

First account on kinorhynchs from Portugal, with the description of two new species: *Echinoderes lusitanicus* sp. nov. and *E. reicherti* sp. nov.

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Abstract The first exploration of the kinorhynch meiofauna in Portuguese marine waters has revealed the existence of two undescribed species of the cyclorhagid genus *Echinoderes*. In the present contribution we describe *Echinoderes lusitanicus* sp. nov. and *Echinoderes reicherti* sp. nov., both collected from subtidal regions of the coast of Algarve in the southernmost region of Portugal. *Echinoderes lusitanicus* sp. nov. is recognized by the presence of tubes on segment 2 in subdorsal and ventrolateral positions, on segment 5 in lateroventral positions, on segment 8 in lateral accessory positions, and on segment 10 in laterodorsal positions. Spines are present in middorsal position on segments 4 to 8, and in lateroventral positions on segments 8 and 9. The females have minute lateral terminal accessory spines. The second species, *E. reicherti* sp. nov., is characterized by tubes on segment 2 in subdorsal and ventrolateral positions, on segment 5 in lateroventral positions, and segment 8 in sublateral positions. In addition, the species

possesses acicular spines in middorsal position on segment 4, and in lateroventral positions on segments 6 to 9. Morphological aspects such as tube/spine pattern of the trunk or sexually dimorphic traits are discussed and compared to other *Echinoderes* species showing close resemblance.

Key words Cyclorhagida, Echinoderidae, Kinorhyncha, Algarve, meiofauna, taxonomy

Introduction

Phylum Kinorhyncha is a group of meiobenthic animals of microscopic size (less than 1 mm in length) that is found exclusively in marine or estuarine realms (Higgins 1988; Sørensen & Pardos 2008; Neuhaus 2013). These animals live interstitially in sediments such as sand, shell gravel and mud, from coarse to fine granulometry. Although described already in the middle of the XIXth century, by Dujardin (1851), only a little more than 200 species have been described so far (Neuhaus, 2013).

Our knowledge about the Portuguese kinorhynch fauna is extremely limited. The occurrence of this group along the Portuguese coast lines is hitherto merely known from surveys on general meiofaunal abundance and total biomass (e.g., Bianchelli et al. 2010). More recently, *Echinoderes dujardinii* Claparède, 1863, *Echinoderes hispanicus* Pardos et al., 1998 and a third undescribed species of *Echinoderes* from the Algarve, the southernmost region of Portugal, were used in a comparative study on the myoanatomy of this genus (Herranz et al., 2014a). However, any attempt to identify or describe new species of kinorhynchs from Portugal has never been done. Hence, the Portuguese coast lines represent a huge gap in our knowledge about the distribution of Kinorhyncha along the Iberian Peninsula - a topic that has been studied over the last 24 years (Sánchez et al., 2012 and references therein). Comprehensive sampling in this region rendered a list of more than 11 genera and 29 species from Spanish territory only, with the most speciose genus being *Echinoderes*. Hence, given the high biodiversity found in this area, one could expect as well a high diversity of kinorhynchs inhabiting Portuguese coast lines.

Aiming at increasing the knowledge about the biogeography and biodiversity of Kinorhyncha, we inspected the Portuguese coastline for the first time in search of this marine group. In this study, meiofaunal samples were taken in the area between Faro and Vale do Lobo, in the Algarve. The samples were taken as part of several field samplings performed in Portugal between February 2012 and September 2014, in an attempt to better characterize the kinorhynch meiofauna present in Portuguese marine waters. Here, we focus on the description of two new species of *Echinoderes*, which together represent the first kinorhynch species described from Portugal.

Materials and methods

The study sites are situated in the Algarve, the southernmost region of Portugal, in the area between Faro and Albufeira (Figure 1). Samples were taken at various subtidal localities, and three of them yielded the specimens used in the present study. Stations 21-10-2013-1B (36° 54'24''N 007°53'58''W) and 21-10-2013-2B depth (36° 54'27''N 007°52'13''W) are both subtidal, located at 96 m and 100 m depth, respectively, off Faro. The sediment consists of mud at both stations, which were sampled with a Higgins meiobenthic dredge on October 21, 2013. Station 28-10-2013-2B (36° 59'52''N 008°09'19''W) is also subtidal but located at 35 m depth off Vale do Lobo, about 8 km west of Faro. The sediment consisted of mud, which was sampled with a triangular dredge on October 28, 2013.

The samples were brought to the Centro de Ciências do Mar (CCMAR) lab, at the University of Algarve, and processed alive. Kinorhynchs were extracted with the “bubbling and blot”-method (Higgins 1988), following the procedure described by Sørensen & Pardos (2008). Kinorhynch specimens were picked up and observed live, and subsequently fixed in 4% paraformaldehyde buffered with phosphate buffer saline solution (PBS). Before fixation some specimens were exposed to freshwater to make them protrude their heads. Specimens for light microscopy (LM) were dehydrated through a graded series of glycerin, and mounted in Fluoromount G between two cover slips attached to a plastic H-S slide. They were examined and photographed with an Olympus BX51 microscope, equipped with a drawing tube and an Olympus DP22

camera connected to a DP2-SAL camera controller. Line art illustrations were based on camera lucida drafts of mounted specimens that subsequently were scanned and drawn in Adobe Illustrator CS6. Measurements were made with the software embedded in the DP2-SAL controller. All dimensions reported in the tables are based on LM measurements. If a dimension mentioned in the text is estimated from scanning electron microscopical (SEM) images, this will be indicated clearly. Specimens for scanning electron microscopy were dehydrated through a graded series of alcohol and subsequently transferred to acetone through a graded ethanol/acetone series, critical point dried, mounted on aluminum stubs, sputter coated with a platinum/palladium mix and examined with a JEOL JSM-6335F Field Emission scanning electron microscope. All examined material is deposited at the Natural History Museum of Denmark.

Taxonomy

Class Cyclorhagida (Zelinka, 1896) Sørensen et al., 2015

Order Echinorhagata Sørensen et al., 2015

Family Echinoderidae Bütschli, 1876

Genus *Echinoderes* Claparède, 1863

***Echinoderes lusitanicus* sp. nov.**

Type material

Holotype, adult female, collected from mud on October 21, 2013, at station 13.10.21.2B (Figure 1), at 100 m depth off Faro, on the south coast of Portugal (36° 54'27''N 007°52'13''W), mounted in Fluoromount G, deposited at the Natural History Museum of Denmark (NHMD), under catalogue number ZMUC KIN-000924. Paratypes, four adult specimens (cat. nos. ZMUC KIN-000925 to KIN-000928) mounted in Fluoromount G, and four adult specimens (cat. no. ZMUC KIN-000929) mounted on one SEM stub, collected at the same locality as holotype, deposited at NHMD. Additional paratypes include six adult specimens (cat. nos. ZMUC KIN-000930 to KIN-000935) mounted in Fluoromount G, and five adult specimens (cat. no.

ZMUC KIN-000936) mounted on one SEM stub, collected near the locality of the holotype from mud on October 21, 2013, at station 13.10.21.1B (Figure 1), at 96 m depth (36° 54' 24''N 007°53'58''W).

Diagnosis

Echinoderes with tubes on segment 2 in subdorsal and ventrolateral positions, on segment 5 in lateroventral positions, segment 8 in lateral accessory positions, and on segment 10 in laterodorsal positions; spines are present in middorsal position on segments 4 to 8, and in lateroventral positions on segments 8 and 9. Males with three penile spines, females with minute lateral terminal accessory spines.

Etymology

The species name '*lusitanicus*' is derived from Lusitania – the Latin name for the ancient Iberian Roman province that includes approximately all of modern Portugal south of the Douro river, as well as a part of Spain.

Description

Adults with head, neck and eleven trunk segments (Figure 2A, B, 3A and 4A). Trunk with rectangular overall appearance; dorsal and lateral body regions with dense, uniform covering of cuticular hairs. Lateral terminal accessory spines in females conspicuously short (Figure 3I and 4H). For complete overview of measures and dimensions, see Table 1. Distribution of cuticular structures, i.e., sensory spots, glandular cell outlets and tubes, is summarized in Table 2.

The head consists of a retractable mouth cone and an introvert. In specimens mounted for SEM, the introvert was either fully or partly retracted, or alternatively too dirty to allow complete examination of scolid arrangements. Hence, the following description of the head appendages is only partial. The mouth cone is equipped with nine outer oral styles, each consisting of two joined units, arranged as one style anterior to each introvert sector, except the middorsal sector 6. The styles alternate slightly in lengths,

between longer and shorter ones. A pair of lateral spikes is located at the base of each style, and a fringe consisting of four long spikes is located even more posteriorly.

Introvert Ring 01 has ten primary spinoscalids that each consists of a basal sheath and an end piece (Figure 4B). The basal sheath carries a proximal fringe with four fringe tips, and distally, along its margin, another fringe also with four tips. Introvert Rings 2, 3, and 4, with 10, 20 and 10 scalids, respectively. Scalids in these rings consist of a basal sheath with fringed posterior margin and a pointed end piece; the scalids become gradually shorter from Ring 02 towards Ring 04 (Figure 4B). It was not possible to examine scalid arrangements or morphology posterior to Ring 04.

The neck has 16 placids, measuring 11 μm in length (Figure 2A, B). Midventral placid broadest, measuring 10 μm in width, whereas all other are narrower, measuring 6 μm in width. A total of six trichoscalids are present, located in sectors 2, 4, 5, 7, 8 and 10. All trichoscalids attach to trichoscalid plates (Figure 2A, B).

Segment 1 consists of a complete cuticular ring. Sensory spots are located medially on the segment in subdorsal and laterodorsal positions (Figure 2A and 4C). The sensory spots are small and rounded, with a single long cuticular hair extending from the lateral margin of each sensory spot. Glandular cell outlets type 1 are present in lateroventral positions (Figure 2B). Cuticular hairs absent. Posterior segment margin is straight. The segment terminates into a pectinate fringe (Figure 4C). Fringe with relatively long and thin, sometimes undulating fringe tips along the dorsal, lateral and lateroventral parts of the segment margin; ventral parts of the margin with considerably shorter fringe tips (Figure 2B and 4D).

Segment 2 consists of a complete cuticular ring, with tubes located in subdorsal (ca. 15 μm from SEM) and ventrolateral positions (Figure 2A,B, 3B,E and 4C,D). Sensory spots are located in middorsal, laterodorsal and ventromedial positions, and glandular cell outlets type 1 in ventromedial positions (Figure 2B and 3E). Sensory spots on this and all following segments are small and rounded. Secondary pectinate fringe present on anterior part of segment, consisting of short, thin, flexible fringe tips. On this and all following segments, cuticular hairs are densely distributed dorsally and laterally, forming a uniform

covering between the secondary fringe and the pectinate fringe. The cuticular hairs emerge from a short elevation of the cuticle and their length increases from anterior to posterior on the segment, i.e., posteriormost cuticular hairs are slightly longer than the anteriormost. The hair covering is interrupted on the ventral side of the segment, where only a ventromedial round patch is present. Pectinate fringe of posterior margin with relatively long and thin, sometimes undulating fringe tips along the whole segment margin.

Segment 3, and remaining segments, consist of one tergal and two sternal plates (Figure 2B and 3A). Segment with sensory spots in subdorsal and ventromedial positions, and glandular cell outlets type 1 in ventromedial positions (Figure 2A, B). Cuticular hairs densely distributed over tergal plate, between secondary fringe and intersegmental joint line. Hairs on sternal plates arranged in a round pattern covering only paraventral areas. Posterior segment margin with pectinate fringe as on preceding segment.

Segment 4 with short acicular spine in middorsal position (Figure 2A and 3C). Sensory spots and glandular cell outlets type 1 present in ventromedial positions only (Figure 2B). Secondary fringe, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 5 with short acicular spine in middorsal position and tubes in lateroventral positions (Figure 2A,B, 3C,F and 4E). Sensory spots present in sublateral and ventromedial positions. Glandular cell outlets, secondary fringe, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 6 with acicular spine in middorsal position (Figure 2A and 3C). This acicular spine is slightly longer than that of preceding segment. Sensory spots (Figure 4E), glandular cell outlets (Figure 3F), secondary fringe, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 7 with long acicular spine in middorsal position (Figure 2A and 3D). This acicular spine is approximately twice as long as the acicular spines of segments 4 and 5. Sensory spots are located in paradorsal and ventromedial positions, and glandular cell outlets type 1 are located in ventromedial positions. Secondary fringe, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 8 with long acicular spine in middorsal position, tubes in lateral accessory positions, and short spines in lateroventral positions (Figure 2A, B, 3D, G, and 4F). Sensory spots are located in paradorsal positions only, and glandular cell outlets type 1 in ventromedial positions. Secondary fringe, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 9 with small short spines in lateroventral positions (Figure 2B, 3G, H and 4F, G). Sensory spots present in paradorsal, midlateral and ventrolateral positions. Glandular cell outlets type 1 located in ventromedial positions. A pair of minute sieve plates is located in lateral accessory positions (Figure 2B and 3G, H). Hairs, secondary fringe, posterior segment margin and its pectinate fringe of as on preceding segment.

Segment 10 with minute laterodorsal tubes located at the posterior segment margin (Figure 2A, C). These tubes are slightly longer in males than in females. Sensory spots present in subdorsal and ventrolateral positions. Glandular cell outlets type 1 located in paraventral positions. Cuticular hair covering as on preceding segments. Posterior segment margin of tergal plate straight; sternal plates extend posteriorly into a midventral triangular projection (Figure 2B). Fringe tips in pectinate fringe of posterior margin as on preceding segment.

Segment 11 with lateral terminal spines (Figure 2A-D, 3H-J and 4H-J); lateral terminal spines in males conspicuously longer than in females (male average LTS length = 50 μm ; female average LTS length = 20 μm). Males with three pairs of penile spines: dorsal and ventral penile spines are long, flexible and slender, medial one is much shorter and thicker (Figure 2C, D, 3J, and 4I, J). Females with rather minute lateral terminal accessory spines (Figure 2A-B, 3I, and 4H). Sensory spots present in paradorsal positions. Cuticular hair covering dense on both tergal and sternal plates. Tergal plate projects slightly beyond sternal plates, and terminates into two short tergal extensions with interrupted medial margins.

Notes on diagnostic and taxonomic features

Echinoderes lusitanicus sp. nov. can be distinguished from all other kinorhynchs by its presence of lateroventral tubes/spines on segments 5, 8 and 9 only, combined with the presence of subdorsal tubes on segment 2. No other kinorhynch show this particular tube/spine pattern. The presence of lateroventral tubes and/or spines on only segments 5, 8 and 9 is shared with four other species only: *Echinoderes caribiensis* Kirsteuer, 1964, *E. eximus* Higgins & Kristensen, 1988, *E. skipperae* Sørensen & Landers, 2014 and *E. tchefouensis* Lou, 1934. *Echinoderes caribiensis* is a shallow water species from mangroves in Venezuela (Kirsteuer 1964), and it truly possesses lateroventral tubes or spines on segments 5, 8 and 9, but since it otherwise does not have any spines on segments 1 to 10, it cannot in any way be confused with *E. lusitanicus* sp. nov. *Echinoderes tchefouensis* is widely distributed in the West Pacific, from Korea to Malaysia (Sørensen et al. 2012; Sørensen et al., unpubl. obs.), while *E. eximus* is known from West Greenland (Higgins & Kristensen 1988). Besides their similarity in lateroventral spine pattern, *E. tchefouensis* and *E. eximus* do not resemble *E. lusitanicus* sp. nov. in any other way. First, the lateral spine patterns are actually not identical, because in *E. tchefouensis* and *E. eximus* the lateral spine on segment 8 is displaced to the lateral accessory position (Sørensen et al. 2012; Sørensen pers. obs.). Furthermore, these species have much longer lateral terminal accessory spines, no tubes on segment 2, and in addition, *E. tchefouensis* has a pair of very large and conspicuous glandular cell outlets type 2 on segment 8 (Sørensen et al. 2012). The species that shows most resemblance to *E. lusitanicus* sp. nov. is *E. skipperae*. The two species share the same tube/spine distribution in the lateral series, and *E. lusitanicus* sp. nov. also show, at least to some extent, the same rectangular overall trunk shape that is highly characteristic for *E. skipperae* (Sørensen & Landers, 2014). However, the new species does not have the conspicuously strong pachycycli that are found in *E. skipperae*, and the dorsal spine patterns are also different, since *E. lusitanicus* sp. nov. has middorsal spines on segments 4 to 8, whereas *E. skipperae* has them on segments 4, 6 and 8 only. Furthermore, *E. lusitanicus* sp. nov. has subdorsal tubes on segment 2, which are lacking in *E. skipperae*. Instead, the latter has subdorsal tubes on this segment, which are absent in *E. lusitanicus* sp. nov.

Two other morphological features attract special attention in *E. lusitanicus* sp. nov. Both regard sexually dimorphic characters, namely the extraordinary short lateral terminal accessory spines in females, and the significant difference in length of lateral terminal spines between males and females. The easy way to discriminate between the sexes among species of *Echinoderes* is to look for the presence of either penile spines, present in males only, or for lateral terminal accessory spines that are restricted to females (see e.g., Sørensen and Pardos 2008; Neuhaus 2013). In *E. lusitanicus* sp. nov. though, the sexes can also be discriminated by the lengths of the lateral terminal spines. In females, the lateral terminal spines are short and measure between 17 and 22 μm , which is less than 10% of the trunk length. Oppositely, the lateral terminal spines in males are more than twice as long, measuring 45 to 57 μm , which is around 20% of the trunk length. To our knowledge, a similar sexual dimorphism is not known for any other species of Echinoderidae.

The other sexually dimorphic specialty regards the lateral terminal accessory spines. As a general rule, females in species of Echinoderidae can be distinguished from males by the presence of lateral terminal accessory spines. There are exceptions though, since lateral terminal accessory spines actually are missing in females of several species belonging to the *E. coulli*-group (see Sørensen 2014), but also of species such as *E. capitatus* Zelinka 1928 and *E. isabelae* G^oOrdoñez et al. 2008. A recently described species from the *E. coulli*-group, *E. komatsui* Yamasaki and Fujimoto, 2014, shows an interesting intermediate condition in regard to the presence of lateral terminal accessory spines. Here, the species actually has lateral terminal accessory spines, and they are restricted to females only, but the spines are highly reduced. In females of *E. lusitanicus* sp. nov. the lateral terminal accessory spines are even more reduced and appear only as a pair of short spikes. This apparent reduction of a female secondary sexual character is interesting, and suggests that *E. lusitanicus* sp. nov. belongs to a lineage of *Echinoderes* that moves towards a reduction or complete loss of lateral terminal accessory spines. This could either indicate that *E. lusitanicus* sp. nov. is closely related with species of the *E. coulli*-group, or support that species of

Echinoderes at least twice went through a gradual reduction and subsequent loss of lateral terminal accessory spines.

***Echinoderes reicherti* sp. nov.**

Type material

Holotype, adult female, collected from mud on October 28, 2013, at station 13.10.28.2B (Figure 1), at 35 m depth off Vale do Lobo, about 8 km west of Faro, on the south coast of Portugal (36° 59'52''N 008°09'19''W), mounted in Fluoromount G, deposited at the Natural History Museum of Denmark (NHMD), under catalogue number ZMUC KIN-000937. Paratypes, sixteen adult specimens (cat. nos. ZMUC KIN-000938 to KIN-000953) mounted in Fluoromount G, and eleven adult specimens mounted on two SEM stubs (cat. nos. ZMUC KIN-000954 to KIN-000955), collected at the same locality as holotype, deposited at NHMD.

Diagnosis

Echinoderes with tubes on segment 2 in subdorsal and ventrolateral positions, on segment 5 in lateroventral positions, and segment 8 in sublateral positions; spines are present in middorsal position on segment 4, and in lateroventral positions on segments 6 to 9. Glandular cell outlets type 2 present in sublateral positions of segment 2. Males with three penile spines, females with slender lateral terminal accessory spines.

Etymology

The species is named after Prof Dr Heinrich Reichert, an enthusiastic supporter of the study of meiofauna and lesser-known invertebrates.

Description

Adult with head, neck and eleven trunk segments (Figure 5A,B, 6A and 7A). For complete overview of measures and dimensions, see Table 3. Distribution of cuticular structures, i.e., sensory spots, glandular cell outlets and tubes, is summarized in Table 4.

All specimens mounted for SEM had their heads retracted, which made detailed examination of mouth cone and introvert structures impossible.

The neck has 16 placids (Figure 5A, B), measuring 11 μm in length. Midventral placid broadest, measuring 13 μm in width, whereas all other are narrower, measuring 7 μm in width. A total of six trichoscalids are present, and located in sectors 2, 4, 5, 7, 8 and 10. The trichoscalids attach to trichoscalid plates.

Segment 1 consists of a complete cuticular ring. Sensory spots are located in subdorsal, laterodorsal and ventromedial positions (Figure 5A, B and 7B). All sensory spots are rounded, with a central pore. A cuticular hair emerges on each side of the subdorsal sensory spots. Glandular cell outlets type 1 are present in ventrolateral positions (Figure 5B and 7B,B'). A few cuticular hairs are present, scattered around the segment, located along a medial band. Each hair emerges through a rounded perforation site. The segment terminates into a pectinate fringe. Fringe with very short fringe tips along the whole segment margin.

Segment 2 consists of a complete cuticular ring with subdorsal (ca. 11.5 μm from SEM) and ventrolateral tubes (ca. 11.7 μm from SEM) (Figure 5A,B, 6B,D and 7C,H). Sensory spots are located in subdorsal – which are flanked by a cuticular hair on each side – and ventromedial positions (Figure 5A, B). Glandular cell outlets type 2 are present in sublateral position (Figure 5B and 6D). Cuticular hairs bracteate, present in a median band around the segment. Posterior segment margin is straight along the dorsal lateral edges. Secondary pectinate fringe present on anterior part of segment, consisting of short, thin, flexible fringe tips. On this and all following segments, pectinate fringe of posterior margin with relatively long and thin, sometimes undulating fringe tips along the lateral and lateroventral parts of the segment margin; ventral parts of the margin with slightly shorter fringe tips.

Segment 3, and remaining segments, consist of one tergal and two sternal plates (Figure 5A, B and 6A). Segment with sensory spots in subdorsal positions, which are flanked by a cuticular hair on each side. Glandular cell outlets not observed. Cuticular hairs as on preceding segment, but covering more densely the median area of the segment. Secondary fringe and pectinate fringe of posterior margin as on preceding segment.

Segment 4 with small acicular spine in middorsal position (Figure 5A, 6C and 7I). Sensory spots and glandular cell outlets not observed. Secondary fringe, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 5 with lateroventral tubes (ca. 12.4 μm from SEM) (Figure 5B, 6E and 7D, E). Sensory spots present in subdorsal – which are flanked by a cuticular hair on each side – and ventromedial positions (Figure 5A, B). Glandular cell outlets are not observed. Secondary fringe, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 6 with small acicular spine in lateroventral position (Figure 5B, 6F and 7E). Sensory spots present in paradorsal and midlateral positions. Secondary fringe, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 7 with small acicular spine in lateroventral position (Figure 5B, 6F and 7E, F) and with sensory spots in subdorsal, midlateral and ventromedial positions (Figure 5A, B). Secondary fringe, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 8 with sublateral tube (Figure 5B, 6G and 7F) and small acicular spine in lateroventral positions (Figure 5B, 6G and 7F, G). Sensory spots present in paradorsal position (Figure 5A), and glandular cell outlets type 1 in paradorsal positions (Figure 5A). Secondary fringe, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 9 with small acicular spine in lateroventral position (Figure 5B, 6G and 7G). Sensory spots present in laterodorsal, midlateral and ventrolateral position (Figure 5A,B). Glandular cell outlets as on

preceding segment. A pair of small sieve plates is located in the lateral accessory positions (Figure 5B). Secondary fringe, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 10 with minute tubes located at the posterior segment margin (Figure 5A, C and 7J, K); tubes are slightly longer in males (ca. 8.8 μm from SEM) than in females (ca. 2.5 μm from SEM). Sensory spots present in subdorsal and ventrolateral positions (Figure 5A, B). Two glandular cell outlets type 1 located in middorsal position, and one additional pair in paraventral positions (Figure 5A, B). Cuticular hair covering as on preceding segments but less dense. Posterior segment margin of tergal plate straight; sternal plates with curved posterior margin, which extend posteriorly into a midventral triangular projection. Fringe tips in pectinate fringe of posterior margin as on preceding segment.

Segment 11 with lateral terminal spines (Figure 5, 6H, I and 7L). Males with three pairs of elongate and thin penile spines (Figure 5C, D, 6I and 7L). Females with lateral terminal accessory spines (Figure 5A, B and 6H). Sensory spots and glandular cell outlets not observed. Cuticular hairs present only at the most posterior part of the segment. Tergal plate projects beyond sternal plates, and terminates into two pointed tergal extensions with interrupted medial margins (Figure 6H-I).

Notes on diagnostic and taxonomic features

Echinoderes reicherti sp. nov. is distinguished from all other congeners by the unique combination of a number of specific characters. Indeed, the presence of subdorsal and ventrolateral tubes, as well as sublateral glandular cell outlets type 2 on segment 2 combined with the presence of a middorsal spine on segment 4 is a pattern not present in any other kinorhynch.

The presence of lateroventral and subdorsal tubes on segment 2 is shared with four other species only: *Echinoderes abbreviatus* Higgins, 1983, *E. adrianovi* Herranz, Sánchez, Pardos & Higgins, 2014, *Echinoderes kanni* Thormar & Sørensen, 2010 and *Echinoderes lusitanicus* sp. nov. Both *E. adrianovi* and *E. kanni*, from the Floridian West coast and the Solomon Islands, respectively, possesses a pattern of lateral tubes/spines that is almost identical to that of *E. reicherti* sp. nov. (cf. Thormar & Sørensen, 2010; Herranz

et al., 2014b). However, in *E. adrianovi* and *E. kanni* the tube in segment 8 is located in lateral accessory positions rather than in sublateral as observed in *E. reicherti* (in fact, the latter condition is known only from *Echinoderes astridae* Sørensen, 2014). In addition, *E. adrianovi* and *E. kanni* possess middorsal spines between segments 4-8 and thus, they cannot be confused with *E. reicherti*. The other new species described here, *E. lusitanicus* is easily distinguished from *E. reicherti* because it possesses tubes and/or spines on only segments 5, 8 and 9. The presence of ventrolateral tubes in *E. abbreviatus* was not mentioned in the species' original description (Higgins 1983), but the presence of such tubes was confirmed after a recent reexamination of the type material (Sørensen et al., submitted). However, with its short and stout lateral terminal accessory spines, and middorsal spines on segments 4, 6 and 8 only, *E. abbreviatus* cannot be confused with *E. reicherti* sp. nov.

Another feature on segment 2 that attracts attention is the presence of glandular cell outlets type 2 in sublateral position. Such characteristic is for now shared with *E. abbreviatus* (observation by Sørensen et al., submitted) from Belize, as well as *E. cernunnos* Sørensen, Rho, Min, Kim & Chang, 2012 and *E. obtuspinosus* Sørensen, Rho, Min, Kim & Chang, 2012, two Korean species. However, all 3 species possess glandular cell outlets type 2 also in subdorsal, laterodorsal and ventrolateral position, which makes *E. reicherti* sp. nov. easily distinguishable (Higgins, 1983; Sørensen et al., 2012). A fifth species with glandular cell outlets type 2 in sublateral positions on segment 2 is currently being described from the Gulf of Mexico (Sørensen et al., submitted). Interestingly, this species also possesses only a single middorsal spine (on segment 4), but it also differs from *E. reicherti* sp. nov. by having one additional pair of glandular cell outlets type 2 in subdorsal positions on segment 2, only tubes in lateroventral positions on segment 2 (opposite to subdorsal and ventrolateral in *E. reicherti* sp. nov.), and tubes in lateral accessory positions on segment 8 (opposite to sublateral in *E. reicherti* sp. nov.).

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Figure legends

Figure 1. Map showing the approximate position of the sampling area at the Portuguese coast. Inset to the lower left shows a close-up of the sampling area between Faro and Albufeira. Two sampling localities (dots) yielded specimens of *Echinoderes lusitanicus*, while *Echinoderes reicherti* was found only in one locality (star).

Figure 2. Line art illustrations of *Echinoderes lusitanicus* sp. nov. (A) Female, dorsal view; (B) Female, ventral view; (C) Male, segments 10 to 11, dorsal view; (D) Male, segments 10 to 11, ventral view.

Figure 3. Light micrographs showing overviews and details of trunk morphology in *Echinoderes lusitanicus* sp. nov. A-I. Female holotype (KIN-000924). J. Male paratype (KIN-000925). (A) Ventral overview, anterior faces up; (B) Segments 1 to 3, dorsal view; (C) Segments 4 to 6, dorsal view; (D) Segments 7 and 8, dorsal view; (E) Segments 2 to 3, ventral view; (F) Segments 5 and 6, ventral view; (G) Segments 8 to 10, ventral view; (H) Segments 9 to 11, ventral view, showing female morphology; (I) Right half of segment 11, ventral view, showing female morphology; (J) Right half of segment 11, ventral view, showing male morphology. Abbreviations: gco1, glandular cell outlet type 1; lat, lateral accessory tube; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tube; mds (and arrowheads), middorsal spine; pe, penile spine; sdt, subdorsal tube; si, sieve plate; vlt, ventrolateral tube.

Figure 4 Scanning electron micrographs showing overviews and details in head and trunk morphology of *Echinoderes lusitanicus* sp. nov. paratypes (KIN-000936). (A) Lateroventral overview of whole specimen, anterior faces up; (B) Introvert, sector 4; (C) Segments 1 to 3, subdorsal view; (D) Segments 2 and 3, ventral view; (E) Segments 5 to 6, ventral view; (F) Segments 8 to 10, lateroventral view; (G) Segment 9, ventral view; (H) Detail of right half of segment 11 in female showing lateral terminal accessory spine; (I) Partial view of segment 11 showing a penile spine, lateral view; (J) Detail of segment 11 in male showing the

penile spines, laterodorsal view. Abbreviations: lat, lateral accessory tube; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tube; pe, penile spine; psp, primary spinoscalid; sdt, subdorsal tube; sp₂₋₄ spinoscalid of ring 2 to 4; vlt, ventrolateral tube; ss, sensory spot.

Figure 5. Line art illustrations of *Echinoderes reicherti* sp. nov. (A) Female, dorsal view; (B) Female, ventral view; (C) Male, segments 10 to 11, dorsal view; (D) Male, segments 10 to 11, ventral view.

Figure 6. Light micrographs showing overviews and details of trunk morphology in *Echinoderes reicherti* sp. nov. A-H. Female holotype (KIN-000937). I. Male paratype (KIN-000943). (A) Ventral overview, anterior faces up; (B) Segments 2 to 3, dorsal view; (C) Segments 4 to 5, dorsal view; (D) Segments 1 to 3, ventral view; (E) Segments 4 to 6, ventral view; (F) Segments 6 to 7, ventral view; (G) Segments 8 to 10, ventral view; (H) Segments 10 and 11 showing female morphology, ventral view; (I) Segment 11, ventral view, showing male morphology. Abbreviations: gco₂, glandular cell outlet type 2; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tube; mds, middorsal spine; pe, penile spine; sdt, subdorsal tube; slt, sublateral tube; vlt, ventrolateral tube.

Figure 7. Scanning electron micrographs showing overviews and details in trunk morphology of *Echinoderes reicherti* sp. nov. paratypes (KIN-000954). (A) Ventrolateral overview of whole specimen, anterior faces up; (B) Segment 1, laterodorsal view. The square outlines the region where a glandular cell outlet type 1 is located, as shown in B'; (B') Detail of a glandular cell outlet type 1 located in segment 1; (C) Segments 2 to 3, ventral view; (D) Segments 4 to 6, lateroventral view; (E) Segments 5 to 7, ventral view; (F) Segments 7 to 8, lateroventral view; (G) Segments 8 to 9, ventral view; (H) Close-up showing subdorsal tube on segment 2; (I) Close-up showing middorsal spine on segment 4; (J) Close-up showing laterodorsal tube on segment 10 in female; (K) Close-up showing laterodorsal tube on segment 10 in male; (L) Segment 11 in female, dorsal

view. Abbreviations: ldt, laterodorsal tube; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tube; mds, middorsal spine; sdt, subdorsal tube; slt, sublateral tube; vlt, ventrolateral tube.

Table 1. Measurements (μm) from light microscopy of adult *Echinoderes lusitanicus* sp. nov., including number of measured specimens (n) and standard deviation (SD). Abbreviations: ac: acicular spine; (f), female; LA: lateral accessory; LTS: lateral terminal spine; LV: lateroventral; (m), male; MD: middorsal; MSW-8: maximum sternal width, measured on segment 8 in this species; S: segment lengths; SW-10, standard width, always measured on segment 10; TL: trunk length; tu, tube.

Character	n	Range	Mean	SD
TL	10	213 – 294	248	25.87
MSW-8	10	44 – 48	46	1.45
MSW-8/TL	10	15.3 – 22.5%	18.8%	2.18%
SW-10	10	36 – 48	40	3.56
SW-10/TL	10	13.6 – 20.7%	16.3%	2.20%
S1	10	23 – 29	26	1.66
S2	10	22 – 27	24	1.73
S3	10	25 – 30	28	1.43
S4	10	24 – 34	28	2.75
S5	10	26 – 35	30	2.69
S6	10	30 – 37	33	2.13
S7	10	32 – 39	36	2.51
S8	10	32 – 43	38	3.17
S9	10	35 – 41	41	2.27
S10	10	28 – 36	32	2.50
S11	10	15 – 28	20	3.61
MD4 (ac)	10	18 – 32	25	4.79
MD5 (ac)	10	18 – 44	30	9.70
MD6 (ac)	10	25 – 52	45	8.21
MD7 (ac)	10	45 – 58	54	3.65
MD8 (ac)	10	55 – 68	61	4.89
LV 2 (tu)	2	17 – 18	18	0.71
LV 5 (tu)	3	16	16	0.00
LV 8 (ac)	3	9 – 13	11	2.08
LA 8 (tu)	3	17 – 18	18	0.58
LV 9 (ac)	10	15 – 18	16	1.06
LTS (ac)(f)	4	17 – 22	20	2.16
LTS/TL (f)	4	6.6 – 10.3%	7.9%	1.66%
LTS (ac)(m)	6	45 – 57	50	4.15
LTS/TL (m)	6	19.4 – 22.7%	20.8%	1.35%

Table 2. Summary of nature and location of sensory spots, glandular cell outlets and spines arranged by series in *Echinoderes lusitanicus* sp. nov. Abbreviations: LA: lateral accessory; LD: laterodorsal; LV: lateroventral; MD: middorsal; ML: midlateral; PD: paradorsal; PV: paraventral; SD: subdorsal; SL: sublateral; VL: ventrolateral; VM: ventromedial; ac, acicular spine; (f), female condition of sexual dimorphic character; gco1, glandular cell outlet type 1; ltas, lateral terminal accessory spine; lts, lateral terminal spine; (m), male condition of sexual dimorphic character; pe, penile spines; si, sieve plate; ss, sensory spot; tu, tube.

Position Segment	MD	PD	SD	LD	ML	SL	LA	LV	VL	VM	PV
1			ss	ss				gco1			
2	ss		tu	ss					tu	gco1,ss	
3			ss							gco1,ss	
4	ac									gco1,ss	
5	ac					ss		tu		gco1,ss	
6	ac					ss				gco1,ss	
7	ac	ss								gco1,ss	
8	ac	ss					tu	ac		gco1	
9		ss			ss		si	ac	ss	gco1	
10			ss	tu					ss		gco1
11		ss			pe(m)		ltas(f)	lts			

Table 3. Measurements (μm) from light microscopy of adult *Echinoderes reicherti* sp. nov., including number of measured specimens (n) and standard deviation (SD). Abbreviations: ac: acicular spine; LTAS, lateral terminal accessory spine; LTS: lateral terminal spine; LV: lateroventral; MD: middorsal; MSW-7: maximum sternal width, measured on segment 7 in this species; S: segment lengths; SL: sublateral; SW-10, standard width, always measured on segment 10; TL: trunk length; tu, tube.

Character	n	Range	Mean	SD
TL	10	210 – 257	243	15.01
MSW-7	10	48 – 54	51	1.76
MSW-7/TL	10	19.6 – 25.7%	21.1%	1.77%
SW-10	10	38 – 49	43	3.23
SW-10/TL	10	16.0 – 20.5%	17.9%	1.56%
S1	10	22 – 24	22	0.70
S2	10	27 – 31	28	1.34
S3	10	22 – 27	26	1.51
S4	10	24 – 30	27	1.73
S5	10	28 – 31	29	0.92
S6	10	28 – 32	30	1.06
S7	10	31 – 34	32	0.79
S8	10	33 – 36	34	0.82
S9	10	32 – 36	34	1.33
S10	10	33 – 39	36	1.70
S11	10	31 – 36	33	1.72
MD4 (ac)	9	21 – 28	24	2.55
LV 6 (ac)	9	17 – 21	20	1.24
LV 7 (tu)	10	18 – 23	20	1.65
LV 8 (ac)	10	20 – 25	23	1.64
SL 8 (tu)	1	14	-	-
LV 9 (ac)	10	20 – 26	23	1.55
LTS (ac)	10	86 – 133	122	13.41
LTS/TL	10	41.0 – 54.3%	50.0%	3.71%
LTAS	5	33 – 37	35	1.67

Table 4. Summary of nature and location of sensory spots, glandular cell outlets and spines arranged by series in *Echinoderes reicherti* sp. nov. Abbreviations: LA: lateral accessory; LD: laterodorsal; LV: lateroventral; MD: middorsal; ML: midlateral; PD: paradorsal; PV: paraventral; SD: subdorsal; SL: sublateral; VL: ventrolateral; VM: ventromedial; ac, acicular spine; (f), female condition of sexual dimorphic character; gco1/2, glandular cell outlet type 1 or 2; ltas, lateral terminal accessory spine; lts, lateral terminal spine; (m), male condition of sexual dimorphic character; pe, penile spines; si, sieve plate; ss, sensory spot; tu, tube.

Position Segment	MD	PD	SD	LD	ML	SL	LA	LV	VL	VM	PV
1			ss	ss					gco1	ss	
2			tu,ss			gco2			tu	ss	
3			ss								
4	ac										
5			ss					tu		ss	
6		ss			ss			ac			
7			ss		ss			ac		ss	
8		gco1,ss				tu		ac			
9		gco1		ss	ss		si	ac	ss		
10	gco1,gco1		ss	tu					ss		gco1
11					pe(m)		ltas(f)	lts			