary is between 20 and 24 million worms if the densities found in sampled sites are representative of worm density throughout the inhabitable part of the estuary.

Discussion

Taxonomic and distributional implications

Diopatra aciculata was originally described from a single specimen collected from Port Phillip Bay, Australia (Knox & Cameron, 1971). The species went unreported for more than 20 years before being re-described by Paxton (1993) who reported a distribution mostly along the south-eastern coast of Australia from several large, marine-dominated estuaries and embayments, including Port Phillip Bay, Botany Bay, Hobson Bay, Barker's Inlet and Swan Estuary. These estuaries have conditions very similar to those that predominate in most of the Knysna Estuary (cf. Paxton, 1993; Allanson *et al*., 2000*a*; Largier *et al*., 2000). In South Africa, the distribution of *D. aciculata* has only been confirmed for Knysna and Swartkops estuaries on the south and south-east coasts of the country, respectively (van Rensburg, 2019; Elgetany *et al*., 2020). No large-bodied *Diopatra* species was detected during extensive biodiversity surveys conducted in the Knysna Estuary in the 1940s and 1990s (Day *et al*., 1951; Allanson *et al*., 2000*b*). An unidentified *Diopatra* was first recorded in Knysna about 15 years ago (Napier *et al*., 2009) and it was only recently confirmed to be *D. aciculata* (van Rensburg, 2019; Elgetany *et al*., 2020). It is therefore probable that *D. aciculata* arrived in the estuary during the last 15–25 years. By contrast, *Diopatra neapolitana*, the morphologically similar species (Dağli *et al*., 2005; van Rensburg, 2019; Elgetany *et al*., 2020; current study) used as bait in the Mediterranean and Portugal (Cunha *et al*., 2005; Dağli *et al*., 2005; Pires *et al*., 2012*a*; de Carvalho *et al*., 2013; Arias *et al*., 2016) was reported in the Swartkops Estuary in the 1950s (Macnae, 1956, 1957) where it was also later reported to be used as bait (van der Westhuizen and Marais, 1977). Given the morphological and molecular evidence that confirmed the presence of *D. aciculata* in Swartkops Estuary (van Rensburg, 2019; Elgetany *et al*., 2020; current study), it is likely that *D. neapolitana* were misidentified there.

Historically, in South Africa, Day (1967) reported *D. neapolitana* at only two isolated localities, once in Namibia and once in Durban harbour, disregarding reports from Swartkops (Macnae, 1956, 1957). Later the species was reported from Sundays River Estuary 25 km east of Swartkops (McLachlan *et al*., 1984) and more recently documented to have a continuous distribution along the southern African coast (Branch *et al*., 2016). However, the distribution from northern Namibia to southern Mozambique, as defined by Branch *et al*. (2016), has not been confirmed and is likely a consequence of conflating descriptions and distributions of *D. neapolitana* and an indigenous subspecies, *Diopatra neapolitana capensis* (cf. Day, 1967; Branch *et al*., 2016). If reports of *D. neapolitana* in Swartkops Estuary (Macnae, 1956, 1957) were actually of *D. aciculata*, then other reports (e.g. Day, 1967; McLachlan *et al*., 1984; Branch *et al*., 2016) may also represent misidentifications, especially since the cosmopolitan distribution of *D. neapolitana* has been questioned (Paxton, 1998; Berke *et al*., 2010; Fauchald *et al*., 2012), with records from Japan referred to *Diopatra sukogokai* (Paxton, 1998). If this is the case, then records of *D. aciculata* in South Africa predate its description in Australia. Consequently, it is not certain if

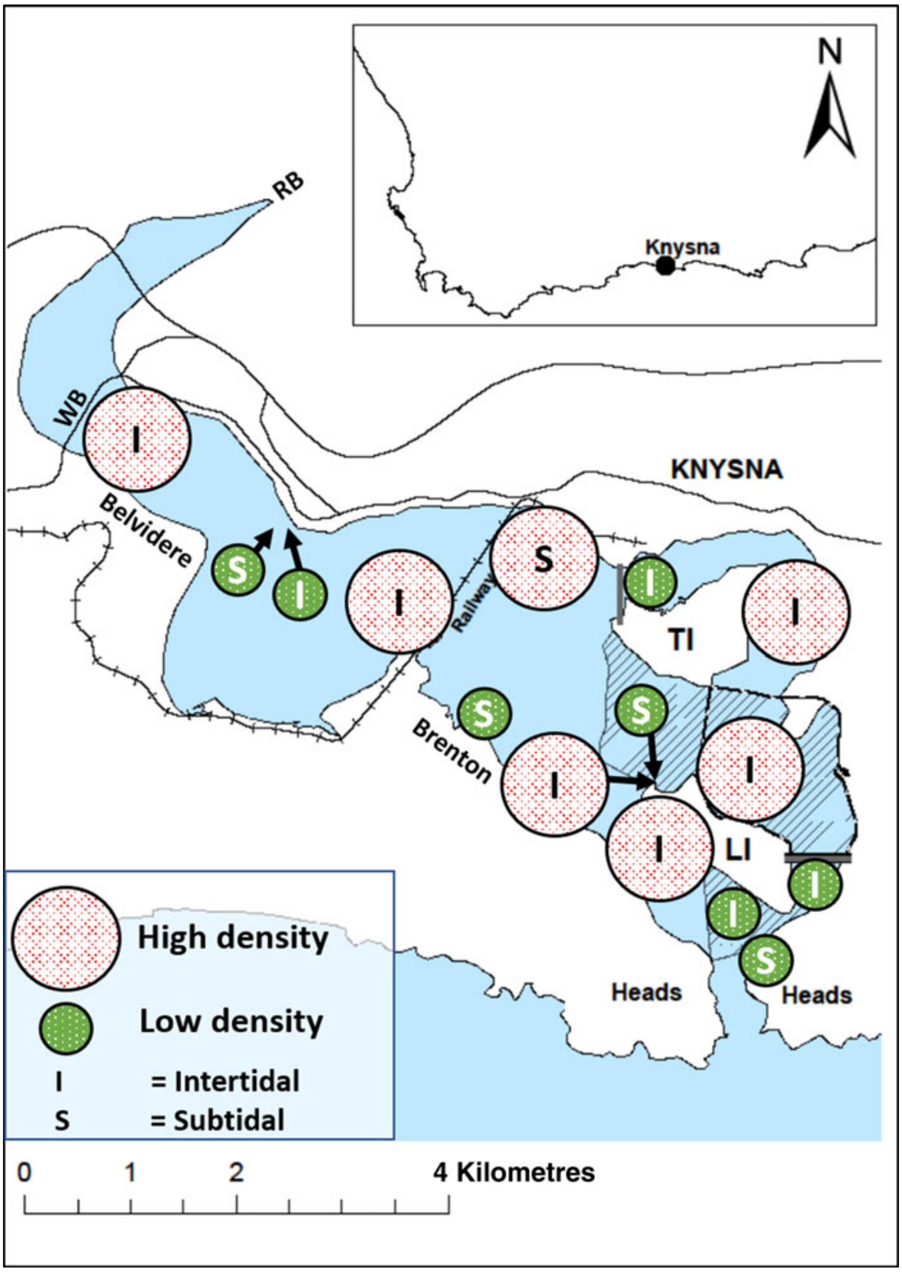

Fig. 9. Visual representation of *D. aciculata* density and distribution throughout the Knysna Estuary. Large red circles show high density group with >0 medians. Smaller green circles show low density group with medians = 0. S = Subtidal sites; I = Intertidal sites.

D. aciculata is native in Australia and non-indigenous in South Africa, or vice versa, and it should be considered cryptogenic until molecular analyses can provide more insight into population structure of the continental populations. There is, however, no doubt that the species is undergoing range expansion in South Africa, but until the native range is elucidated, it is unclear if this expansion is extralimital or invasive (sensu Robinson *et al.*, 2016). However, recent evidence confirms the alien presence of *D. neapolitana* in India (Parameswaran, 1973; Elgetany *et al.*, 2020) and Brazil (Bergamo *et al.*, 2019), thus other identifications outside the Mediterranean, such as in Vietnam (Tue *et al.*, 2012) and Mozambique (MacNae & Kalk, 1958) may well be correct. Morphologically *D. aciculata* and *D. neapolitana* are very similar and exceedingly difficult to tell apart, although the longer dorsal cirri, wider bodies and longer antennae of *D. aciculata* (Elgetany *et al.*, 2020) may be the best distinguishing characters in accordance with recent findings. The presence of spermaductal papillae as described for *D. neapolitana* (Arias *et al.*, 2016) has also not yet been observed in *D. aciculata*.

Distribution, density and population estimate

Diopatra aciculata occurred throughout the estuary, from sites near the mouth to about 12 km upstream, and seems to be absent where freshwater conditions start to predominate (Figures 2 & 9, cf. Largier *et al.*, 2000). This distribution is not surprising. Although *Diopatra* are essentially marine-adapted, many species, including *D. aciculata*, occur in estuaries (van Der Westhuizen & Marais, 1977; Cunha *et al.*, 2005; Rodrigues *et al.*, 2009; Arias *et al.*, 2010; Pires *et al.*, 2010; Pires *et al.*, 2012*b*; de Carvalho *et al.*, 2013; Arias & Paxton, 2014), particularly large, marine-dominated estuaries and embayments (Paxton, 1993). Furthermore, absence of the species from areas where estuarine conditions are freshwater dominated (Largier *et al.*, 2000) reflects known low tolerance by *Diopatra* species for very low salinities (Hakkim, 1975; Freitas *et al.*, 2015; Pires *et al.*, 2015).

Although *Diopatra aciculata* was found at sites throughout most of the estuary, distribution was patchy; densest patches were found in areas that are well flushed during low tide, while few or no worms were found in areas with little or no flowing water during low tide (van Rensburg, 2017 Pers. Obs.). We therefore hypothesize that the patchy distribution within the estuary could reflect a preference for certain microhabitats, especially with regards to water flow. This was previously demonstrated by Mangum *et al.* (1968) who found a positive correlation between population density of *Diopatra cuprea* (Bosc, 1802) and current velocity. The apparent preference for flowing water may be related to feeding behaviour; *Diopatra* species are considered to be discreetly mobile, never completely leaving their permanent tubes, but instead waiting for food to pass within easy reach of their tube openings (Fauchald & Jumars, 1979; Jumars *et al.*, 2015). Such species probably rely on higher water flow rates to bring more food towards them.

Species of the genus *Diopatra* can often attain high densities (Paxton & Bailey-Brock, 1986; Conti & Massa, 1998; Harwell & Orth, 2001; Cunha *et al.*, 2005; Dağli *et al.*, 2005; Rodrigues *et al.*, 2009; Berke *et al.*, 2010; Arias & Paxton, 2015; Arias *et al.*, 2016). For example, mean densities of the similarly sized *D. neapolitana* range from 20–200 worms m^{-2} in different parts of the Mediterranean (Dağli *et al.*, 2005; Berke *et al.*, 2010; Arias & Paxton, 2015; Arias *et al.*, 2016). At a mean of 3.47 worms m^{-2}, densities of *D. aciculata* in Knysna are much lower, but the maximum of 51 worms m^{-2} suggests great potential for an increase in population size.

We estimated that 20–24 million *D. aciculata* may currently occur in Knysna Estuary, suggesting that it has the potential to play a significant role in the Knysna Estuary ecosystem. Twenty million individuals could undoubtedly have a trophic impact either as a resource for fish (e.g. van der Westhuizen & Marais, 1977) and birds (e.g. Perez-Hurtado *et al.*, 1997) or as a predator of other invertebrates (e.g. Tue *et al.*, 2012). Furthermore, *Diopatra* tubes may affect biodiversity by providing or modifying habitats for other organisms (Woodin, 1981; Harwell & Orth, 2001; Thomsen & McGlathery, 2005; Berke *et al.*, 2010; Thomsen *et al.*, 2011). Other *Diopatra* have been classified as sediment stabilizers (Bailey-Brock, 1984; Luckenbach, 1986), thus, *D. aciculata* may displace important indigenous bioturbating bait species such as *Arenicola loveni* (Reichardt, 1988; Huttel, 1990; Philippart, 1994; Napier *et al.*, 2009; Berke *et al.*, 2010; Pillay *et al.*, 2012; Simon *et al.*, 2019) and sandprawn, *Callichirus kraussi* (Branch & Pringle, 1987; Siebert & Branch, 2006, 2007; Pillay *et al.*, 2007, 2012; Henninger & Froneman, 2013) while also facilitating mudprawn *Upogebia africana* which prefers more stable sediments (Wynberg & Branch, 1994; Siebert & Branch, 2005). By contrast, by trapping seeds or plants in their tubes, *D. aciculata* may facilitate the recovery or maintenance of seagrass (see Harwell & Orth, 2001; Thomsen & McGlathery, 2005) such as *Zostera capensis* Setch, 1933 that have declined in the Knysna Estuary in the last 50 years (see Allanson *et al.*, 2000*b*; cf. Day *et al.*, 1951; Maree, 2010). Restoration of *Z. capensis* may provide increased habitat for fish (Whitfield *et al.*, 1989) and invertebrates (Mead *et al.*, 2013), including the endangered Knysna seahorse *Hippocampus capensis* (Teske *et al.*, 2007) and the critically endangered limpet *Siphonaria compressa* (Allanson & Herbert, 2005), but also reduce densities of invertebrate species that were absent when *Zostera* were abundant (Allanson *et al.*, 2000*b*). The current density of *D. aciculata* may still be too low to cause significant effects. Potential impacts may therefore still be mitigated, especially if continued population growth can be controlled.

Whatever the origin of the species, it was not present in the Knysna Estuary before the late 1990s, and population growth control should still be considered since it may negatively affect the ecosystem. The easiest way to do so may be by encouraging increased harvesting by bait collectors since bait polychaetes, including *D. neapolitana* (Dağli *et al.*, 2005) are susceptible to over fishing (Gaigher, 1979; Baird *et al.*, 1981; Britz *et al.*, 2001; Simon *et al.*, 2020). This solution should, however, be considered with caution since intensive bait collecting does not only affect target species, but can also indirectly harm the habitat and associated biota by trampling and physical disturbance of the environment (Wynberg & Branch, 1991, 1994, 1997; Pillay *et al.*, 2010; Simon *et al.*, 2019). Furthermore, as *D. aciculata* can only be collected manually from the intertidal zone (van Rensburg, 2017 Pers. Obs.), subtidal populations can provide a continuous supply of recruits to re-populate the intertidal baited areas.

The identification of a potentially invasive polychaete species that is conspicuous, harvested as bait and which occupies one of the most important estuaries in South Africa highlights the gaps in our knowledge of such species in the country (see Britz *et al.*, 2001; Simon *et al.*, 2019; Simon *et al.*, 2020). The appearance of *D. aciculata* in Knysna Estuary was probably not considered unusual because of its close resemblance to *D. neapolitana*, a species already thought to occur widely (and naturally) in South Africa, including on the south coast (Macnae, 1956, 1957; McLachlan *et al.*, 1984; Branch *et al.*, 2016). Very few studies in South Africa have investigated the taxonomy (Lewis & Karageorgopoulos, 2008; Kara *et al.*, 2018) or population structure (Gaigher, 1979; van Herwerden, 1989; Kara *et al.*, 2018; Simon *et al.*, 2020) of bait polychaetes and we have no understanding of their ecological impacts within their ecosystems. Thus, further research on exploited bait polychaetes is needed. Additionally, further research on *D. aciculata* should be conducted on global, regional and local scales, particularly to determine (1) the complete distribution in South Africa, (2) if the species is invasive or native, (3) its ecological and trophic impacts and (4) a more accurate population estimate and extirpation viability assessment.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315420000740.

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