VIE ET MILIEU - LIFE AND ENVIRONMENT, 2020, 70 (1): 33-45

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *SCOLELEPIS NEGLECTA* (POLYCHAETA: SPIONIDAE)

VICTOR SURUGIU [1*], *ANDREI ȘTEFAN* [2], *OANA PAULA POPA* [2]

[1] *"Alexandru Ioan Cuza" University of Iaşi, Faculty of Biology, B-dul Carol I, No. 20A, 700507 Iaşi, Romania*
[2] *"Grigore Antipa" National Museum of Natural History, Şos. Kiseleff, No. 1, 011341 Bucharest 1, Romania*
** Corresponding author: vsurugiu@uaic.ro*

COI GENE SEQUENCES
GENETIC DISTANCES
MORPHOLOGY
POLYCHAETES
SCOLELEPIS

ABSTRACT. – *Scolelepis neglecta* Surugiu, 2016 is a polychaete species that was described from the Cantabrian coast of Asturias (northern Spain) based only on anterior fragments from museum collections. Recently collected live material of *S. neglecta* from the intertidal muddy sand flats of the Villaviciosa estuary (Asturias, Bay of Biscay, northern Spain), has enabled us to report some new, previously unknown morphological characters, such as the shape of the pygidium, the shape, number and distribution of the notopodial hooded hooks, and to assess the validity of *S. neglecta* by the means of molecular analyses. In order to reveal the taxonomic relationships between *S. squamata* from the Black Sea, *S. neglecta* from northern Spain and other species of *Scolelepis*, we have compared sequences of the mitochondrial cytochrome oxidase subunit 1 (COI) gene obtained in the present study with those available in GenBank or in BOLD Systems databases. Molecular phylogeny reconstruction revealed a clear separation of *S. neglecta* as a stand-alone clade. The molecular analysis also indicated that in the Black Sea exist two genetically distinct species of *Scolelepis*, both previously referred to as *S. squamata*, but none of them identical to *S. squamata* whose sequences exist in GenBank. These species are herein designated as *Scolelepis* cf. *cirratulus* and *Scolelepis* cf. *mesnili*, respectively, until new material for both species, especially from the type localities, will be available for molecular studies. The lowest genetic distance values were found between *S. neglecta* and *Scolelepis* cf. *cirratulus* from the Black Sea and the highest ones were found between *S. neglecta* and *S. goodbodyi* from Brazil and *S. squamata* from the west coast of North America. Apart from providing COI sequences for *S. neglecta* and for two species of *Scolelepis* from the Black Sea, this is the first study which attempts to provide inferences about the phylogenetic relationships between species belonging to the genus *Scolelepis*.

INTRODUCTION

The genus *Scolelepis* represents one of the largest and most difficult genera of the family Spionidae. It groups spionids that share the presence of a pointed prostomium, branchiae starting from chaetiger 2 and continuing to near end of body, the lack of ciliated grooves on palps, and a cushion-like pygidium without cirri (Meißner & Götting 2015). The members of *Scolelepis* are unique among Spionidae in having ungrooved prehensile palps (Dauer 1983). The eggs have thick reticulated shells and membrane vesicles (Joyner 1962, Meißner & Götting 2015, Surugiu 2016). The diagnostic characters that have proven to be relevant in distinguishing species of *Scolelepis* are those related to the shape of the prostomium, the presence and size of lateral wings on the peristomium, the shape of basal palpal sheaths, the presence or absence of notochaetae on chaetiger 1, the shape of postchaetal lamellae of chaetiger 1, the degree of branchial fusion, the shape and the distribution of hooded hooks, the nature of notopodial capillaries, the ratio of the length of the hood to the main fang, the shape of the anterior and posterior neuropodial postchaetal lamellae, the shape of the posterior notopodial postchaetal lamellae, and the shape of the pygidium (Maciolek 1987, Delgado-Blas 2006, Meißner & Götting 2015, Surugiu 2016). Scanning Electron Microscopy of the palp ciliation pattern also proved to be very useful for the identification of *Scolelepis* species (Williams 2007, Surugiu 2016). For unequivocal identification of many species belonging to the genus *Scolelepis* an integrative taxonomic approach should be used.

At present, *ca*. 86 species of *Scolelepis* are recognized (Surugiu 2016), but only for few of them there are gene sequences available in GenBank. Most of the gene sequences of *Scolelepis* species existing in GenBank come from material from outside European waters, especially based on COI gene fragments. Apart from a parsimony analysis of morphological characters carried out by Sigvaldadóttir *et al*. (1997) between the genera of the family Spionidae, which also included *Scolelepis squamata* (Abildgaard, *in* O.F. Müller, 1806) and *Scolelepis tridentata* (Southern, 1914), there are no other studies to estimate the phylogenetic relationships between the species of the genus *Scolelepis*.

Only three species of *Scolelepis* have previously been reported in the Black Sea: *Scolelepis squamata* (as *Nerine cirratulus*), *Scolelepis tridentata* (as *Pseudomalacoceros tridentata*) and *Scolelepis cantabra* (Rioja 1918) (as

Pseudomalacoceros cantabra) (Marinov 1977, Kisseleva 2004, Kurt Şahin & Çinar 2012).

Among the European species, *S. squamata* is one of the most problematic due to the substantial degree of variation of its morphological characters. By comparing specimens of *Scolelepis cirratulus* (Delle Chiaje, 1829) from the Black and the Mediterranean seas and syntypes of *S. mesnili* (Bellan & Lagardère, 1971) from the Atlantic coast of France with *S. squamata* from the North Sea and the Atlantic coast of Spain, Surugiu (2016) came to the conclusion that most of the characters used to distinguish these species were size-dependent and that they all belong to the same species *S. squamata*. Also, as a result of the re-assessment of *S. squamata*, a new species *S. neglecta* Surugiu, 2016 was described from the Cantabrian coast of Spain which used to be confused with *S. squamata* and *S. mesnili*. The original description of *S. neglecta* was based on 53 incomplete specimens hosted in three different museum collections.

During a field trip to the Villaviciosa estuary (northern Spain, ~185 km away from the type locality of *S. neglecta*) in April 2015, one of the authors had the occasion to collect some specimens of *S. neglecta*, out of which one was complete. Repeated sampling, which took place in September 2016 in the same location, yielded additional material for observations on live specimens and for molecular analyses.

Therefore, the objectives of this study are: (i) to describe and to illustrate previously unreported morphological features for *S. neglecta*, (ii) to provide molecular data to support *S. neglecta*, (iii) to check the identity of polychaetes identified as *Scolelepis squamata* from the Black Sea, and (iv) to assess for the first time the phylogenetic relationship between different species of *Scolelepis* based on partial mitochondrial COI gene sequences.

MATERIAL AND METHODS

Sample collection and processing: Collection of material took place in June 2014, April 2015, May 2016 and September 2016 from intertidal and shallow subtidal sandy areas at low tide in different localities on the coasts of Spain (Galicia and Asturias) and Romania (Fig. 1; Table I).

Samples of sediment were collected at the low water mark with the aid of a shovel, to a depth of 10-20 cm. Collected sediments were carefully washed in the field on a 500 µm mesh sieve and *Scolelepis* worms retained in the residue were hand-picked from the mesh with live insect handling forceps. Immediately, the worms were relaxed in 7.5 % magnesium chloride solution in tap water for 10-20 minutes. The material was transported to the laboratory and examined alive under light microscopes. For molecular analyses specimens were fixed in 96 % ethanol. Voucher specimens were fixed with a 10 % formaldehyde solution made with filtered sea water, rinsed in fresh water and preserved in 70 % ethanol.

All specimens are deposited in the polychaete collection of the "Grigore Antipa" National Museum of Natural History, Bucharest, Romania (MGAB). In addition to the freshly collected material, specimens deposited in the Biological Research Collection of Marine Invertebrates, Departamento de Biologia, Universidade de Aveiro, Portugal (DBUA) and in the Senckenberg Museum, Frankfurt, Germany (SMF) were examined.

Morphological analysis: For light microscopy (LM), specimens were rinsed in tap water and examined under a Nikon SMZ800 stereomicroscope. Maximum length

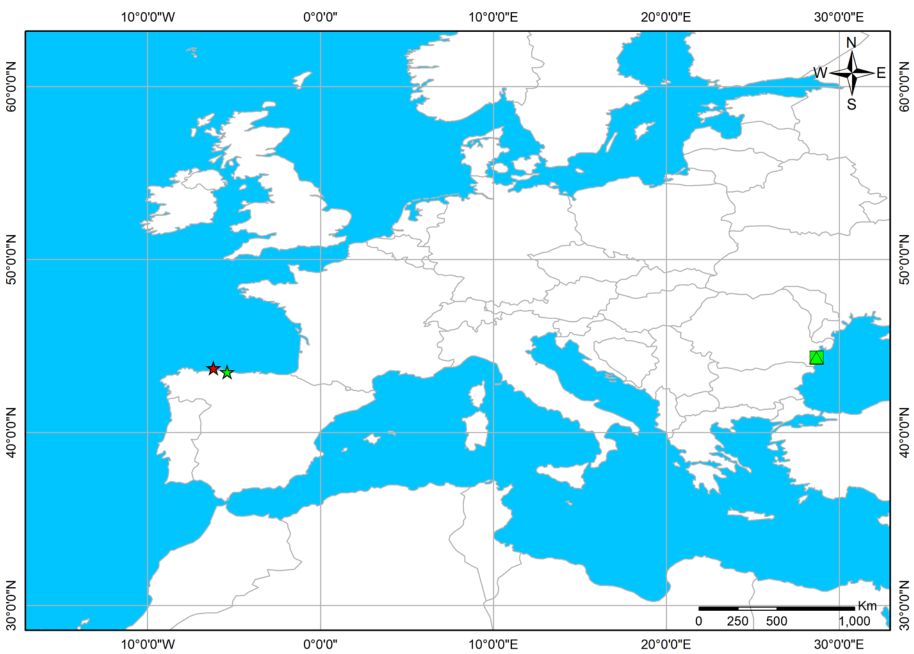

Fig 1. – Map showing the type locality of *Scolelepis neglecta* Surugiu, 2016 from the Cantabrian Sea, Spain (red star) and *Scolelepis* spp. sampling sites for genetic studies (green figures, see details in Table I). Green square, *Scolelepis* cf. *cirratulus* from the Black Sea, Romania; green triangle, *Scolelepis* cf. *mesnili* from the Black Sea, Romania; green star, *S. neglecta* from the Villaviciosa estuary, Spain.

Table I. – Taxa, sampling localities, collection dates and GenBank accession numbers of COI sequences analyzed in the present study. Sequences obtained in the present study are given in bold.

No	Species	Collection locality	Coordinates	Date of collection	Voucher	GenBank accession numbers
1.	*Scolelepis* cf. *cirratulus*	Cap Midia, Black Sea, **Romania**	44.342444° N, 28.691000° E	29 Jun. 2014	Sc1	**MN378522**
2.	*Scolelepis* cf. *cirratulus*	Cap Midia, Black Sea, **Romania**	44.342444° N, 28.691000° E	29 Jun. 2014	Sc2	**MN378523**
3.	*Scolelepis* cf. *cirratulus*	Cap Midia, Black Sea, **Romania**	44.342444° N, 28.691000° E	29 Jun. 2014	Sc3	**MN378524**
4.	*Scolelepis* cf. *cirratulus*	Cap Midia, Black Sea, **Romania**	44.342444° N, 28.691000° E	29 Jun. 2014	Sc4	**MN378525**
5.	*Scolelepis* cf. *mesnili*	Cap Midia, Black Sea, **Romania**	44.342444° N, 28.691000° E	29 Jun. 2014	Sm1	**MN378526**
6.	*Scolelepis* cf. *mesnili*	Cap Midia, Black Sea, **Romania**	44.342444° N, 28.691000° E	29 Jun. 2014	Sm2	**MN378527**
7.	*Scolelepis* cf. *mesnili*	Cap Midia, Black Sea, **Romania**	44.342444° N, 28.691000° E	29 Jun. 2014	Sm3	**MN378528**
8.	*Scolelepis* cf. *mesnili*	Cap Midia, Black Sea, **Romania**	44.342444° N, 28.691000° E	29 Jun. 2014	Sm4	**MN378529**
9.	*Scolelepis neglecta* Surugiu, 2016	La Ermita, Villaviciosa estuary, Asturias, Bay of Biscay, **Spain**	43.520454° N, 5.398028° W	20 Sep. 2016	SnE1	**MN378530**
10.	*Scolelepis neglecta* Surugiu, 2016	El Puntal, Villaviciosa estuary, Asturias, Bay of Biscay, **Spain**	43.524520° N, 5.391519° W	20 Sep. 2016	SnP1	**MN378531**
11.	*Scolelepis neglecta* Surugiu, 2016	El Puntal, Villaviciosa estuary, Asturias, Bay of Biscay, **Spain**	43.524520° N, 5.391519° W	20 Sep. 2016	SnP3	**MN378532**
12.	*Scolelepis neglecta* Surugiu, 2016	El Puntal, Villaviciosa estuary, Asturias, Bay of Biscay, **Spain**	43.524520° N, 5.391519° W	20 Sep. 2016	SnP4	**MN378533**
13.	*Scolelepis neglecta* Surugiu, 2016	El Puntal, Villaviciosa estuary, Asturias, Bay of Biscay, **Spain**	43.524520° N, 5.391519° W	20 Sep. 2016	SnP5	**MN378534**
14.	*Scolelepis neglecta* Surugiu, 2016	El Puntal, Villaviciosa estuary, Asturias, Bay of Biscay, **Spain**	43.524520° N, 5.391519° W	20 Sep. 2016	SnP6	**MN378535**
15.	*Scolelepis neglecta* Surugiu, 2016	El Puntal, Villaviciosa estuary, Asturias, Bay of Biscay, **Spain**	43.524520° N, 5.391519° W	20 Sep. 2016	SnP7	**MN378536**
16.	*Scolelepis squamata* (Abildgaard, *in* O.F. Müller, 1806)	Bamfield Inlet, Bamfield, British Columbia, **Canada**	48.820° N, 125.150° W	03 Jun. 2008	BAMPOL0017	HM473679
17.	*Scolelepis squamata* (Abildgaard, *in* O.F. Müller, 1806)	Dixon Island, Bamfield, British Columbia, **Canada**	48.850° N, 125.120° W	04 Jun. 2008	BAMPOL0179	HM473680
18.	*Scolelepis squamata* (Abildgaard, *in* O.F. Müller, 1806)	Great Nicobar Island, **India**	6.9327° N, 93.9316° E	Mar. 2012	CASMB B-Quarry 1	KC208487
19.	*Scolelepis squamata* (Abildgaard, *in* O.F. Müller, 1806)	British Columbia, **Canada**	48.858° N, 125.159° W	28 May 2010	BP2010-136	HQ932541

Table I. – Continued.

No	Species	Collection locality	Coordinates	Date of collection	Voucher	GenBank accession numbers
20.	*Scolelepis squamata* (Abildgaard, *in* O.F. Müller, 1806)	Katchemak Bay, Cook Inlet, Alaska, **USA**	59.493° N, 151.646° W	15 May 2011	11BIOAK-1640	MF120984
21.	*Scolelepis squamata* (Abildgaard, *in* O.F. Müller, 1806)	Katchemak Bay, Cook Inlet, Alaska, **USA**	59.493°N, 151.646° W	15 May 2011	11BIOAK-1651	MF121271
22.	*Scolelepis squamata* (Abildgaard, *in* O.F. Müller, 1806)	Kasitsna Bay Dock, Cook Inlet, Alaska, **USA**	59.468° N, 151.553° W	19 May 2011	11BIOAK-1610	MF121430
23.	*Scolelepis squamata* (Abildgaard, *in* O.F. Müller, 1806)	Kasitsna Bay Dock, Cook Inlet, Alaska, **USA**	59.468° N, 151.553° W	19 May 2011	11BIOAK-1609	MF121675
24.	*Scolelepis foliosa* (Audouin & Milne Edwards, 1833)	Cabedelo, Minho, Viana do Castelo, Lima estuary, **Portugal**	41.682° N, 8.834° W	21 May 2012	LPCSED1-003	KF369182
25.	*Scolelepis foliosa* (Audouin & Milne Edwards, 1833)	Cabedelo, Minho, Viana do Castelo, Lima estuary, **Portugal**	41.683° N, 8.834° W	01 Apr. 2014	LCAS1-03	KR916933
26.	*Scolelepis foliosa* (Audouin & Milne Edwards, 1833)	Cabedelo, Minho, Viana do Castelo, Lima estuary, **Portugal**	41.682° N, 8.834° W	21 May 2012	LPCSED4	KR916934
27.	*Scolelepis foliosa* (Audouin & Milne Edwards, 1833)	Cabedelo, Minho, Viana do Castelo, Lima estuary, **Portugal**	41.683° N, 8.834° W	01 Apr. 2014	LCAS1-02	KR916935
28.	*Scolelepis foliosa* (Audouin & Milne Edwards, 1833)	Cabedelo, Minho, Viana do Castelo, Lima estuary, **Portugal**	41.682° N, 8.834° W	21 May 2012	LPCSED1-002	KR916936
29.	*Scolelepis foliosa* (Audouin & Milne Edwards, 1833)	Cabedelo, Minho, Viana do Castelo, Lima estuary, **Portugal**	41.683° N, 8.834° W	01 Apr. 2014	LCAS1-01	KR916937
30.	*Scolelepis chilensis* (Hartmann-Schröder, 1962)	Campos Basin, **Brazil**	22.760° S, 41.880° W	15 Jan. 2012	389	KU900481
31.	*Scolelepis goodbodyi* (Jones, 1962)	Campos Basin, **Brazil**	22.96° S, 42.01° W	15 Jan. 2013	452	KU900483
32.	*Scolelepis acuta* (Treadwell, 1914)	Campos Basin, **Brazil**	22.760° S, 41.880° W	15 Jan. 2012	468	KU900484
33.	*Scolelepis acuta* (Treadwell, 1914)	Scripps Coastal Reserve Beach, San Diego, California, **USA**	32.8664° N, 117.254° W	11 Apr. 2011	Su13BW42	KR911911
34.	*Scolelepis acuta* (Treadwell, 1914)	Scripps Coastal Reserve Beach, San Diego, California, **USA**	32.8664° N, 117.254° W	08 Jan. 2013	Su13BW44	KR911912
35.	*Scolelepis acuta* (Treadwell, 1914)	Scripps Coastal Reserve Beach, San Diego, California, **USA**	32.8664° N, 117.254° W	20 Aug. 2013	Su13BW53	KR914681
36.	*Scolelepis acuta* (Treadwell, 1914)	Scripps Coastal Reserve Beach, San Diego, California, **USA**	32.8664° N, 117.254° W	21 Aug. 2013	Su13BW40	KR914682
37.	*Scolelepis acuta* (Treadwell, 1914)	Scripps Coastal Reserve Beach, San Diego, California, **USA**	32.8664° N, 117.254° W	08 Jan. 2013	Su13BW45	KR914683
38.	*Scolelepis acuta* (Treadwell, 1914)	Scripps Coastal Reserve Beach, San Diego, California, **USA**	32.8664° N, 117.254° W	20 Aug. 2013	Su13BW49	KR914686

Table I. – Continued.

No	Species	Collection locality	Coordinates	Date of collection	Voucher	GenBank accession numbers
39.	*Scolelepis acuta* (Treadwell, 1914)	Scripps Coastal Reserve Beach, San Diego, California, **USA**	32.8664° N, 117.254° W	20 Aug. 2013	Su13BW50	KR914688
40.	*Scolelepis acuta* (Treadwell, 1914)	Scripps Coastal Reserve Beach, San Diego, California, **USA**	32.8664° N, 117.254° W	08 Jan. 2013	Su13BW46	KR914689
41.	*Scolelepis acuta* (Treadwell, 1914)	Scripps Coastal Reserve Beach, San Diego, California, **USA**	32.8664° N, 117.254° W	08 Jan. 2013	Su13BW43	KR914690
42.	*Scolelepis daphoinos* Zhou, Ji & Li, 2009	Yangkou sandy beach, Qingdao, **China**	36.23° N, 120.67° E	30 Aug. 2008	OUC POLYN6 0804	GU362687
43.	*Scolelepis eltaninae* Blake, 1983	Ross Sea slope, **Antarctica**	72.0819° S, 173.0488° E	24 feb. 2008	Polychaeta2C2_St122	KF713383
44.	*Scolelepis kudenovi* Hartmann-Schröder, 1981	Casuarina Beach, Lizard Island, Great Barrier Reef, Queensland, **Australia**	14.67944° S, 145.44694° E	21 Aug. 2013	LizSpio 75	KP636516
45.	*Spio blakei* Maciolek, 1990	Off Casuarina Beach, Lizard Island, Queensland, **Australia**	14.67944° S, 145.44694° E	17 Aug. 2013	KJ271	KP636499
46.	*Spio blakei* Maciolek, 1990	Off Casuarina Beach, Lizard Island, Queensland, **Australia**	14.67944° S, 145.44694° E	17 Aug. 2013	LizSpio 74 MI	KP636500
47.	*Spio blakei* Maciolek, 1990	Off Casuarina Beach, Lizard Island, Queensland, **Australia**	14.67944° S, 145.44694° E	17 Aug. 2013	LizSpio 70	KP636501
48.	*Malacoceros fuliginosus* (Claparède, 1868)	Helgoland, North Sea, **Germany**	?	26 Sep. 2005	Mf_5	EF432012
49.	*Malacoceros fuliginosus* (Claparède, 1868)	Helgoland, North Sea, **Germany**	?	26 Sep. 2005	Mf_4	EF432013
50.	*Malacoceros fuliginosus* (Claparède, 1868)	Helgoland, North Sea, **Germany**	?	26 Sep. 2005	Mf_3	EF432014
51.	*Malacoceros fuliginosus* (Claparède, 1868)	Helgoland, North Sea, **Germany**	?	26 Sep. 2005	Mf_1	EF432015
52.	*Malacoceros fuliginosus* (Claparède, 1868)	Helgoland, North Sea, **Germany**	?	26 Sep. 2005	Mf_2	EF432016
53.	*Malacoceros indicus* (Fauvel, 1928)	Mangrove Beach, Lizard Island, Queensland, **Australia**	14.67972° S, 145.4636° E	22 Aug. 2013	KJ269	KP636508
54.	*Malacoceros indicus* (Fauvel, 1928)	Off Casuarina Beach, Lizard Island, Queensland, **Australia**	14.67944° S, 145.44694° E	17 Aug. 2013	KJ270	KP636509
55.	*Capitella neoaciculata* Silva & Seixas, 2017	Maracaípe Mangrove, Pernambuco, **Brazil**	8.31° S, 35.12° W	20 May 2015	PE27	KX121857

(excluding palps) and width (as the distance between the distalmost structures on widest chaetiger seen on anterior end in dorsal view, excluding chaetae) of worms were measured using a calibrated eyepiece graticule mounted on a stereomicroscope to the nearest 10 µm. Photographs were taken from fixed specimens in the laboratory at the Faculty of Biology, University of Iași using a Leica DFC450C camera coupled to a Leica M205A stereomicroscope or to a Leica DM750 compound microscope. Images of multiple layers of worms were stacked using Zerene Stacker v.1.04 software. Before photography, specimens were slightly stained with an aqueous solution of methylene blue (MBW).

For scanning electron microscopy (SEM), a formalin-fixed specimen was dehydrated through an ethanol series of increasing concentration and then acetone, critical-point dried in carbon dioxide, mounted on aluminium stub, coated with gold and examined with a Vega Tescan SBH scanning electron microscope at the Faculty of Biology, University of Iaşi.

Final plates were prepared using Adobe® Photoshop® CC v.14.0 software. Complete information about samples is given below, in the 'Material examined' section along with the description of the species. Complete specimens are indicated by "cs", while anterior, middle and posterior fragments are designated as "af", "mf" and "pf", respectively.

Molecular analysis: Seven specimens of *S. neglecta* were collected from 'La Ermita' and 'El Puntal' intertidal muddy sand flats (Villaviciosa estuary in the northern Spain) and kept in 96 % ethanol (Table I). We also analyzed eight putative specimens of *S. squamata* that were collected from the Romanian coast of the Black Sea (Table I). For the molecular characterization of available *Scolelepis* species we used the COI gene fragment, amplified by universal primers HCO2198 and LCO1490 (Folmer *et al.* 1994). The PCR reaction was performed in a total volume of 50 µl containing DNA template, 1X Green GoTaq® Flexi Buffer, 2.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.5 µm of each primer and 1.5 units of GoTaq® DNA polymerase (Promega, Madison, USA). The PCR products were isolated from samples presenting clean and visible bands on ethidium bromide stained agarose gel, using the FavorPrep™ Gel/PCR Purification Kit (FAVORGEN® Biotech Corp., Changzhi, Taiwan) and following the producer specifications. For sequencing, Macrogen (Seoul, South Korea) services were used. The sequences were manually aligned and edited in CodonCode Aligner v.3.7.1 (CodonCode Corporation, Dedham, MA, USA). Genetic polymorphism and haplotype identification were assessed in DnaSP v.5 (Librado & Rozas 2009).

COI fragments were aligned together with previous published sequences of polychaetes from GenBank (see Table I), using CLUSTAL W (Thompson *et al.* 1994). Two sequence alignment sets were used: one set containing the initial fragment of 563 bp consisting of only *Scolelepis* species and *Capitella neoaciculata* Silva & Seixas, 2017 (as outgroup) for the phylogenetic tree reconstruction and a second set containing a fragment trimmed to 498 bp consisting of *Scolelepis*, *Malacoceros*, *Spio* and *Capitella* species for the genetic distances calculation. DNA sequences were translated to protein sequences under the 'Invertebrate Mitochondrial' genetic code table in MEGA7 (Kumar *et al.* 2016) and checked for stop codons.

The model and parameters for the Bayesian Analysis were determined using jModelTest2 (Darriba *et al.* 2012), according to the hierarchical likelihood ratio test criterion (hLRT). F81+G model of molecular evolution was the best nucleotide substitution model for phylogenetic relationships assessed by Bayesian inference using MrBayes v3.2.7, implemented in CIPRES Science Gateway (https://www.phylo.org/) (Miller *et al.* 2010). We performed two parallel runs for 10 million generations sampled every 1000th generation. FIGTREE v1.4.4 (Rambaut 2014) and Inkscape v0.92.4 were used for visualizing trees and producing publication-quality figures.

Genetic distances between the analyzed species were also calculated in MEGA7 using the Kimura 2-parameter model (Kimura 1980). All sequences obtained in the present study were submitted to GenBank, with accession numbers listed in Table I.

RESULTS

Morphology

Spionidae Grube, 1850
Genus *Scolelepis* Blainville, 1828
Subgenus *Scolelepis* Blainville, 1828
Type-species: *Lumbricus squamata* Müller, 1806, by monotypy.
***Scolelepis* (*Scolelepis*) *neglecta* Surugiu 2016**
(Figs 2-3)
Scolelepis (*Scolelepis*) *neglecta* Surugiu, 2016: 161-168, figs 5-8, table 1.

Material (described below)

SPAIN – **Asturias** • 1 cs, 10 af, 4 mf (fixed in 10 % formaldehyde, of which 1 af on SEM stub); Ría de Villaviciosa, Marina of El Puntal; 43.52467°N, 5.39175°W; 25 Apr. 2015; coll. V. Surugiu; intertidal muddy sand flats with *Zostera noltei*; MGAB PLY 0158 • 3 af, 6 mf (poorly preserved with 4 % glutaraldehyde); Ría de Villaviciosa, Marina of El Puntal; 43.52452°N, 5.39152°W; 20 Sep. 2016; coll. V. Surugiu; intertidal sand flats; MGAB PLY 0160 • 4 af, 1 mf, 1 pf (fixed in 96 % ethanol); Ría de Villaviciosa, Marina of El Puntal; 43.52452°N, 5.39152°W; 20 Sep. 2016; coll. V. Surugiu; intertidal sand flats; DNA voucher specimens: SnP1, SnP3-SnP7; GenBank accession numbers: MN378531 to MN378536; MGAB PLY 0163 • 2 af, 1 mf (fixed in 96 % ethanol); Ría de Villaviciosa, La Ermita; 43.52045°N, 5.39803°W; 20 Sep. 2016; coll. V. Surugiu; intertidal muddy sand flats; DNA voucher specimen SnE1; GenBank accession number MN378530; MGAB PLY 0164. – **Galicia** • 1 af, 2 mf; Pontevedra, central part of the north shore of the Ría de Vigo, Praia de Meira; 42.28537° N, 8.72604° W; 24

May 2016; coll. V. Surugiu; intertidal fine sands; MGAB PLY 0159. – **Cantabria** • 1 af; Santander; 43.48503° N, 3.75387° W; depth 25 m; 31 Aug. 2017; coll. Instituto de Hidráulica Ambiental de Cantabria; stn. AB-AC10, fine to very fine sand, 1.86 % organic matter; MGAB PLY 0161.

PORTUGAL • 7 af; shelf off Cascais; 38.681667°N, 9.483333°W; depth 40 m; 1997; RV *Andrómeda/Auriga*, cruise GUIA97, Stn. G13, Smith-McIntyre grab; DBUA 0170.01 • 2 af; shelf off Cascais; 38.681667°N, 9.483333°W; depth 40 m; Jun. 1998; RV *Andrómeda/Auriga*, cruise GUIA98, Stn. G13, Smith-McIntyre grab; DBUA 0170.03.

Comparative material (*Scolelepis *cf.* mesnili*)

ROMANIA – **Black Sea** • 4 cs (fixed in 10 % formaldehyde), 5 af (fixed in 96 % ethanol); Cap Midia-Digul de Nord; 44.342444°N, 28.691000°W; depth 0.5-0.8 m; 29 Jun. 2014; coll. V. Surugiu; fine sand with shells; DNA voucher specimens: Sm1-Sm4; GenBank accession numbers: MN378526 to MN378529; MGAB PLY 0061 • 8 cs, 1 af (fixed in 10 % formaldehyde); Năvodari-Digul de Sud; 44.323722° N, 28.639278° E; depth 0.5-0.8 m; 29 Jun. 2014; coll. V. Surugiu; fine sand with shells; MGAB PLY 0062.

Comparative material (*Scolelepis *cf.* cirratulus*)

ROMANIA – **Black Sea** • 3 cs, 1 af (fixed in 10 % formaldehyde), 4 af (fixed in 96 % ethanol); Cap Midia-Digul de Nord; 44.342444°N, 28.691000°W; depth 0.5-0.8 m; 29 Jun. 2014; coll. V. Surugiu; fine sand with shells; DNA voucher specimens: Sc1-Sc4; GenBank accession numbers: MN378522 to MN378525; MGAB PLY 0061.

ITALY – **Tyrrhenian Sea** • 5 cs; Campania, N. of Napoli, Gulf of Gaeta, Licola; 1966-1968, coll. J. Dörjes, SMF 12914.

Description (Iberian specimens)

The only complete specimen 57 mm long, 1.60 mm wide (at chaetiger 11) for 153 chaetigers. The longest anterior fragment 58 mm for 133 chaetigers. The widest anterior fragment 2.08 mm. Body widest at the level of anterior 8-48 chaetigers, then gradually tapering to both ends; body suboval in cross section. Color of living specimens yellowish with branchiae and postchaetal neuropodial lamellae red; prostomium whitish; proboscis pinkish; ventral median longitudinal groove bordered by greenish lines; dorsal and ventral medial longitudinal blood vessel red.

Prostomium anteriorly trilobate, medially acuminate, antero-laterally rounded, posteriorly extended as a short, bluntly rounded, attached, slightly raised caruncle, extending to posterior margin of chaetiger 1, with transverse constriction in posterior third (Fig. 2A). Occipital antenna absent. Juveniles with 4 dark-brown eyes in a trapezoidal arrangement, almost in a transverse line; antero-lateral smaller, rounded, posterior elongated transversely. Nuchal organs J-shaped, on sides of caruncle, just behind bases of palps (Fig. 2A, D).

Peristomium short, distinctly separated from prostomium by a shallow groove, forming well-developed dorsolateral wings (Fig. 2A, C), distinctly separated from chaetiger 1 (Fig. 2B, C). Eversible proboscis sac-like, inflated. Palps very short, tapering, deciduous, as long as 3-6 chaetigers (Fig. 2A-C); ciliation of palps consisting of two antero-longitudinal widely spaced bands of transverse rows of short, non-motile cilia, both on elevated 12–13 μm in height lobes, those on medial side approximately 14-16 μm wide, those on lateral side subdivided into two closely applied side-by-side rows of approxi-

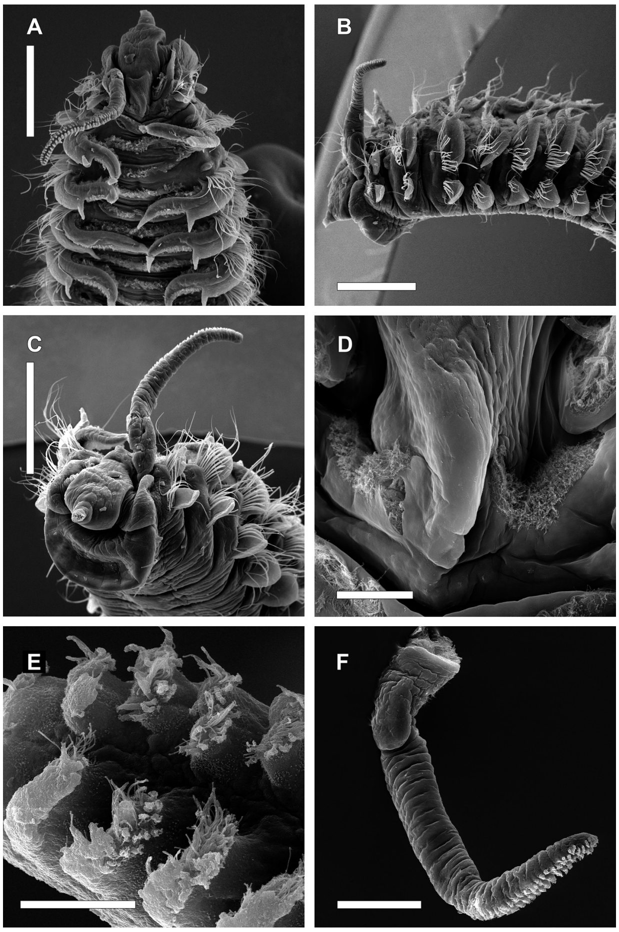

Fig. 2. – Adult morphology of *Scolelepis neglecta* Surugiu, 2016 from the Villaviciosa estuary, Asturias, Bay of Biscay, northern Spain (MGAB PLY 158). SEM micrographs. **A**: Anterior end, dorsal view, right palp missing, showing nuchal organs and dorsal primary and secondary transverse ciliary bands. **B**: Same, left lateral view. **C**: Same, antero-lateral view. **D**: Posterior part of caruncle and J-shaped nuchal organs. **E**: Distal section of left palp, antero-lateral view, showing ciliation pattern. **F**: Right palp, lateral view, showing tightly fused, smooth sheath. Scale bars: A-C = 500 μm; D = 50 μm; E = 20 μm; F = 200 μm.

mately 7-8 µm wide each (14-17 µm in total with a distance of 1-2 µm between them) (Fig. 2E). Ratio of lateral to medial rows approximately 1.15-1.25, with a distance of 5-14 µm separating them. Palp sheaths conspicuous, rounded, short, smooth, tightly adhering to lateral bases of palps (Fig. 2F).

Branchiae from chaetiger 2 to last segment, completely developed by chaetigers 3-30; ciliation along inner edge of branchiae; tips of branchiae acuminate, glandular, devoid of cilia. In anterior chaetigers, branchiae fused to notopodial postchaetal lamellae for approximately half of their length (Fig. 2A); in middle chaetigers branchiae fused for 20-35 % of their length; on last chaetigers, branchiae fused only basally to postchaetal lamellae.

Chaetiger 1 well developed, capillary chaetae present in both rami, fewer and thinner than those in following chaetigers; notopodial lamellae elongate, digitiform, tapered subdistally; neuropodial lamellae shorter, acute-ovate (Fig. 3A); notopodial capillaries with indistinct double rows and a dorsal superior tuft of thin and long capillaries numbering up to 10, longer than ventral ones and as long as or slightly shorter than those of dorsal superior tuft on following chaetigers; neuropodial capillaries more numerous, arranged in two distinct rows, 7-9 per row, plus a ventral inferior tuft, all shorter than those of following chaetigers.

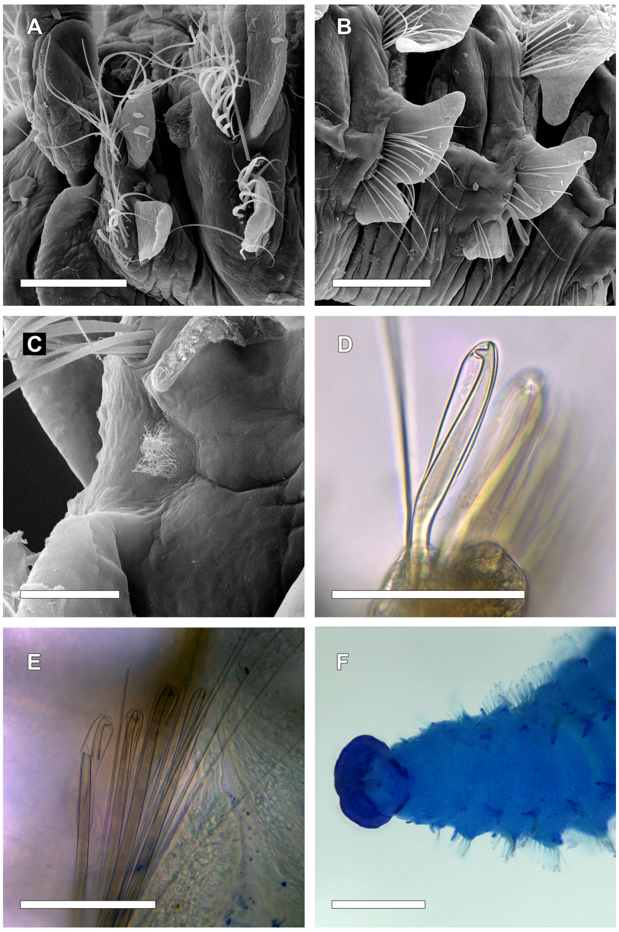

Fig. 3. – Adult morphology of *Scolelepis neglecta* Surugiu, 2016 from the Villaviciosa estuary, Asturias, Bay of Biscay, northern Spain (MGAB PLY 158). SEM (A-C) and LM (D-F) micrographs. **A**: Chaetigers 1 & 2, left lateral view. **B**: Chaetigers 35 & 36, left lateral view, showing the first appearance of hooded hooks. **C**: Close-up view of the lateral ciliated organ of chaetiger 1. **D**: Neuropodial hooded hook from chaetiger 81 in lateral view. **E**: Notopodial hooded hooks from chaetiger 144 in lateral (in the left) and frontal view (in the right). **F**: Posterior end, dorsal view, stained with MBW. Scale bars: A-B = 200 µm; C = 50 µm; D-E = 100 µm; F = 500 µm.

Dorsal transverse ciliation as primary ciliary bands on mid-part of each chaetiger (nototrochs), continuous with ciliation of branchiae (Fig. 2A); additional short row of cilia on outer edge of branchiae between tips of notopodial lamellae and subdistal portion of branchiae. Secondary dorsal transverse rows of cilia, located on anterior part of chaetigers, present in anteriormost 23-48 chaetigers, gradually vanishing in following chaetigers. In middle and posterior chaetigers dorsal surface smooth with low transverse folds uniting branchiae (from about chaetiger 25). Ventral surface smooth with a shallow median groove.

Notopodial postchaetal lamellae well-developed from chaetiger 2, on anterior chaetigers elongated, narrow, attached to branchiae, with outer margin smooth, entire, with free tips acuminate (Fig. 2A), in middle and posterior chaetigers becoming gradually shorter, wider and attached only at base of branchiae, with lower portion projecting ventrally towards neuropodial postchaetal lamella (Fig. 3B). Notopodial prechaetal lamellae subtriangular, with stubby tip, best developed from chaetigers 2-3 to around chaetigers 17-38, then becoming lower and broader inconspicuous folds (Fig. 2B).

Neuropodial postchaetal lamellae in anteriormost 2-5 chaetigers cordate, as long as wide, in following chaetigers becoming progressively more rounded, semicircular, entire, wider than long; slight notch developing in inferior 1/3 of lamella on chaetigers 14-39 (Fig. 3B); notch becoming deeper in middle chaetigers, dividing lamella into two separate lobes, upper lobe being more than twice as large as lower subtriangular lobe, lower lobe located at level of ventral inferior bundle of capillaries. In posteriormost chaetigers gap between lobes wider, setting lobes further apart, upper lobe becoming narrow, rounded, with upper portion elongated and directed towards ventral portion of notopodial postchaetal lamella, lower part reduced to triangular cirrus. Neuropodial prechaetal lamellae absent.

Lateral ciliated organs present between notopodial and neuropodial postchaetal lamellae from chaetiger 1, ovate in anterior chaetigers (Fig. 3A, C), becoming more elongated transversely from around chaetiger 33 onward (Fig. 3B).

Anterior chaetigers with capillaries only, arranged in three distinct groups in both noto- and neuropodia. Notopodial capillary chaetae elongate, narrow, arranged in double rows (7-11 per row) and a dorsal superior tuft of 2-10 longer and thinner capillaries; capillaries of anterior row unilimbate, with fibrose cores, of approximately same length and width as those of posterior row; capillaries of posterior row unilimbate, with uniformly granulated cores. Number and length of notopodial capillaries gradually decreasing towards posterior end.

Neuropodial capillaries similar in morphology to those of notopodia, although shorter and broader, with narrow limbation, with granulated cores (when viewed in direct light) or appearing fibrous (when viewed in reflected light), also arranged in double vertical rows (6-16 per row) plus a ventral inferior bundle of 1-4 long, unilimbate capillaries in position of sabre chaetae; capillaries of anterior row slightly shorter and broader than those of posterior row.

Neuropodial hooded hooks first present in posterior row of chaetigers 26-44, up to 16 per fascicle, accompanied in middle and posterior chaetigers by 1-3 alternating slender capillary chaetae in superior position and by 1-2 shorter slender limbate capillaries in ventral inferior tuft. Neuropodial hooded hooks with bluntly rounded main fang surmounted by a smaller bluntly rounded apical tooth, main fang at right angle to shaft; shaft long, with small constriction and bend just below hood insertion (Fig. 3D). Hood elongate, more than 5 times length of main fang, with apical-rostral slit-like opening. Ventral sabre chaetae absent.

Notopodial hooded hooks first present in chaetigers 95-128, numbering up to 4 in a bundle, accompanied by 4-8 very long, slender, alimbate capillary chaetae. Notopodial hooded hooks with bluntly rounded main fang surmounted by a small bluntly rounded or slightly incised apical tooth, main fang at right angle to shaft; shaft very long, slender, gently curved, without constriction (Fig. 3E). Two prepygidial achetous segments.

Pygidium ventral cushion-like rounded pad (Fig. 3F).

Remarks

The material collected in Villaviciosa estuary is in agreement with the description of the species provided by Surugiu (2016). We have also noticed some previously unreported features such as the coloration of living specimens, the presence of eyes only in unpreserved juvenile specimens, the presence of notopodial hooks in far posterior segments, and the shape of the pygidium.

Habitat and ecology

Scolelepis neglecta inhabits intertidal to subtidal (down to 40 m depth) fine sands ($Q_{50} = 0.25 \pm 0.05$ mm; So = 1.52-1.83; with organic matter content of 0.25-7.47 %), pH 8, salinity 25-32 PSU, and dissolved oxygen 7.5 ml l^{-1} (Viéitez 1976, Anadón *et al*. 1997, present study). It can occur together with *Tellina tenuis* da Costa, 1778, *Abra tenuis* (Montagu, 1803), *Ensis directus* (Conrad, 1843), *Diopatra neapolitana* Delle Chiaje, 1841, *Diopatra marocensis* Paxton *et al*., 1995, *Nephtys hombergii* Savigny *in* Lamarck, 1818, *Nephtys cirrosa* Ehlers, 1868, *Leiochone clypeata* Saint-Joseph, 1894, *Cirriformia tentaculata* (Montagu, 1808), and *Phylo foetida* (Claparède, 1868) (present study).

Molecular analysis

A fragment of 498 bp from the COI gene was analyzed in 55 specimens belonging to the *Scolelepis*, *Spio* and *Malacoceros* genera, with *Capitella neoaciculata* as the outgroup, using MEGA7 with the Kimura 2-parameter model to calculate genetic distances between and within analyzed species (Tables II-III).

With regard to the interspecific genetic distances (Table II), the main finding was the fact that four specimens from the Black Sea that were considered to be adults of *S. squamata* proved to be not conspecific (0.229) with another four specimens that were presumed to be juveniles of the same species. These two species also differed from populations of *S. squamata* from North America (both by a genetic distance of 0.292). Therefore, we herein designate the two species of *Scolelepis* from the Black Sea as *Scolelepis* cf. *cirratulus* and *Scolelepis* cf. *mesnili*, respectively, until additional material from the type localities of both species will be available for molecular studies. The highest value recorded between *Scolelepis* species was found between *S. goodbodyi* (Jones, 1962) and *S. kudenovi* Hartmann-Schröder, 1981 (0.393). The lowest value was found between *S. foliosa* (Audouin & Milne Edwards, 1833) from Portugal and *Scolelepis* cf. *cirratulus* from the Black Sea (0.111). *S. neglecta* is genetically closest to *Scolelepis* cf. *cirratulus* from the Black Sea (0.199) and is most distantly related with *S. goodbodyi* from Brazil (0.316).

For *S. neglecta* we identified a value of 0.005 for intraspecific genetic variation (Table III), while for *Scolelepis* cf. *cirratulus* this value was lower (0.002) and for *Scolelepis* cf. *mesnili* this value was higher (0.012). The values for the other *Scolelepis* species fall within this range, with the exception of *S. acuta* (Treadwell, 1914) (0.043).

Six haplotypes were detected in the seven analyzed specimens of *S. neglecta*. Also, all observed nucleotide substitutions were synonymous and did not result in differences in the aminoacid sequence.

For the Bayesian inference, the best substitution model was found to be the General Time Reversible one and the rates among sites were Gamma-distributed (using 6 discrete Gamma categories) with invariant sites. The obtained tree categorized all *Scolelepis* species as a monophyletic group, with *S. neglecta* specimens clustered together on the same clade and clearly distinct from all other *Scolelepis* species (Fig. 4). Sequence data of *S. chilensis* (Hartmann-Schröder, 1962) and *S. acuta* from Brazil appears to be conspecific, but different from

S. acuta from California, USA. *Scolelepis* cf. *mesnili* from the Black Sea form a separate clade from all other analyzed *Scolelepis* species.

DISCUSSION

In literature there are no previous results about molecular phylogenetic relationship among *Scolelepis* species. The overall mean genetic distance between the eleven analyzed species of *Scolelepis* in the present study was 0.211, a value similar to the one identified by other studies on polychaetes (Schulze *et al*. 2000, Blank & Bastrop 2009, Barroso *et al*. 2010, Carr *et al*. 2011, Magalhães *et al*. 2014, Silva *et al*. 2017, Ye *et al*. 2019).

The present molecular data provide strong support to the description of *S. neglecta* which was previously based only on morphological data.

Surprisingly, the analyzed data indicate that specimens identified as *S. squamata* from the Great Nicobar Island (India) are conspecific with geographically distant populations of *S. squamata* from British Columbia (Canada) and Alaska (USA). However, since the type locality of *S. squamata* is the island of Helgoland in the North Sea, we doubt that the populations from the Indian and Pacific Oceans belong to the same species as *S. squamata* from the Northeast Atlantic. In order to elucidate the taxonomic relationships between these populations, further morphological and genetic analyses should be carried out on material originating from type localities of different species of *Scolelepis*.

Due to the absence of reliable morphological characters to distinguish between species, *S. cirratulus* and *S. mesnili* were considered by Surugiu (2016) as subjective synonyms of *S. squamata*. However, the sequence data of the mitochondrial COI fragments showed that under *S. squamata* in the Black Sea there are in fact two genetically distinct species, both also distinct from *S. squamata* from the Pacific coast of North America. Revealed genetic differences are equal with the overall genetic distance calculated for analyzed *Scolelepis* species (0.22). *Scolelepis* cf. *cirratulus* from the Black Sea comprised specimens with large body size (body width > 1.9 mm), long palps (reaching chaetigers 14-24), hooks in neuropodia starting from chaetigers 33-42, hooks in notopodia starting from chaetigers 58-90, one rounded api-

Table II. – Interspecific distances between analyzed species using Kimura 2-parameter model implemented in MEGA7 (below diagonal) and SD (standard deviation, above diagonal), with a bootstrap of 1000 replicates.

Species	N specimens	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *Capitella neoaciculata*	1		0.035	0.039	0.036	0.036	0.036	0.036	0.040	0.037	0.045	0.038	0.034	0.036	0.036	0.034
2. *Malacoceros fuliginosus*	5	0.399		0.027	0.021	0.021	0.021	0.021	0.024	0.022	0.031	0.022	0.022	0.023	0.028	0.025
3. *Malacoceros indicus*	2	0.448	0.272		0.027	0.027	0.026	0.027	0.030	0.025	0.031	0.028	0.027	0.024	0.028	0.028
4. *Scolelepis acuta*	10	0.403	0.209	0.283		0.018	0.022	0.021	0.021	0.021	0.030	0.020	0.022	0.022	0.027	0.024
5. *Scolelepis chilensis*	1	0.401	0.196	0.266	0.159		0.022	0.021	0.023	0.021	0.033	0.021	0.023	0.025	0.026	0.023
6. *Scolelepis* cf. *cirratulus*	4	0.428	0.195	0.258	0.227	0.195		0.020	0.024	0.015	0.034	0.023	0.023	0.022	0.028	0.023
7. *Scolelepis daphoinos*	1	0.406	0.205	0.285	0.202	0.185	0.183		0.023	0.020	0.032	0.022	0.024	0.023	0.026	0.022
8. *Scolelepis eltaninae*	1	0.443	0.235	0.304	0.193	0.209	0.233	0.205		0.025	0.033	0.023	0.025	0.023	0.027	0.026
9. *Scolelepis foliosa*	6	0.432	0.203	0.246	0.198	0.182	0.111	0.172	0.244		0.034	0.024	0.022	0.023	0.026	0.022
10. *Scolelepis goodbodyi*	1	0.535	0.355	0.319	0.340	0.359	0.368	0.359	0.361	0.363		0.036	0.034	0.030	0.034	0.039
11. *Scolelepis kudenovi*	1	0.440	0.196	0.276	0.183	0.190	0.214	0.198	0.220	0.216	0.393		0.025	0.022	0.029	0.025
12. *Scolelepis* cf. *mesnili*	4	0.370	0.223	0.269	0.218	0.213	0.229	0.223	0.243	0.217	0.388	0.238		0.024	0.027	0.025
13. *Scolelepis neglecta*	**7**	**0.430**	**0.228**	**0.236**	**0.224**	**0.232**	**0.199**	**0.211**	**0.227**	**0.201**	**0.316**	**0.206**	**0.225**		0.027	0.027
14. *Scolelepis squamata*	8	0.418	0.297	0.284	0.288	0.268	0.292	0.265	0.275	0.258	0.358	0.310	0.292	**0.273**		0.026
15. *Spio blakei*	3	0.403	0.247	0.277	0.245	0.209	0.232	0.197	0.262	0.209	0.423	0.244	0.254	**0.255**	0.263	

Table III. – Intraspecific distances between analyzed species using Kimura 2-parameter model implemented in MEGA7, with a bootstrap of 1000 replicates.

Species	N specimens	d	S.E.
Spio blakei	3	0.003	0.002
Malacoceros fuliginosus	5	0.005	0.002
Malacoceros indicus	2	0.002	0.002
Scolelepis squamata	8	0.010	0.002
Scolelepis foliosa	6	0.005	0.002
Scolelepis acuta	10	0.043	0.005
Scolelepis* cf. *cirratulus	**4**	**0.002**	**0.001**
Scolelepis* cf. *mesnili	**4**	**0.012**	**0.003**
Scolelepis neglecta	**7**	**0.005**	**0.002**

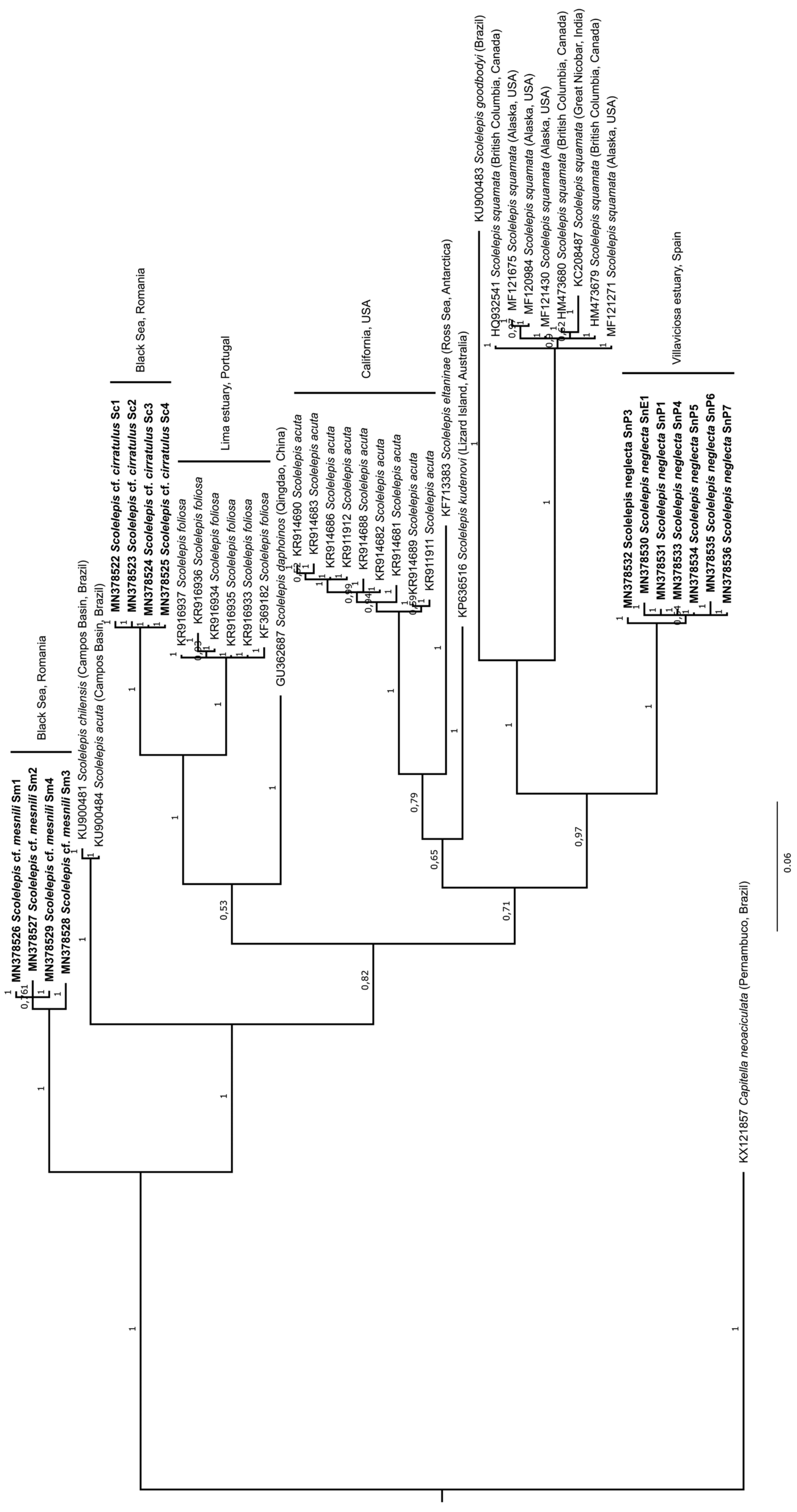

Fig. 4. – Phylogenetic relationships of *Scolelepis neglecta* based on Bayesian Inference for COI nucleotide sequences. Sequences produced in this study are presented in bold.

cal tooth above main fang, obtuse angle between main fang and shaft, and rounded tips of postchaetal notopodial lamellae. *Scolelepis* cf. *cirratulus* from the Black Sea appeared closely related genetically to *S. foliosa* from Portugal, but were clearly distinct morphologically from the description of *S. foliosa*. Specimens of *Scolelepis* cf. *mesnili* were considered to be juveniles of *S. squamata* because of their smaller body size (body width < 1.5 mm), shorter palps (reaching chaetigers 9-15), hooks in neuropodia starting from chaetigers 23-26, hooks in notopodia starting from chaetigers 37-57, one or two apical teeth above main fang, acute to right angle between main fang and shaft, and sharply pointed tips of postchaetal notopodial lamellae. However, genetic analyses showed that they formed a distinct clade. *Scolelepis* cf. *mesnili* seems to correspond morphologically to *S. mesnili* (Bellan & Lagardère, 1971). Therefore, the reinstatement of *S. mesnili* and *S. cirratulus* cannot be ruled out. In this new light, the type material of *S. foliosa*, *S. squamata*, *S. cirratulus* and *S. mesnili* should be re-evaluated in order to clarify to which taxa the two species from the Black Sea belong or whether one or both of them represent an yet undescribed species.

Acknowledgements. – Our sincere thanks are to N Anadón and A Arias (Universidad de Oviedo, Spain), to A Palanca-Soler and L C Miramontes-Sequeiros (Universidad de Vigo, Spain) and to N Palanca-Catán (Universidad de Valapraíso, Chile) for providing generous help in field collections, to Ravara (Universidade de Aveiro, Portugal) for facilitating the study of the DBUA museum's polychaete collection, to D Fiege for arranging the loan of specimens deposited in the Senckenberg Museum Frankfurt (Germany), to A Puente (Instituto de Hidráulica Ambiental de Cantabria, Spain) for providing specimens from Santander, to A Lobiuc (CERNESIM, Romania) and I Gostin ("Alexandru Ioan Cuza" University of Iaşi, Romania) for assistance with SEM and to E Baltag ("Alexandru Ioan Cuza" University of Iaşi, Romania) for preparing Figure 1. We would like also to thank the two anonymous reviewers for their valuable comments and suggestions that greatly improved the text.

REFERENCES

Anadón N, Anadón A, Santos-Salas C, Álvarez-Claudio C 1997. Macrozoobentos de la Ría de Villaviciosa (Asturias, Norte de España). *Bol Cienc Nat* 44: 201-206.

Barroso R, Klautau M, Solé-Cava AM, Paiva PC 2010. *Eurythoe complanata* (Polychaeta: Amphinomidae), the 'cosmopolitan' fireworm, consists of at least three cryptic species. *Mar Biol* 157(1): 69-80.

Bellan G, Lagardère F 1971. *Nerine mesnili*, n. sp., Spionidien méconnu des plages sableuses de la Province Lusitaniene. *B Soc Zool Fr* 96(4): 571-579.

Blainville H 1828. Dictionnaire des Sciences naturelles, dans lequel on traite méthodiquement des différents êtres de la nature, considérés soit en eux-mêmes, d'après l'état actuel de nos connaissances, soit relativement à l'utilité qu'en peuvent retirer la médecine, l'agriculture, le commerce et les arts. Suivi d'une biographie des plus célèbres naturalistes. Vol 57, F.G. Levrault, Strasbourg & Paris: 628 p.

Blank M, Bastrop R 2009. Phylogeny of the mud worm genus *Marenzelleria* (Polychaeta: Spionidae) inferred from mitochondrial DNA sequences. *Zool Scr* 38: 313-321.

Carr CM, Hardy SM, Brown TM, Macdonald TA, Hebert PD 2011. A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS ONE* 6(7): e22232.

Darriba D, Taboada GL, Doallo R, Posada D 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods*, 9(8): 772.

Dauer DM 1983. Functional morphology and feeding behavior of *Scolelepis squamata* (Polychaeta: Spionidae). *Mar Biol* 77: 279-285.

Delgado-Blas VH 2006. Partial revision of *Scolelepis* (Polychaeta: Spionidae) from the Grand Caribbean region, with the description of two new species and a key to species recorded in the area. *Contrib Zool* 75(1-2): 75-97.

Folmer O, Black M, Hoen W, Lutz R, Vrijenhoek R 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotech* 3(5): 294-299.

Grube AE 1850. Die Familien der Anneliden. *Arch Naturgesch Berlin* 16(1): 249-364.

Joyner A 1962. Reproduction and larval life of *Nerine cirratulus* (Delle Chiaje) Family Spionidae. *Proc Zool Soc Lond* 138(4): 655-666.

Kimura M 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16: 111-120.

Kisseleva MI 2004. Polychaetes (Polychaeta) of the Azov and Black Seas. Kola Science Centre of the Russian Academy of Science Press, Apatity: 409 p. [in Russian]

Kumar S, Stecher G, Tamura K 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Mol Biol Evol* 33(7): 1870-1874.

Kurt Şahin G, Çinar ME 2012. A check-list of polychaete species (Annelida: Polychaeta) from the Black Sea. *J Black Sea/Medit Environ* 18(1): 10-48.

Librado P, Rozas J 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25(11): 1451-1452.

Maciolek NJ 1987. New species and records of *Scolelepis* (Polychaeta: Spionidae) from the east coast of North America. *Proc Biol Soc Wash* 7: 16-40.

Magalhães WF, Seixas VC, Paiva PC, Elias R 2014. The multitentaculate Cirratulidae of the genera *Cirriformia* and *Timarete* (Annelida: Polychaeta) from shallow waters of Brazil. *PLoS ONE* 9(12): e112727

Marinov T 1977. Fauna na Bŭlgaria. Mnogochetinesti chervey (Polychaeta). Vol 6, Bŭlgarskata Akademia na Naukite, Sofia: 258 p. [In Bulgarian]

Meißner K, Götting M 2015. Spionidae (Annelida: 'Polychaeta': Canalipalpata) from Lizard Island, Great Barrier Reef, Australia: the genera *Malacoceros*, *Scolelepis*, *Spio*, *Microspio*, and *Spiophanes*. *Zootaxa* 4019(1): 378-413.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. 2010 Gateway Computing Environments Workshop (GCE), New Orleans, LA: 1-8.

Müller OF, Abildgaard PC, Vahl M, Holten JS, Rathke J 1806. Zoologia Danica seu Animalium Daniae et Norvegiae rariorum ac minus notorum. Descriptiones et historia. Vol IV, N. Christensen, Havniae [Copenhagen]: 46 p.

Rambaut A 2014. FigTree v1.4.2. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. Available at: http://tree.bio.ed.ac.uk/software/figtree/

Schulze SR, Rice SA, Simon JL, Karl SA 2000. Evolution of poecilogony and the biogeography of North American populations of the polychaete *Streblospio*. *Evolution* 54: 1247-1259.

Sigvaldadóttir E, Mackie ASY, Pleijel F 1997. Generic interrelationships within the Spionidae (Annelida: Polychaeta). *Zool J Linn Soc* 119*:* 473-500.

Silva CF, Seixas VC, Barroso R, Di Domenico M, Amaral ACZ, Paiva PC 2017. Demystifying the *Capitella capitata* complex (Annelida, Capitellidae) diversity by morphological and molecular data along the Brazilian coast. *PLoS ONE* 12(5): e0177760.

Surugiu V 2016. On the taxonomic status of the European *Scolelepis* (*Scolelepis*) *squamata* (Polychaeta: Spionidae), with description of a new species from southern Europe. *Zootaxa* 4161(2): 151-176.

Thompson JD, Higgins DG, Gibson TJ 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22(22): 4673-4680.

Viéitez JM 1976. Ecología de Poliquetos y moluscos de la playa de Meira (Ría de Vigo). I. Estudio de las comunidades. *Invest Pesq* 40(1): 223-248.

Williams JD 2007. New records and description of four new species of spionids (Annelida: Polychaeta: Spionidae) from the Philippines: the genera *Dispio*, *Malacoceros*, *Polydora*, and *Scolelepis*, with notes on the palp ciliation patterns of the genus *Scolelepis*. *Zootaxa* 1459: 1-35.

Ye L, Yao T, Wu L, Lu J, Wang J 2019. Morphological and molecular diagnoses of *Polydora brevipalpa* Zachs, 1933 (Annelida: Spionidae) from the shellfish along the coast of China. *Chin J Oceanol Limnol* 37: 713-723.

Received on March 12, 2020
Accepted on September 28, 2020
Associate editor: J Orignac