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Description of a new species of *Mesochaetopterus* (Annelida, Polychaeta, Chaetopteridae), with re-description of *M. xerecus* and an approach to the phylogeny of the family

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Running title: New *Mesochaetopterus* from the NW Mediterranean

A large chaetopterid polychaete, *Mesochaetopterus rogeri* sp. nov. is described as new from the Mediterranean Sea. The analyses of partial sequences from the nuclear 18S rRNA (643bp) and the mitochondrial Cytochrome Oxidase I (577bp) genes of representative individuals of all known chaetopterid genera indicated the initial assignment of the new species into *Mesochaetopterus*. These analyses also supported the monophyly of the family and revealed two well-supported clades: *Chaetopterus / Mesochaetopterus* and *Spiochaetopterus /* *,Phyllochaetopterus*. *Mesochaetopterus rogeri* sp. nov. was close to *M. xerecus*, here re-described from newly collected material. *Mesochaetopterus rogeri* sp. nov. was characterized by: 1) two long tentacles with dorsal transversal black bands with alternating widths (sometimes with two additional longitudinal light-brown bands); 2) A region with nine chaetigers (up to 12), with 13 - 19 modified chaetae in the 4th; 3) B region with three flat segments, with accessory feeding organs in the 2nd and 3rd; 4) sandy straight tubes, 2.5 m long or more, vertically embedded in the sand. In the Bay of Blanes, *M. rogeri* sp. nov. occurs between 6 and 9 (up to 30) m deep, with a patchy distribution (< 1 ind. m^{-2}), maximum densities in April/June (likely due to recruitment events) and minimum in September/November (likely a behavioural response to increasing sediment dynamics). Although it was originally thought that *M. rogeri* sp. nov. could be an introduced species, we argue that it is probably a native of the Mediterranean, which has been overlooked by scientists up to now.

ADDITIONAL KEYWORDS: 18S rRNA - behaviour - *Chaetopterus* – Chaetopteridae – Cytochrome Oxidase I – distribution – genetics – Mediterranean Sea - *Mesochaetopterus* – *Phyllochaetopterus* - phylogeny – *Spiochaetopterus*.

INTRODUCTION

During recent years, scuba divers have occasionally reported the presence of "strange" large worms having a pair of long palps with a characteristic striped "black and white" colour pattern in shallow sandy bottoms of the Iberian Mediterranean shoreline, mainly along the Catalan littoral. Up to now, the only available data on their presence were these incidental reports, sometimes accompanied by pictures of the living specimens "in situ" (Fig. 8A, B). The oldest known reference, a worm from Almería, SW Iberian Penninsula, appeared in an encyclopaedia in 1979, labelled as "Spionidae" (George & George, 1979, fig.8, pag. 353}.

Routine monitoring of the brine discharge from the Blanes desalination plant in the shallow sandy bottoms facing the beach at Punta del Tordera (Catalan coast, NW Mediterranean) revealed a stable population, allowing the collection of enough appropriate material.

In this study, we used a partial nuclear gene (18S rRNA) and a mitochondrial gene, Cytochrome Oxidase I (COI), aiming to confirm the assignment of the Catalan specimens into the correct genus within the Chaetopteridae (Annelida, Polychaeta), as well as to assess the phylogenetic relationships within the four known genera of the family: *Chaetopterus*, *Mesochaetopterus*, *Spiochaetopterus* and *Phyllochaetopterus*.

The Catalan specimens were revealed to be an unknown species of *Mesochaetopterus*. Thus they are described as a new species, *M. rogeri* sp. nov, in spite of lacking data on the morphology and chaetal arrangement of the posterior-most segments (due to logistic difficulties of extracting complete worms). They were compared with all known species of the genus, particularly with *Mesochaetopterus xerecus* Petersen and Fanta, 1969, which closely resembles the new species both in size and in having striped palps (Petersen & Fanta, 1969). In the case of *M. xerecus*, however, all material was lost (including the types). For this study, new material collected at Baranaguá Bay (Paraná, Brazil) was used both in genetic and morphologic analyses. In addition, we provide a brief diagnosis of the soft body of the species, together with a full description and pictures of its chaetal arrangement, which was poorly described and illustrated in the original description.

The seasonal trends of the population in Punta del Tordera, habitat preferences, and behaviour of of *Mesochaetopterus rogeri* sp. nov. are also provided, compared with the known traits of the other known large-sized species of *Mesochaetopterus* and discussed, to attempt to explain the presence of such a large unknown worm in the widely investigated

shallow-waters of the Mediterranean Sea.

MATERIAL AND METHODS

Sampling procedure

All observations on living worms and sampling were performed by SCUBA diving. Most sampling attempts were carried out off Punta del Tordera on the Catalan shoreline of the Mediterranean coast of the Iberian Peninsula (Fig. 1) from October 2001 to September 2004. As a first collection method, we used a PVC plate introduced obliquely into the sediment to cut the worm's tube, trying to prevent the worms from withdrawing inside the tube. The body in *Mesochaetopterus* is divided into three differentiated parts: the nine to ten anterior-most segments, the two to three mid-body segments, and an undefined (but usually very numerous) number of posterior segments; these form the regions A, B and C, respectively. Using the PVC plate, the quick reaction of the worm only allowed for the collection of anterior fragments containing the A region and, at most, part of the B (as well as part of the tube). Successive attempts using either irritating or anaesthetizing compounds, sucking or impelling pumps of different types, did not result in collection of either entire specimens or larger fragments. The last attempt, carried out in September 2004, involved the archaeological research vessel "Tethys", from the Centre d'Arqueologia Submarina of the Museu d'Arqueologia de Catalunya. The vessel is equipped with powerful suction devices, which allowed us to excavate up to 2.5 m deep into the sediment. However, the tube extended deeper into the sediment and only a large fragment of a worm (with ten segments of region C, in addition to A and B regions) was collected, after several hours of underwater working. Being the most complete available fragment, this specimen was selected as the holotype of the new species.

The newly collected specimens of *Mesochaetopterus xerecus* were found in the Ilha do Mel, Baranaguá Bay (Paraná, Brazil). The worms were collected by researchers from the Centro de Estudos do Mar (Pontal do Sul, Brazil) from intertidal soft sediments at low tide using a shovel.

Genetic analysis, DNA extraction and sequencing

For the genetic analyses, all newly collected specimens were preserved directly in 100% ethanol. Total genomic DNA was extracted from individuals using the QIAamp DNA Minikit (Qiagen). A fragment of 18S rRNA nuclear gene was amplified by polymerase chain reaction (PCR) using newly designed primers (Chae18SF: 5'-AAACGGCTACCACATCCAAG-3', Chae18SR: 5'-AACTAAGAACGGCCATGCAC-3'). Cycle parameters consisted of a first denaturing step at 94ºC for 2 min, followed by 35 cycles of 1 min at 94ºC, 1 min at 55ºC and 1 min at 72ºC, and a final extension at 72ºC for 7 min. Mitochondrial cytochrome oxidase I (COI) gene was amplified using primers HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO1490 (5'-TCAACAAATCATAAAGATATTGG-3') (Folmer et al., 1994). The amplification profile was optimized for each extraction, optionally with a touchdown of five cycles of 94°C for 60 seg, 45°C for 90 seg, 72°C for 60 seg, and then 35 cycles of 94°C for 30–40 seg, 51°C for 30–90 seg, 72°C for 60 seg, with an initial single denaturation step at 94°C for 60–120 seg and a final single extension step at 72°C for 5–7 min. Both gene amplifications were carried out in 20 μL of total volume with 1X reaction buffer (Genotek), 2 mM $MgCl_2$, 250 μM dNTPs, 0.25μM of each primer, 1U Taq polymerase (Genotek) and 20-30 ng genomic DNA. PCR were performed in a Primus 96 plus (MWG Biotech). PCR products were cleaned with the QIAquick PCR Purification Kit (Qiagen) or ethanol precipitation and sequenced with the BigDye Sequencing Kit ABI Prism. PCR products were purified by ethanol precipitation and analyzed on an ABI 3700 automatic sequencer (Applied Biosystems) from the 'Serveis Cientifico-Tècnics' of the 'Universitat de Barcelona'. Representatives of all known genera of Chaetopteridae (i.e. *Chaetopterus*, *Mesochaetopterus*, *Spiochaetopterus* and *Phyllochaetopterus*) were used for the genetic analysis and the new sequences obtained were deposited in the EMBL (Table 1). For 18S rRNA, the GenBank sequences of *Chaetopterus variopedatus* from the Atlantic Ocean (U67324), *C. pugaporcinus* (DQ209224), *Mesochaetopterus taylori* (DQ209217) and *Prionospio sp.* (DQ209226) were also used. As well as, for COI, we have included sequences from GenBank of *Chaetopterus pugaporcinus* (DQ209257), *Mesochaetopterus taylori* (DQ209251), *Phyllochaetopterus socialis* (DQ209247) and *Prionospio sp.* (DQ209266). *Prionospio sp.* was used as outgroup. Specimens belonging to the two morphotypes of *Mesochaetopterus rogeri* sp. nov. (i.e. with or without reddish bands in palps) were specifically selected for the analyses.

Sequence analysis and phylogenetic reconstruction

Sequences were edited and aligned with SeqMan II (DNASTAR, Inc., Madison, Wis.) and ClustalX (Thompson et al., 1997) under default settings and verified visually. Gblocks software was used to check the alignments (Castresana, 2000), since regions that are not well conserved may not be homologous or may have been saturated by multiple substitutions, and the exclusion of poorly aligned positions and highly divergent regions aids phylogenetic reconstruction. The method makes the final alignment more suitable for phylogenetic analysis by selecting blocks of positions that meet a simple set of requirements regarding the number of contiguous conserved positions, lack of gaps, and the degree of conservation of flanking positions. Genetic divergence estimated as the percentage of haplotype sequence differences between species was calculated using the program PAUP* version 4.0b10 (Swofford, 2001).

The homogeneity of base composition across taxa was assessed using the goodness-of-fit (χ^2) test and the incongruence length difference (ILD) test (Farris *et al.,* 1994) was used to assess analytical differences between genes; both tests are implemented in PAUP* ver. 4.0b10. In the latter test, only parsimony-informative characters were included and heuristic searches were performed with ten random stepwise additions with TBR branch swapping and 1000 randomizations. In order to assess the degree of saturation, Ts, Tv and Ts+Tv *versus* genetic divergence for all pairwise comparisons in each gene independently were plotted.

Each gene was analysed individually and both genes were joined creating a new data set. Phylogenetic trees were inferred by Bayesian inference (BI) using Mr Bayes 3.1.2 (Huelsenbeck & Ronquist, 2001), since this method appears to be the best for inferring phylogenetic relationships between species (Alfaro *et al.,* 2003; Carreras-Carbonell *et al.,* 2005). The computer program MODELTEST version 3.06 (Posada & Crandall, 1998) was used to choose the best-fit evolution model under the Akaike information criterion (AIC) for each gene separately and then subsequently used in the BI analyses (Posada & Buckley, 2004). The Markov chain Monte Carlo (MCMC) algorithm with four Markov chains was run for 1,500,000 generations, sampled every 100 generations, resulting in 15,000 trees. The first 1500 trees were eliminated since they did not reach stationarity for the likelihood values and the rest were used to construct the consensus tree and obtain the posterior probabilities of the branches.

Morphological observations

Light microscope (LM) micrographs were made with a Zeiss Axioplan (body and tube) and a Zeiss Stemi 2000-c (chaetae) stereomicroscopes equipped with the SPOT hardware and software (SP100 KAF1400 digital camera and software version 2.1.) from Diagnostic Instruments Inc. For the LM micrographs of chaetae, each parapodia of the A region was dissected from the body and isolated in a Petri dish. Parapodia were carefully dissected and representatives of each chaetal type were removed with fine forceps under a compound microscope, prior to mounting on a slide. LM micrographs of uncini were made by dissecting a fragment from a parapodium and squashing it directly onto a slide. The Type Series of the new species and the newly collected specimens of *Mesochaetopterus xerecus* are deposited in the Museo Nacional de Ciencias Naturales (MNCN) of Madrid.

Ecological data

Data on population abundances in the Punta del Tordera were collected monthly by SCUBA during routine brine discharge monitoring from February 2002 to January 2004. To quantify the abundance of worms, the number of tentacle pairs protruding from the tube was noted along transects of 50 m long and 2 m wide. The abundances were monitored at two locations (south and facing the Tordera River), with two random sites per location and eight random transects per site. The seasonal pattern is represented as monthly averages of density (individuals m^{-2}). Water temperature was registered in parallel with observations on the worms. Data on wave height were obtained from the mooring buoy (DATAWELL Waverider) controlled by the Laboratori d'Ingenieria Maritima of the Universitat Politècnica de Catalunya, located at 1 mile off Punta del Tordera (41° 38.81' N; 02° 48.93' E). Wave height was expressed as the significant wave height in cm (height corresponding to the average of 1/3 of the highest waves). The long-term climatic dataset was measured by Josep Pascual in l'Estartit, about 50 km north of Blanes. Water temperature (at 20 m deep) trends from 1969 to 2003 were estimated by linear regressions by means of the SYSTAT software (version 5.2.1 1990-92, by SYSTAT corporation).

RESULTS

Genetic analysis

A total of 1220 bp was analysed for all genes combined: 643bp for 18S rRNA and 577bp for COI. The first gene presented 54 variable and 24 parsimony informative sites (8.40% and

3.73%, respectively), whereas COI gene showed a greater proportion yielding 277 variable and 205 parsimony informative sites (48% and 35.53%, respectively). The Ts/Tv was 1.46 for 18S rRNA and 1.01 for COI. Saturation tests carried out for each gene independently showed no evidence of sequence saturation in these genes (data not shown). The goodness-of-fit test for each gene showed homogeneous base composition across taxa ($P = 1.00$) and the partition homogeneity test showed no significant heterogeneity between genes ($P_{ILD} = 0.46$). Although there is no generally accepted P value for significant results, most authors agree data should be combined when P values are greater than 0.05 (Cristescu & Hebert, 2002; Russello & Amato, 2004). The models selected according to the AIC and applied to the tree reconstruction were as follows: TrN+I (γ=0.002) for the 18S rRNA and GTR+I+G (γ=3.38) for COI.

We obtained a tree for each gene independently and combining both genes. Phylogenetic reconstruction using 18S rRNA has shown that the species selected to represent each chaetopterid genera form a monophyletic group with two well-supported clades corresponding to: 1) *Chaetopterus* and *Mesochaetopterus*, and 2) *Spiochaetopterus* and *Phyllochaetopterus* (Fig. 2A). The genetic distance between the two clades (mean 3.25%) was three times greater than within clades (mean 1.36 and 1.24% between *Chaetopterus* and *Mesochaetopterus* and *Spiochaetopterus* and *Phyllochaetopterus*, respectively) (Table 2). The mean genetic distance between species from the different Chaetopteridae genera was 2.09%. No genetic differences were found between the two morphotypes of *M. rogeri* sp. nov. (i.e. with and without reddish bands in palps), which were included in the *Chaetopterus* / *Mesochaetopterus* clade, closer to *M. xerecus*. Between this species and *M. rogeri* sp. nov., two diagnostic sites were detected (G to T in 145bp and C to T in 207bp positions), with a genetic distance of 0.31% (shaded box, Table 2). This was less than the distance between *Chaetopterus* and *Mesochaetopterus* genera (mean 1.36%), but was greater than between the two Mediterranean populations of *C. variopedatus* (0.16%). The genetic distance between both Mediterranean and Atlantic *C. variopedatus* populations were greater than that between the two *Mesochaetopterus* species (bold numbers, Table 2).

Phylogenetic tree using COI gene yielded two well-supported clades, one containing all *Chaetopterus* species and the other containing *Mesochaetopterus xerecus* and *M. rogeri* sp. nov. However, *M. taylori* was clustered, with low node-support value, at *Chaetopterus* clade. All *Mesochaetopterus* and *Chaetopterus* species form a well-supported group clearly differentiated from *Phyllochaetopterus socialis* (Fig. 2B). No genetic differences were found

between the two morphotypes of *M. rogeri* sp. nov. (i.e. with and without reddish bands in palps). Between this species and *M. xerecus* the genetic distance was 1.21% (shaded box, Table 2). This was greater than between the two Mediterranean populations of *C. variopedatus* (0.35%). However, the genetic distance between both Mediterranean and Atlantic *C. variopedatus* populations were greater than that between the two *Mesochaetopterus* species (bold numbers, Table 2).

The phylogenetic tree combining both genes was reconstructed, yielding high node-support values. Two well-supported clades were found. The first one included *Chaetopterus* and *Mesochaetopterus*, with the species belonging to each genus being clearly grouped and forming two different and highly supported groups. The second clade only included *Phyllochaetopterus socialis*, since COI gene could not be sequenced for any *Spiochaetopterus* species (Fig. 2C).

Systematic account

ORDER CANALIPALPATA
SUBORDER SPIONIDA
FAMILY CHAETOPTERIDAE

MESOCHAETOPTERUS ROGERI SP. NOV.
(FIGS. 3, 4, 5, 6)

Diagnosis. Large *Mesochaetopterus* with well-developed peristomium, with a pair of long peristomial palps with successive series of dorsal transversal black stripes, alternating one thick and wide with one to several thin narrow ones, sometimes with two longitudinal dorsal and ventral orange to light-brown stripes and a lateral black stripe (most often present in the first basal half of the palp) and two longitudinal ciliated grooves. Second pair of palps and eyes absent. A region with nine (10-12) chaetigerous segments. Parapodia with notopodial lobes only, bearing capillary and lanceolate (dorsally) to oar-like and sickle-like (ventrally) chaetae. Fourth notopodia with 18(13-19), lanceolate, knife-like and stout modified chaetae. Ventral glandular shield long, pale brownish. B region with three elongated, flattened segments having biramous parapodia. Notopodia wing-like, bearing about 15 capillary chaetae. Neuropodia unilobed (segment 1) and bilobed (segments 2 and 3), bearing uncini with eight (nine) teeth. Associated feeding organs in segments 2 and 3. C region with all

segments nearly similar, bearing biramous parapodia with unilobed chaetigerous notopodia and bilobed uncinigerous neuropodia. Associated feeding organs absent. Tube longer than 2.5 m, parchment-like internally, externally fully covered by sand grains. Tube ending unknown.

A region. Holotype with nine segments, ranging from nine (N = 14), ten (N = 2) to 12 (N = 1), 0.8 cm to 1 cm long. Prostomium small, triangular, light brown dorsally, with a rounded, entire anterior border. Eyespots absent. Peristomium extended, twice as long as and completely surrounding the prostomium, contracted in fixed worms. Two peristomial lips, separated by a mid-ventral notch with a variable brownish dorsal pigmentation. Two long dorsally-grooved palps arise dorsally just behind the junction of the latero-posterior peristomial borders, up to five times as long as the A region in preserved worms (up to 15 cm long "in vivo"). Palps with a characteristic colour pattern composed of: 1) two longitudinal orange to light-brown stripes (one dorsal and one ventral), covering the whole palp in the last third; 2) several successive series of dorsal or dorso lateral transversal black stripes, alternating one thick and wide with one to several thin and narrow ones (less than one third the thickness and from half to one third the width of the broad stripes); and 3) a longitudinal black stripe of variable length usually in the first basal half of the palps, just at the lateral limits of the orange ventral bands. Longitudinal orange bands absent in some specimens. Two longitudinal ciliated grooves (one dorsal and one ventral) on each palp. Second pair of small antennae or palps absent. Eyes absent. Mouth as a vertical slit, below the prostomium and surrounded by the peristomium. Anterior end of the dorsal ciliated groove just behind the prostomium, between the basis of the palps, forming a small triangular lip. Dorsal ciliated faecal groove running from the mouth, through the median line, to the posterior end. Ventral plastron long, pale brownish in colour (often darker posteriorly), restricted to the ventral side of A region, without secretory crescents but showing a characteristic distinct epithelium.

Parapodia of A region uniramous, short, with notopodia only. Chaetae yellowish to pale orange (up to dark brown in A4), occurring dorso-laterally on two irregular question mark shaped rows on segments A1-A3 and A5-A6, and in a single, irregular, question mark shaped row on segments A4 and A7-A12. Chaetal arrangement changing in segments A1, A2 to A6 (except A4) and A7 to A12; notopodia having from 15 (A1) to 70 (A7-A12) long and fine lanceolate dorsal chaetae, becoming progressively capillary when more dorsal; notopodia of A1 with about 30 small lanceolate, oar-like chaetae latero-ventrally; notopodia of A2 to A3 and A5 t A6 with up to 35 oar-like chaetae latero-ventrally, the ventral-most chaetae twice as wide and long as the lateral ones, and twice as wide as the A1 ventral chaetae; from A7 to

A12, the ventral notopodial oar-like chaetae being replaced with sickle-like chaetae (up to 60 chaetae per parapodia).

A4 notopodia with up to 20 yellowish, transparent, finely pointed capillary chaetae in dorsal position (d); four to five yellowish, transparent knife-like chaetae more than five times wider than the lanceolate ones (ld1); one to three dark yellow to brownish knife-like chaetae, stouter that the previous ones, with the tip of the curved edge slightly serrated (ld2); 13 to 19 (typically 18) asymmetrical, knoblike, stout modified chaetae having serrated tips, the ventral chaetae (v) smaller than the lateral ones (lv1 to lv2). Modified chaetae dark brown, often partly embedded in the notopodia.

B region. Always with three segments, 2.5 to 3.5 cm long, as a flat plate-like region. Flattened and elongated segments with their flanks dorsally glandular, all them similar in size, longer than C segments; B1 slightly narrower than B2 and B3. Associated feeding organs on posterior part of B2 and B3, only one per segment. Parapodia of B region biramous. Notopodia unilobed: B1 long, pointed, digitiform, distally slightly swollen (d1); B2 and B3 wider than B1, triangular, with a groove on the anterior side, distally slightly swollen and with a dark pigmented band just before the distal swelling (d2, d3). From 11 to 13 extremely long and thin notochaetae, scarcely protruding from the tip of the notopodia; about 11 have tape-like tips, while two or three are capillary. Neuropodia unilobed in B1, with a single low ventral lobe (v1), and bilobed in B2 and B3, with a short, slightly anteriorly oriented dorsolateral lobe, and an elongate, posteriorly oriented ventral lobe (v2, v3). Uncini roughly D-shaped, with a single row of eight to nine minute teeth (most commonly 8); dorsal lobe with fewer uncini (about 60) than ventral lobe (over 400), in B2 and B3. Uncini of dorsal and ventral lobes in three or more irregular rows, with adjacent uncini somewhat displaced up or down in relation to each other. Uncini of dorsal lobes with teeth directed posteriorly, while those of ventral lobes have anteriorly directed teeth. Uncini of dorsal lobes smaller in size than ventral ones.

C region. Known only from the holotype, incomplete, consisting of ten segments for about 5 cm in length. First segment longer than the remaining ones, but shorter than those of B region. Parapodia biramous, as a flat plate-like region with glandular lateral epithelium. Gut markedly protruding from the body plan, dark green in living and recently preserved specimens. Notopodia poorly developed, nearly triangular or wing-like shaped, with five to ten very long and thin chaetae, scarcely protruding from the tip of the notopodia; three to nine

with tape-like tips and one to three capillary. Associated feeding organs absent. Neuropodia all bilobed, similar to B2 and B3 in shape, distribution and number of uncini. Uncini roughly D-shaped, with a single row of eight to nine minute teeth (most commonly eight), slightly smaller in posterior-most segments.

Tube. Known part of the tube straight, completely buried into the sediment, except for the aperture, which protruded 1-2 cm from the sediment surface and was completely coated with sand grains (Fig. 2A). From the surface opening, the tube followed a vertical path downward for more than 2.5 m. Total tube length and shape of end still unknown. Tube structure very similar all along its length, with a relatively thin-walled, parchment-like material embedded with a thick external layer of sand grains, with a few (often only one) ramifications of the main tube at non-regular intervals, shorter than the main tube, filled with sand and closed at the junction by the main tube wall Tube colour changing from yellowish to blackish at around 30 cm deep from the surface of the sediment. Detached fragments of sediment-filled or collapsed tubes of different diameters occurring around the inhabited ones. Tube surrounded by a thick cylinder of sand all along its length, about 6 cm in diameter, more compact than the remaining sediment and particularly evident when drilling around the inhabited tubes.

Etymology. Species name dedicated to Roger Martin (first author's elder son).

Material examined. HOLOTYPE: incomplete specimen, with the A and B regions and only 10 segments of the C region, measuring 110 mm long by 11 mm of maximum width, MNCN 6.01/10145. PARATYPES: 16 incomplete specimens (lacking the C region), 13-15 m deep, Punta del Tordera, Blanes (Girona, Catalunya, Spain, 41.40° N, 2.48° E), coll. D. Martin, MNCN 16.01/10146. One incomplete specimen (lacking the B and C regions), 7-10 m deep at Badalona (Barcelona, Catalunya, Spain, 41.27° N, 2.15° E), coll. L. Dantart and G. Álvarez, MNCN 16.01/10147.

Known geographical distribution. Distributional area of the species (Fig. 1) based on underwater observations: Andalucía, SW Iberian Penninsula: 20-30 m depth on the west coast near Hotel La Parra, Almería, (observed by A. Svoboda, see George & George, 1979); 5 m deep at Cabo de Gata, Almeria (observed by J. Junoy). Valencia, W Iberian Penninsula: 10-15 m deep at Cullera, south the river Jucar (observed by J. Tena team); 11 m deep at Canet d'En

Berenguer (observed by J. Tena team). Alicante, W Iberian Penninsula: 20 m deep at Punta del Rincón de Lois, Benidorm (observed by J. Tena team). Catalunya, NW Iberian Penninsula: 5-7 m deep at Punta de la Mora, Garraf (observed by B. Weitzmann); 10-15 m deep at Badalona (observed by L. Dantart, G. Álvarez and X. Turón); 30 m deep at Mataró (observed by L. Dantart); 20 m deep at Arenys de Mar (observed by B. Weitzmann); 10 m deep at Malgrat (observed by L. Dantart); 6 m deep at Blanes Harbour (observed by D. Martin); 6-10 m at Cala Sant Francesc, Blanes (observed by different divers of the CEAB); 6-15 m deep at Cala S'Aguia, Pinya de Rosa, Blanes (observed by different divers of the CEAB); 10-15 m deep at Cala Pola, Tossa de Mar (observed by the *Caulerpa* team of the CEAB); west of Urbanization Rosamar, Sant Feliu de Guixols, 20-30m (observed by A. Svoboda).

MESOCHAETOPTERUS XERECUS PETERSEN & FANTA 1969
(FIGS. 7, 8, 9, 10, 11)

Diagnosis. Based on Petersen & Fanta (1969) and on observations of newly collected specimens. Large chaetopterid (reaching up to 60 cm long "in vivo") with a well-developed peristomium (about one-fifth of the A region length) surrounding the prostomium, and a pair of long peristomial palps up to twice as long as the A region in preserved worms. Palps with transversal dark pigmented rings (dark-orange to greenish-brown) of different widths, without a clear alternating pattern, and a pair of longitudinal ciliated grooves (one dorsal and one ventral). Second pair of small antennae or palps absent. Eyes present between insertion of the palps and the peristomium. Dorsal ciliated faecal groove running from mouth to posterior end (anus), along the median body line. Ventral plastron occupying the whole A region and up to one third of the first segment of B region.

A region 1 to 1.5 cm long, with 8-14 chaetigerous segments (typically 11-12). Parapodia uniramous, notopodial lobes short; ventral glandular shield long, uniformly greenish in colour. B region of about 3 cm long, with four (up to seven) elongate segments and having associated feeding organs or cupules usually in segments 2 to 4 (up to 7). Parapodia biramous, as a flat plate-like region, with glandular lateral epithelium. Notopodia poorly developed, with a nearly triangular or wing-like shape. Uncinigerous neuropodia unilobed in first segment and bilobed in segments two and three. C region up to 55 cm long for 90 - 120 segments. Parapodia biramous, with unilobed notopodia and bilobed uncinigerous neuropodia. All

segments similar, except for some in the posterior-most pygidial region, which are shorter and have reduced parapodia.

Tube longer than 1 m, parchment-like, externally covered by sand grains (inconspicuous in worms from muddy sandy bottoms), vertical or J-shaped, with a transverse partition with three perforations in the lower part, closed at the lower extremity and ending blindly in a nearly rounded apex.

Males with elongate sperm having a long flagellum. Females with oocytes of about 200 μm in diameter, present in all segment of the C region. Population densities at Ilha do Mel reaching about 100 ind. m^{-2}.

A region. Nine to 14 segments, more frequently nine (N = 3) or 13 (N = 3). Parapodia all uniramous, with notopodia only. Chaetae yellowish to pale orange (dark brown in A4), occurring dorso-laterally in several irregular, question mark shaped rows (a single row in A4). Chaetal arrangement changing in segments: A1, A2-A6 (except the A4), A7-A9 and A10-A14; notopodia of A1 with two types of chaetae, up to 25 very long and fine lanceolate to capillary dorsal chaetae, becoming finer as progressing to the dorsum, and about 30 small lanceolate, oar-like latero-ventral chaetae; notopodia of A2 to A14 with up to 30 very long and fine lanceolate dorsal chaetae, their flattened ends becoming shorter when more dorsal; notopodia from A2 to A3 and A5 to A6 with up to 35 oar-like latero-ventral chaetae, the ventral-most twice wider and longer than the lateral; notopodia from A7 to A9, with up to 30 bayonet ventral chaetae and a few hooked latero-ventral chaetae; the bayonet chaetae becoming smaller and progressively replaced by the hooked ones in posterior-most segments; from A10 to A14, the hooked chaetae fully replacing the bayonet chaetae.

A4 notopodia with up to 15 yellowish, transparent, finely pointed lancet-like dorsal chaetae (d); five yellowish, transparent knife-like chaetae more than twice wider than the lancet-like ones (ld1); two to five dark yellow to brownish knife-like chaetae, stouter that the previous ones, with the tip of the curved edge slightly serrated (ld2, ld3); 12 to 14 (typically 11) asymmetrical, knoblike, stout modified chaetae having serrated tips, the ventral chaetae (v) smaller than the lateral ones (lv1-lv3). Modified chaetae dark brown, often partly embedded in the notopodia.

B region. Usually with four (up to seven) segments having biramous parapodia. 12-16 extremely long, thin notochaetae, scarcely protruding from the tip of the notopodia; about 12 with flattened to lanceolate tips and two to three with pointed tips. Neuropodia unilobed in

segment B1 and bilobed in B2 and B3, with several hundreds of uncini irregularly disposed in two to three rows, roughly triangular, with seven (6-10) long curved teeth plus a few small ones (often difficult to distinguish) in the anterior and posterior ends of the serrated edge. All uncini of B1 similar in size, dorsal uncini of B2 and B3 smaller than ventral ones.

C region. Incomplete, with more than 50 segments, all biramous. Notopodia with about ten long and thin chaetae (shorter than the notochaetae of B region), scarcely protruding from the tip of the notopodia, with flattened to lanceolate tips. Neuropodia all bilobed, with uncini irregularly disposed in two to three rows, similar in shape and teeth arrangement to those of B region. Uncini differing in size both dorso-ventrally and from anterior (smaller) to posterior (larger) sections of each neuropodia, clearer in anterior-most segments and progressively less evident in the posterior-most, becoming basically similar in size around segment C30.

Material examined. Ten incomplete specimens (reaching up to 55 segments in the C region), low intertidal sandy beach, Ilha do Mel, Baranaguá Bay (Paraná, Brazil), 25° 34' S, 48° 20' W, October 30 2001, V. Radashevsky coll., MNCN 16.01/10148

Habitat, behaviour and population density of Mesochaeopterus rogeri *sp. nov.*
Mesochaetopterus rogeri sp. nov. commonly inhabits fine to coarse sandy bottoms (400 μm to 600 μm of grain diameter) between 5 and 30 m deep (but most commonly between 6 and 15 m deep). These locations are often subject to medium-speed currents, which may facilitate the suspension-feeding behaviour of the worm. Different kinds of particles (possibly potential food) transported by the water currents were observed "in situ" when contacting the tentacles; they remained attached to the tentacles' surface and started to be transported towards the mouth with the help of the ciliated grooves.

When undisturbed, the tentacles protruded from the tube opening formed a V, with both tips arranged in spiral (Fig. 3A). Changes in current speed or direction did not trigger any response from the worm, except for the modification of the tentacle position induced by the new situation, which may lead toward a less regular tentacle arrangement in case of stronger currents (Fig. 3B). Any contact with the surrounding sediment induced the worm to retract inside the tube. A slow constant retraction occurred after a subtle contact. If the contact was not repeated, the worm stopped the reaction and re-acquired the typical position after a few minutes. Either repeated or violent contacts with the surrounding sediment caused a fast

retraction inside the tube. However, after several minutes, the worm protruded again to adopt its usual position.

The worms were able to expel from the interior of their tubes both introduced liquids and small particles (such as sand grains) by means of a powerful exhalation current. In normal conditions, with the worms completely inside the tube, there was a regular exhalation/inhalation water flow, probably generated by peristaltic movements of the C region segments. This flow seemed similar to those described as a part of the feeding mode and ventilation system for other chaetopterid polychaetes, including *Mesochaetopterus* (Barnes, 1965; Sendall et al., 1995).

The population density ranged between 1 to 3 ind. 40 m^{-2}, but may reach up to 1 ind. 10 m^{-2} (Fig. 12A). However, they were frequently found in clusters of 2 or 3 ind. m^{-2} separated from their neighbours by large unoccupied areas. Maximum densities tended to occur from April to June, together with rising temperatures, and could perhaps be related to recruitment events, as proposed for Mediterranean soft bottom invertebrates (Sardá et al., 1995, 1999). However, the seasonal pattern of this species differed from the general one proposed by these authors in that remarkable minimum densities occurred between September and November (particularly in October). This could not be explained by an increasing mortality after recruitment events leading to basal adult densities, as previously proposed (Sardá et al., 1995, 1999). In *M. rogeri* sp. nov., the density decrease seemed not to be related to mortality but to an adaptation to survive under the increasing instability of the sediment during the stormy autumnal period characteristic of a Mediterranean environment (Fig. 12A). In fact, the extraordinary length of the tube, as well as the ability to quickly retract inside, could represent an adaptation to such an unstable sedimentary regime. Together with the increase of instability of the water column (as expressed by the increase in wave height, Fig. 12B), the sediments tended to become more and more mobile, to the extent that the worms were not able to protrude above the surface and survived by hiding inside the tube, at a depth where the sediment became stable. This could explain the autumnal decrease in density (Fig. 12A), as the divers could not count the worms that did not protrude from the sediment surface. Moreover, this could also explain the occasional presence of empty ramifications and the huge amount of empty tubes found during the excavation collection. Accordingly, these were probably functional tubes, abandoned by the worms either during their normal growth or as a result of the above-mentioned adaptive process. Once storms ended, the worms built new tube sections to reach the surface so that there would be as many empty tubes as stormy events during the lifetime of each worm.

This mode of life supports the potential influence of *M. rogeri* sp. nov. in structuring the

surrounding sediments, as a sediment stabilizer (dense populations inside extremely long tubes, palisades of empty tubes). Also, the presence of a cylinder of compact sediment surrounding the tube all along its length (i.e. more than 2.5 m deep into the sediment and reaching up to 6 cm in diameter) in *M. rogeri* sp. nov. (also reported for *M. taylori*) has been attributed to an "aerobic halo" generated by the presence of the tube (Sendall et al., 1995). Therefore, a possible significant bioturbating potential can also be attributed to the *M. rogeri* sp. nov. populations.

Morphological comparison between Mesochaeopterus rogeri *sp. nov. and all known species of the genus*

Previous studies on the intra- and interspecific variability in the genus *Mesochaetopterus* show that hard structures (i.e. chaetae and uncini) show a continuity both in shape and size from larvae to juveniles and adults (Bhaud, 2005). This allows the small-sized species of the genus - viz. *M. minutus* Potts, *M. capensis* (McIntosh), *M. laevis* Hartmann-Schroeder, *M. crypticus* Ben-Eliahu, and *M. xejubus* Petersen & Fanta – to be discardes for he purpose of the taxonomical comparison with *Mesochaetopterus rogeri* sp. nov. All these species seldom reach 3.5 cm long (see Table 1 in Nishi, 1999) and the largest uncinal plates are shorter than 70 μm (Bhaud, 2005), while the holotype of *M. rogeri* sp. nov. measured about 9 cm long (lacking most of C region) and their uncinal plates are slightly longer than 110 μm.

Among the large-sized *Mesochaetopterus* species (Table 3), *M. rogeri* sp. nov. resembled *M. taylori* and *M. alipes* in having three parapodia on region B. In addition to geographical distribution and tentacle colour pattern, the new species differs from the former in the number of A4 chaetae, shape of uncini from the B region and tube shape, and from the latter in the shape of A4 chaetae and tube composition. *Mesochaetopterus alipes* was found in Panama and is only known from the original description, which lacked many relevant data. *Mesochaetopterus taylori* was originally described from the Pacific coasts of British Columbia by Potts (1914) and has been subsequently reported along the Pacific and Atlantic coasts of America (Gilbert, 1984; Blake, 1996). The examination of material from Invertebrate Zoology, Royal British Columbia Museum and the Natural History Museum of Los Angeles seems to support the existence of different species along the different geographical locations where the species has been reported, some of them being probably new to science (M. Bhaud, unpublished results). The data for the comparison between *M.*

taylori and *M. rogeri* sp. nov. are thus based on the original description of the species (Table 3).

The possible coincidence of tentacle colour pattern (somewhat based on dark rings or bands), as well as the genetic analyses lead us particularly to compare the new species with *M. xerecus*. However, the original type material of this species was lost and the comparisons are based on newly collected material. In addition, this helped to complete the original description by Petersen and Fanta (1969). However, the striped pattern, as well as the additional reddish longitudinal stripes present in some specimens and the tentacle length, clearly identifies *M. rogeri* sp. nov. In addition, this species may be separated from *M. xerecus* by 1) the chaetal arrangement of the A region (including the A4 segment), and 2) the number and size of teeth, and the shape and outline of uncini from regions B and C (Table 3).

Tube morphology of *M. rogeri* sp. nov. seems to coincide with that of *M. ricketsi*, at least in the known sections (2.5 m and 2 m, respectively). However, both species can be easily distinguished by the number of segments in region B (Table 3).

DISCUSSION

Phylogenetic approach

The main purpose of the molecular analysis was to assign the new species (*M. rogeri* sp. nov.) to its correct genus. A second, but no less relevant consequence was that the phylogenetic relationships between Chaetopteridae genera could also be elucidated. All our analyses placed *M. rogeri* sp. nov. within a *Mesochaetopterus* / *Chaetopterus* clade, always closely related to *M. xerecus*, demonstrating that the new species described here belonged to *Mesochaetopterus*, coinciding with the results of the morphological study.

The 18S rRNA gene is a slowly evolving sequence (Hillis & Dixon, 1991; Avise, 1994) and has been regarded as a very conservative marker of speciation events. With this gene, *M. xerecus* and *M. rogeri* sp. nov. appeared closely related (0.31%), but as distinct clades within the *Mesochaetopterus* clade (Table 2; Fig. 2A). Osborn *et al*. (2007) found similar values between *Chaetopterus* species (ranging from 0.4% to 1.6%). By including specimens of *Chaetopterus variopedatus* from Mediterranean (newly collected in Naples and Banyuls) and Atlantic (from Genbank, collected in Norwich, Norfolk, UK) coasts in the genetic analysis, a result that could be expected from a biogeographical point of view was revealed. While the

genetic distance between the Mediterranean *Chaetopterus* was less than half that of the two species of *Mesochaetopterus*, that between the Mediterranean and Atlantic *Chaetopterus* was more than twice (Banyuls) or even three times (Naples) greater, indicating that they are probably different species, the Atlantic one incorrectly identified as *C. variopedatus*.

When analysing a faster evolving gene (COI), genetic divergence values between species obviously increase, the values between *Chaetopterus* species ranging from 18% to 21% (Osborn *et al*., 2007), similar to those found in the present study. Conversely, the genetic distance between *Mesochaetopterus rogeri* sp. nov. and *M. xerecus* was only 1.21% (i.e. smaller than that between the Mediterranean and Atlantic populations of *C. variopedatus*).

Both 18S and COI genes suggested that the Mediterranean *Chaetopterus* belonged to the same species, while the Atlantic specimens must be considered as different. In fact, several different species of *Chaetopterus* have been described from European coasts, both Mediterranean and Atlantic, some of them being probably valid in addition to *C. variopedatus* (Petersen, 1984). Further studies focused on *Chaetopterus* are needed, involving more individuals and locations, to asses the taxonomical status of the *C. variopedatus* complex.

The monophyly of the Chaetopteridae has been previously demonstrated (Osborn *et al*., 2007), and our results confirmed this finding. However, the monophyly at the genus level seems not to be well resolved. According to our results, *Mesochaetopterus* is a sister group of *Chaetopterus*, both appearing to be monophyletic either using the 18S or the combined 18S and COI, and supported by high node support values (Fig. 2A, 2C). Conversely, *M. taylori* was first joined to the *Chaetopterus* clade when using COI alone, although with low node support values (Fig. 2B). In turn, the monophyly of *Phyllochaetopterus* and *Spiochaetopterus* cannot be assessed, since only one species per genus has been available for the analyses. Furthermore, it was impossible to obtain a COI sequence for the *Spiochaetopterus* species analysed in this paper (*S. solitarius*). In all studied cases, however, the specimens of both genera were closer to each other and clearly more distant from the *Mesochaetopterus* and *Chaetopterus* clades.

Taxonomy, distribution and behaviour of Mesochaeopterus rogeri *sp. nov.*

The fact that we initially thought that *Mesochaetopterus rogeri* sp. nov. could be an introduced exotic species initially lead us to the comparison with *M. xerecus*. Although comparison among all known species of the genus clearly points out the differences among them and *M. rogeri* sp. nov., the large sized Brazilian species apparently had the most similar

morphology (Table 3), this being supported by the genetic analyses. Moreover, the long-living planktonic larvae typical of Chaetopteridae might have easily been introduced to the Mediterranean through a ballast water discharge from a ship coming from Brazil. The discovery of a new population of *M. xerecus* in the Ilha do Mel (Pontal do Sul, Brazil), close to the coasts from where the species was originally described (Rio de Janeiro), allowed us to discard the hypothesis that the Mediterranean species was the same as the Brazilian one. However, taking into account the conspicuous appearance of *M. rogeri* sp. nov. and the long-term background knowledge on the polychaete fauna of the Mediterranean Sea, the possibility of an exotic origin for *M. rogeri* sp. nov. (from a foreign but still unknown region) cannot be dismissed. On the other hand, most known introduced species of marine invertebrates, including polychaetes, tend to occur as concentrated populations in geographically restricted areas, often in sheltered environments, such as harbours or coastal lagoons (Goulletquer et al., 2002). According to numerous underwater observations, *M. rogeri* sp. nov. is currently widely distributed along the Iberian Mediterranean coast, particularly in Catalunya, which is contradictory to a hypothetical introduced origin for the species.

Independently of the origin of the species, a second question occurs when trying to explain the increasing number of records in recent years. Could it be caused by a recent increase in the distributional area of the species? If so, this could be related either to the spreading of the species from warm southern waters (like those at Almería, from where the species was first recorded) due to an increase in seawater temperature (perhaps related to the global warming phenomenon) or to a decline of shellfish harvesting efforts due to over fishing of the smooth venus *Callista chione* (Linnaeus, 1758), this leading to a reduction in habitat disturbance. Although stimulating, exploration of the possible relationships between these hypotheses and the postulated expanding presence of *Mesochaetopterus rogeri* sp. nov. seems not to be possible at present, and falls out of the scope of this study.

However, there is a simple framework allowing explanation of the increase of reports. *Mesochaetopterus rogeri* sp. nov. inhabits "uninteresting" sandy bottoms, which are seldom visited by divers. One of this visits (and the subsequent observation of the worm) occurred once in the 1970 (George & George, 1979), and a gap of about 20 years passed until the second record in 1995. The sand coverage of the tubes and the colour pattern of tentacles may help the species to remain camouflaged within the sediment, so that direct observations are a matter of chance. Nevertheless, the probability of observation rose with the increase in scientific and naturalist scuba divers in recent years: there were fewer than 100 divers

associated to the Catalan Federation of Underwater Activities (FECDAS) in 1954, while the current number exceeds 12000.

From the point of view of traditional soft-bottom sampling, the behaviour and tube structure of *Mesochaetopterus rogeri* sp. nov. may also have contributed to "hide" the presence of the species in the widely investigated shallow-waters of the Mediterranean Sea. Its prompt reaction to any contact in the surrounding sediment, as well as the extreme length of the tube, make its collection virtually impossible. The initial contact of a grab or dredge with the sediment surface triggers a fast retraction inside the tube, so that only fragments of empty tubes are collected (R. Sardá, S. Pinedo and D. Martin, personal observations), as has been repeatedly observed during the long-term monitoring carried out in the Punta del Tordera (Sardá et al. 1995, 1999, 2000; Pinedo et al. 1996). Other large-sized species of *Mesochaetopterus* also build very long tubes, but they are either L-shaped, as in *M. taylori* (Sendall et al., 1995), or J-shaped, as is the case of *M. xerecus* (V. Radashevsky, personal communication; Petersen & Fanta, 1969). Moreover, they rarely extend into the sediment deeper than 30 cm or 1 m, respectively. In addition, they live in tidal environments so that collecting specimens at low tide is an easy task, in comparison to *M. rogeri* sp. nov. In fact, all attempts to collect entire specimens failed, and only the last one involving a large research vessel succeeded in obtaining the longest fragment (i.e. the holotype), which included a few segments of the C region. A similar situation was reported for *M. ricketsi*, whose the tubes extended vertically into the sediments for about 2 m, so that the entire tube was never collected and the orientation and structure of the lower end still remains unknown (MacGinitie and MacGinitie, 1949; Sendall et al., 1995).

Taking this into account, and the fact that *Mesochaetopterus rogeri* sp. nov. seems to be perfectly adapted to the peculiar characteristics of its seasonally unstable sandy habitat, it seems more appropriate to argue that this species is more likely to be a native Mediterranean one that has been overlooked by scientists up to now.

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Table 1. Accession numbers for the newly sampled and sequenced representative species of the family Chaetopteridae. N: number of individuals sequenced.

Species	Collection Locality	N	18S EMBL	COI EMBL
Chaetopterus variopedatus (Renier, 1804)	Naples (Italy)	2	AJ966758	AM503094
	Banyuls-sur-Mer (France)	2	AJ966759	AM503095
	Norwich, Norfolk (UK)	2	U67324	AM503096
Spiochaetopterus solitarius (Rioja, 1917)	Port Vendres (France)	2	AJ966760	nda
Mesochaetopterus xerecus Petersen and Fanta, 1969	Ilha do Mel (Brazil)	3	AJ966763	AM503097
Mesochaetopterus rogeri sp. nov.	Blanes (Spain)	3	AJ966762	AM503098
Phyllochaetopterus socialis Claparède, 1870	Banyuls-sur-Mer (France)	2	AJ966761	DQ209247

Table 2. Matrix of pairwise genetic divergence within and between the species representative of all Chaetopteridae genera. *M: Mesochaetopterus; C: Chaetopterus; S: Spiochaetopterus; P: Phyllochaetopterus;* Atl: Atlantic; Med N: Mediterranean (Naples); Med B: Mediterranean (Banyuls); nda: No data available. Above and below diagonal values for COI and 18S gene values, respectively.

	M. rogeri sp. nov.	*M. xerecus*	*M. taylori*	*C. variopedatus* (Med B)	*C. variopedatus* (Med N)
M. rogeri sp. nov.	-	1.21	22.70	24.26	24.61
M. xerecus	0.31	-	22.18	23.92	24.26
M. taylori	1.56	1.24	-	22.01	22.36
C. variopedatus (BAN)	1.56	1.40	2.18	-	0.35
C. variopedatus (NAP)	1.71	1.56	2.33	0.16	-
C. variopedatus (ATL)	1.40	1.56	2.33	**0.78**	**0.93**
C. pugaporcinus	1.71	1.56	2.33	0.47	0.62
P. socialis	3.27	3.27	3.42	3.42	3.58
S. solitarius	2.80	2.80	3.27	3.11	3.27
Prionospio sp.	4.82	4.67	4.67	4.98	5.13

	C. variopedatus (Atl)	*C. pugaporcinus*	*P. socialis*	*S. solitarius*	*Prionospio sp.*
M. rogeri sp. nov.	22.70	23.92	27.29	nda	29.64
M. xerecus	22.36	23.57	26.92	nda	28.77
M. taylori	21.14	20.80	26.02	nda	29.29
C. variopedatus (BAN)	**19.93**	21.66	27.11	nda	28.94
C. variopedatus (NAP)	**19.93**	21.66	27.11	nda	29.29
C. variopedatus (ATL)	-	19.58	25.49	nda	30.68
C. pugaporcinus	0.93	-	26.39	nda	30.33
P. socialis	3.89	3.27	-	nda	29.01
S. solitarius	3.27	2.96	1.24	-	nda
Prionospio sp.	5.13	5.13	5.91	5.44	-

Table 3. Comparison of the main characteristics of the currently known large-sized *Mesochaetopterus* species. (*) Data emended on the basis of the studied material.

CHARACTERS	M. rogeri sp. nov.	M. taylori (Potts)	M. xerecus Petersen and Fanta*	M. ricketsi Berkeley and Berkeley
Length of living worm (cm)	8.3 (for the A, B, and 10 segments of C region)	60	60	35
Width of worm (cm)	0.8 – 1	1	0.8 - 1.5	1
Tentacle colour	dark pigmented rings and bands, and red longitudinal bands	uniform light yellow	dark pigmented rings	?
Relative length of tentacles : A region	3-5 : 1	1 : 1	2 : 1	2 : 1
Eyes	absent	absent	present	?
Parapodia of the A region	9-12 (9)	9-11 (9)	8-14 (11)	10
Chaetae of the A4 segment	13-19 (18)	10	7-17 (11)	?
Shape of the modified A4 chaetae	short, strong with finely serrated tip	short, strong with truncated tip	short, strong with finely serrated tip	Fimbriated, with oblique edge
Types of chaetae in the a region (except A4)	3	3?	4	?
Parapodia of the B region	3	3	4 (up to 7)	21
Accessory feeding organs	B2	B2-B3	B2-B3 (up to C4)	B21
Glandular lateral epithelium	B1-C?	B1-B3	B1-B4	B region
Length of the largest uncini of the B region (µm)	98 - 111	115	106 - 134	?
Number of teeth on the uncini of the B region	8 (8-9)	9 (7-10)	7 (6-10)	8-10
Relative length of teeth on the uncini of the B region	2/5 of uncini width	1/3 of uncini width	1/2 of uncini width	?
Outline of uncini of the B region	roughly D-shaped, with slightly curved edges	roughly triangular, with markedly curved edges	roughly triangular, with straight edges	roughly D-shaped, with straight edges
Tube	Straight up to 2.5 m deep, sand covered, lower end unknown	L-shaped, parchment-like with sand granins embedded, strait section 10-20 cm deep	Vertical or slightly J-shaped,1 m, parchment-like with sand grains embedded, lower end blid and reounded	Straight up to 2 m deep, sand covered, lower end unknown
Type locality	Mediterranean	California	Brazil	California

Table 3. Cont.

| CHARACTERS	M. alipes Monro	M. japonicus Fijivwara	M. selangolus (Rullier)	M. mexicanus Kudenov
Length of living worm (cm)	80	25	25 (up to 60)	> 9.5
Width of worm (cm)	6	1	1	0.5
Tentacle colour	Opaque white, with traces of brown	Unpigmented	uniform pale brownish	?
Relative length of tentacles : A region	2 : 1	1 : 1	1.5 : 1	?
Eyes	absent	absent	absent	present
Parapodia of the A region	9	9	9-10	13
Chaetae of the A4 segment	?	8 - 13	8 - 15	?
Shape of the modified A4 chaetae	short, strong with truncated edge	Truncated, spatulate, with serrations	Truncated, spatulate, with serrations	Oblique, distally dentate
Types of chaetae in the a region (except A4)	?	?	?	2
Parapodia of the B region	3	2	2	4
Accessory feeding organs	B2	B2	B2	B1-B4
Glandular lateral epithelium	B1	B1 and anterior B2	B1 and anterior B2	?
Length of the largest uncini of the B region (µm)	?	?	72 - 85	140
Number of teeth on the uncini of the B region	8	9 - 10	7 - 8	8 - 9
Relative length of teeth on the uncini of the B region	?	?	1/2 of uncini width	1/2 of uncini width
Outline of uncini of the B region	?	?	roughly D-shaped, with slightly curved edges ans serrated basis	roughly triangular, with markedly curved edges
Tube	Parchment-like	Strait, parchment-like, with bint lower end	J-shaped, parchment-like with embedded sand and periodic annulated rings and perforations at the lower end	Parchment-like
Type locality	Panama	Japan	Malaysia	Mexico

FIGURES
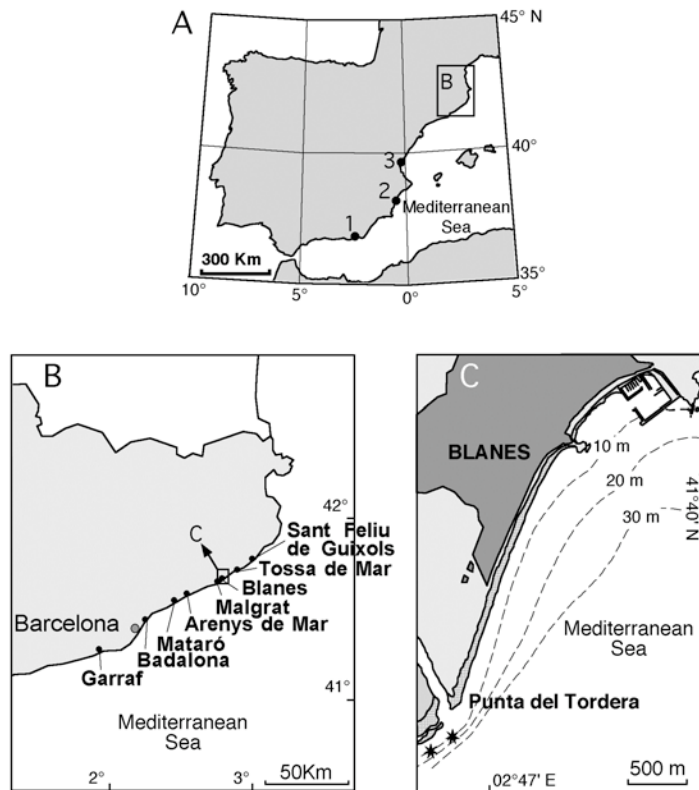
Figure 1. *Mesochaetopterus rogeri* **sp. nov.,** location of reports. A.- Iberian Peninsula. B.- Catalan coasts. C.-
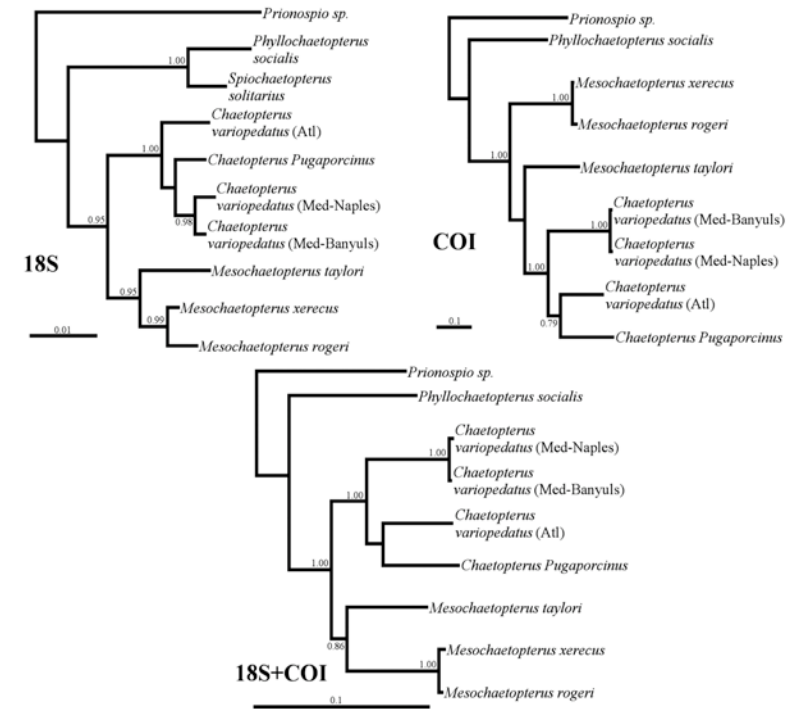Blanes litoral. ✸ = Seasonal monitoring at the Punta del Tordera.

Figure 2. Phylogenetic tree as inferred for the 18S rRNA and COI genes using three methods of analysis.
Numbers above the branches represent bootstrap and posterior probability node support values based on 1000
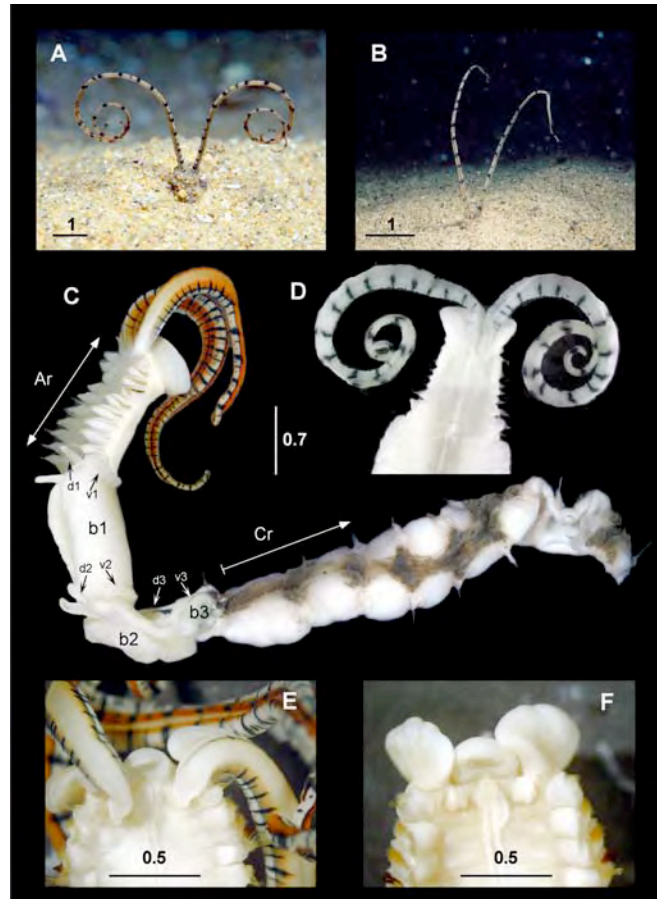replicates (MP/ML/BI). Med: Mediterranean; Atl: Atlantic.

Figure 3. *Mesochaetopterus rogeri* sp. nov. A, B.- Two typical positions of the protruded tentacles in living worms (underwater pictures by L. Dantart). C.- Regions A, B and C (preserved worm, morphotype with reddish bands). D.- Ventral view of region A (preserved worm, morphotype without reddish bands). E.- Detail of the anterior end (dorsal view). F.- Detail of the anterior end (dorsal view, tentacles removed). Ar: A region; b1 to b3: segments of B region; d1 – d3: dorsal rami of B region segments; v1 –v3: ventral rami of B region segments; Cr: C region. Scale bars are cm.
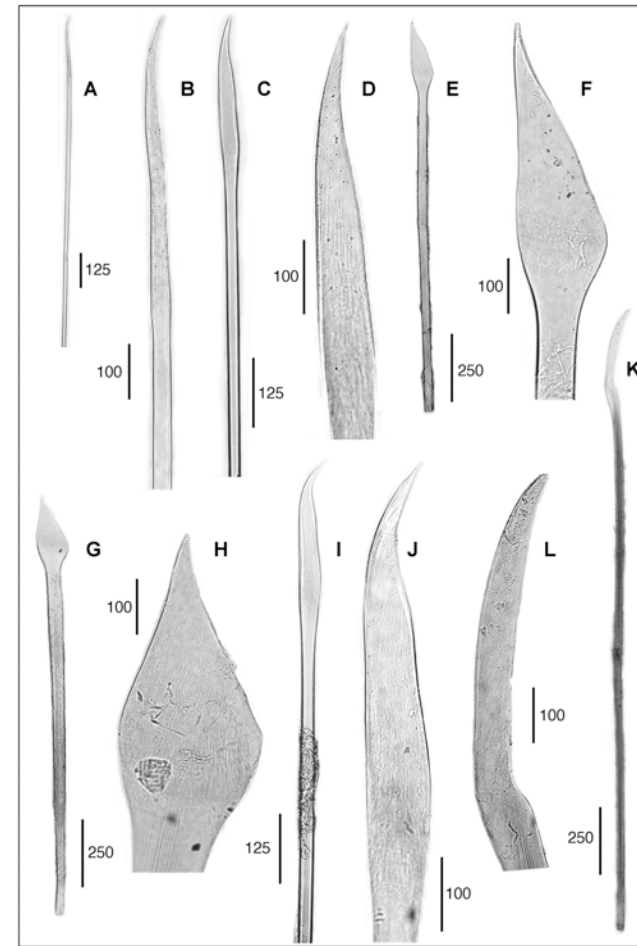
Figure 4. *Mesochaetopterus rogeri* sp. nov., chaetae A.- Dorsal-most capillary from A1. B.- Tip for the dorsal-most capillary from A1. C.- Dorsal fine-lanceolate from A1. D.- Tip of the dorsal fine-lanceolate from A1. E.- Ventral oar-like from A1. F.- Tip of the ventral oar-like from A1. G.- Ventral oar-like from A3. H.- Tip of the ventral oar-like from chaetiger A3. I.- Dorsal fine-lanceolate from A8. J.- Tip of the dorsal fine-lanceolate from A8. K.- Ventral sickle-like from A8. L.- Tip of the ventral sickle-like from A8. Scale bars are μm.
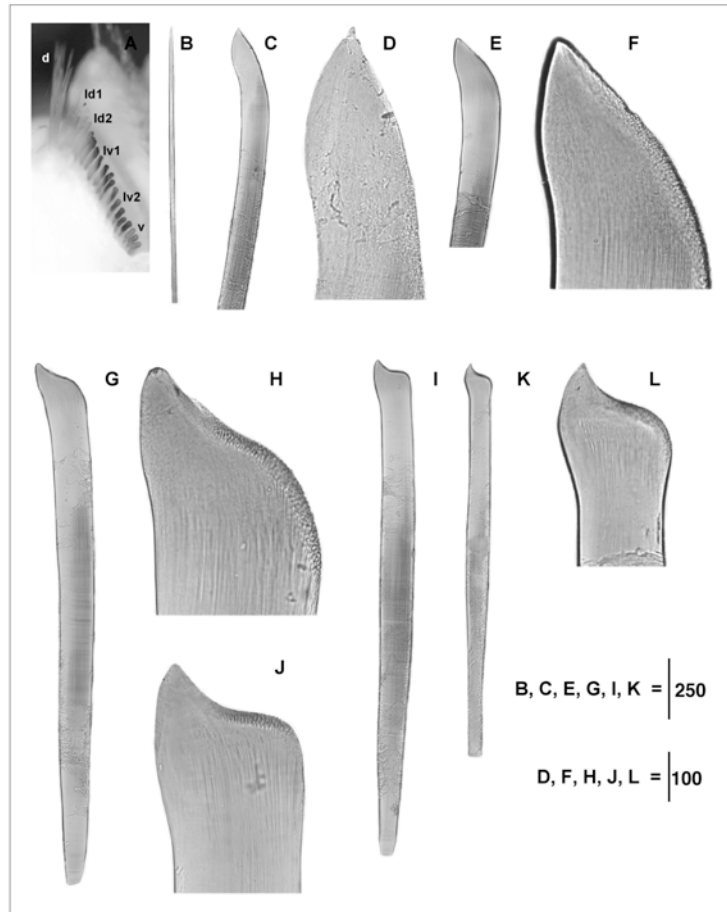
Figure 5. *Mesochaetopterus rogeri* **sp. nov**. A.- Chaetal arrangement of the modified A4 chaetiger. B-L.- Chaetae. B. Tip of the dorsal finely pointed capillary "d". C.- Dorso-lateral transparent knife-like "ld1". D.- Tip of the dorso-lateral transparent knife-like "ld1". E.- Dorso-lateral brownish knife-like "ld2". F.- Tip of the dorso-lateral brownish knife-like "ld2". G, I, K.- Whole view of the typical modified "lv1" (lateral), "lv2" (latero-ventral) and "v" (ventral-most). H, J, L.- Tip of G, I, K, respectively. Scale bars are μm.
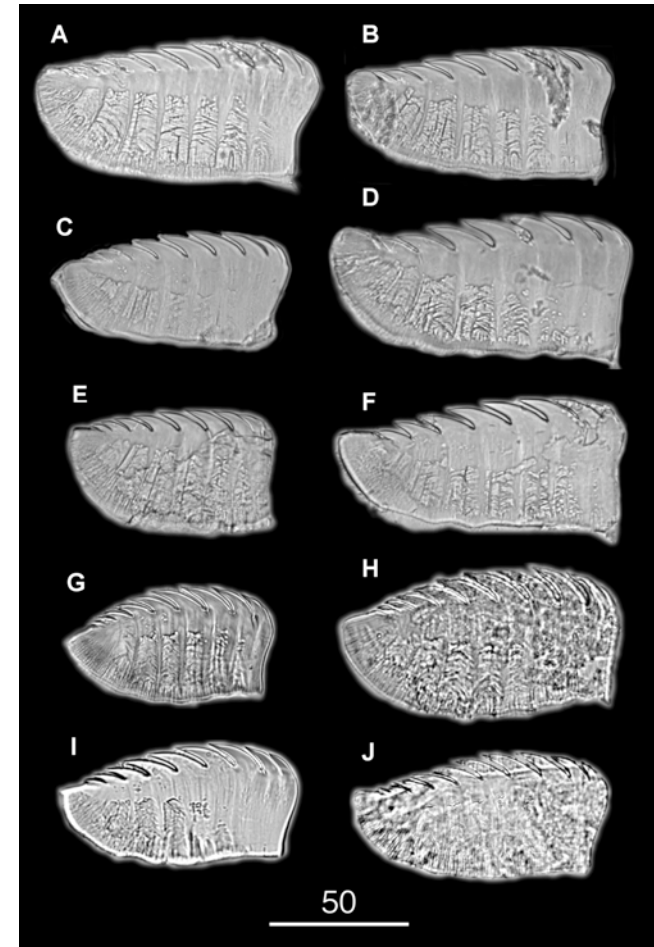
Figure 6. *Mesochaetopterus rogeri* **sp. nov.,** uncini. A.- Dorsal-most from neuropodia B1. B.- Ventral-most from neuropodia B1. C.- From notopodia B2. D.- From neuropodia B2. E.- From notopodia B3. F.- From neuropodia B3. G.- From the anterior region of notopodia C2. H.- From the posterior region of notopodia C2. I.- From the anterior region of neuropodia C2. J.- From the posterior region of neuropodia C2. Scale Bars are μm.
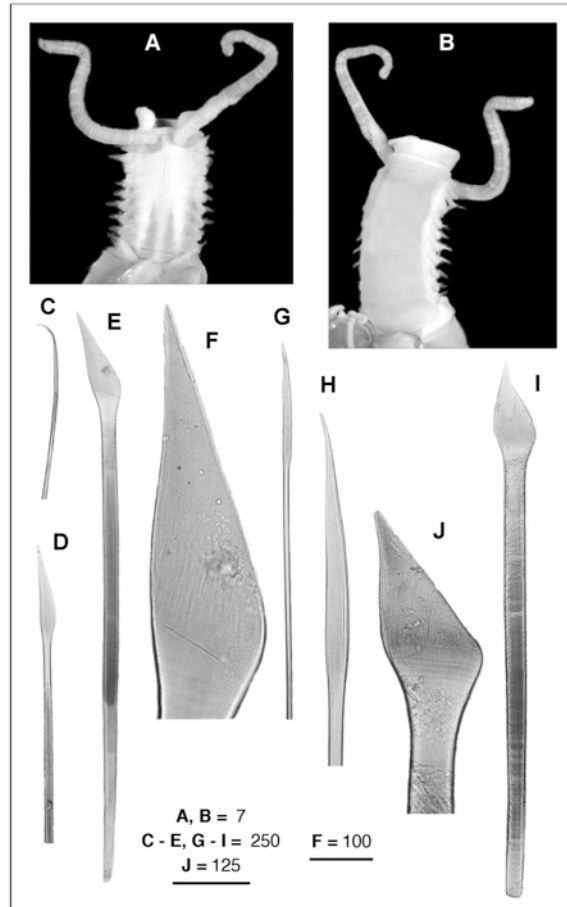
Figure 7. *Mesochaetopterus xerecus.* A.- Dorsal view of region A (preserved). B.- Ventral view of region A (preserved). C - J.- Chaetae. C.- Dorsal-most capillary from A1. D.- Dorso-lateral fine-lanceolate from A1. E.- Ventral oar-like A1. F.- Tip of the ventral oar-like from A1. G.- Dorsal-most fine-lanceolate from A3. H.- Tip for the dorsal-most fine-lanceolate from A3. I.- Ventral oar-like A3. J.- Tip for the ventral oar-like from A3. Scale bars are cm in A, B and μm in C - J.
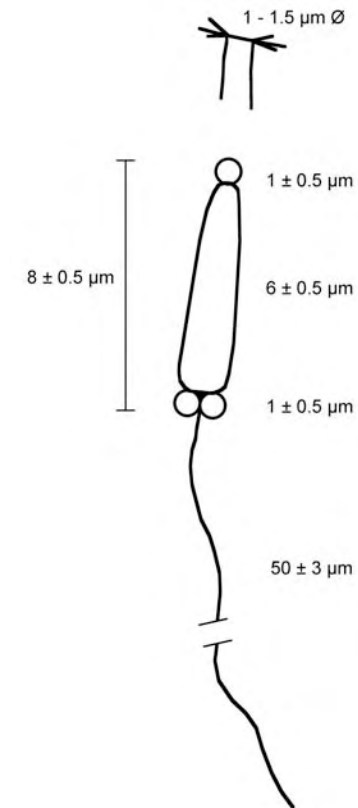

Figure 8. *Mesochaetopterus xerecus,* scheme of a mature sperm.
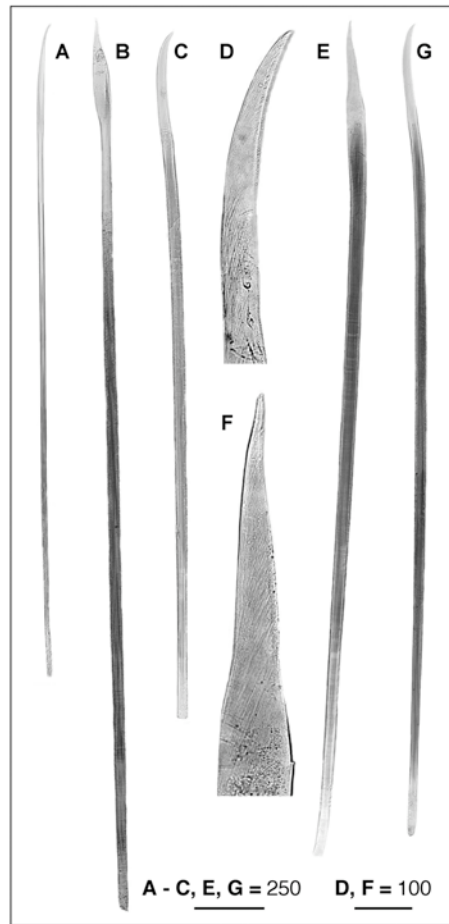
Figure 9. *Mesochaetopterus xerecus,* chaetae. A.- Dorsal-most capillary from A8. B.- Dorso-lateral fine-lanceolate from A8. C.- Latero-ventral sickle-like from A8. D.- Tip of the latero-ventral sickle-like from A8. E.- Ventral-most lancet-like from A8. F.- Tip of the ventral-most lancet-like from A8. G.- Ventral-most sickle-like from A10. Scale bars are μm.
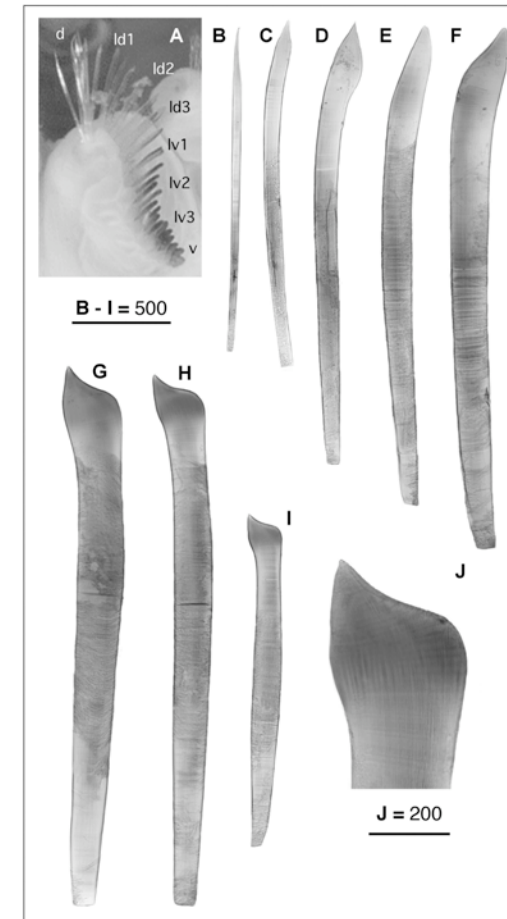

Figure 10. *Mesochaetopterus xerecus.* A.- Chaetal arrangement of the modified A4 chaetiger. B - J.- Chaetae. B. Tip of the dorsal finely pointed lancet-like (d). C.- Dorso-lateral transparent knife-like (ld1). D.- Dorso-lateral transparent knife-like (ld2). E.- Dorso-lateral brownish knife-like (ld3). F.- Latero-ventral brownish knife-like (ld3). G, H, I.- Typical modified: (lv2) lateral, (lv3) latero-ventral and (v) ventral-most. J.- Tip for the ventral-most modified (v). Scale bars are μm.
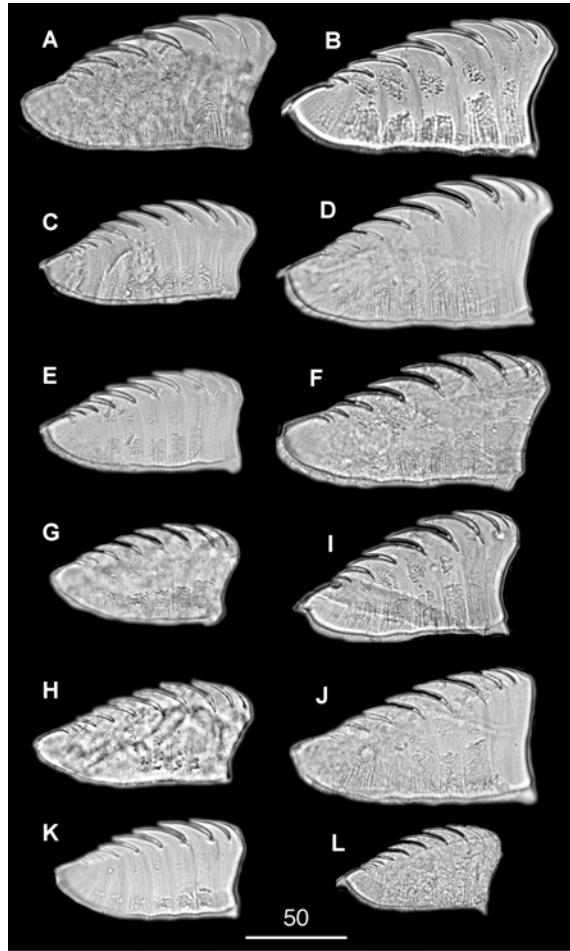
Figure 11.- *Mesochaetopterus xerecus*, uncini. A.- Dorsal-most from neuropodia B1. B.- Ventral-most from neuropodia B1. C.- From notopodia B2. D.- From neuropodia B2. E.- From notopodia B3. F.- From neuropodia B3. G.- From the anterior region of notopodia C2. H.- From the posterior region of notopodia C2. I.- From the anterior region of neuropodia C2. J.- From the posterior region of neuropodia C2. K.- From notopodia C30. L.- From neuropodia C30. Scale Bars are μm.
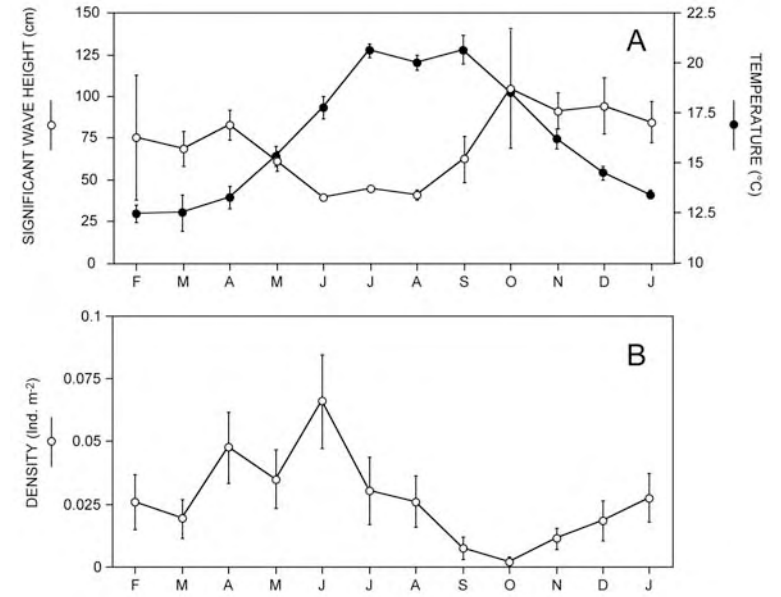

Figure 12. *Mesochaetopterus rogeri* **sp. nov**., seasonal pattern at Punta del Tordera. A.- Water temperature at 20 m deep and significant wave height. B.- Population density.