A new species of *Cirrophorus* (Annelida: Paraonidae) from Mediterranean organically enriched coastal environments, with taxonomic notes on the family

JOACHIM LANGENECK, MICHELE BARBIERI, FERRUCCIO MALTAGLIATI AND ALBERTO CASTELLI

Department of Biology, University of Pisa, via Derna 1, 56126 Pisa, Italy

Cirrophorus nikebianchii sp. nov. is described from brackish-water and organically enriched marine environments of the Mediterranean Sea. The new species is characterized by a very small prostomial antenna and a high number of branchiae pairs. A phylogenetic analysis carried out through the use of three molecular markers (16S rRNA, 18S rRNA and COI) supports the distinction between C. nikebianchii and C. furcatus, a closely related species with which it has been misidentified. Preliminary results obtained show that the genera Cirrophorus and Paradoneis are not reciprocally monophyletic, with uncertain relationships with the remaining genera of Paraonidae. This outcome suggests that the evolutionary history of Paraonidae is less straightforward than previously supposed. Moreover, the uncertainty about the taxonomic status of Paraonides neapolitana, type species of the genus Paraonides, makes the revamping of the taxonomy of Paraonidae more challenging. Awaiting support from studies including more species, and based on morphological and genetic data as well, we suggest to provisionally maintain the current use of Cirrophorus and Paradoneis, and to assign to Paraonella the species traditionally assigned to Paraonides.

INTRODUCTION

The Paraonidae constitutes one of the more diverse and taxonomically complex families among polychaetes. This family has been investigated in the Mediterranean Sea by several authors (Cerruti, 1909; Laubier, 1967; Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Castelli, 1985), but knowledge of the Mediterranean Paraonidae is far from being complete, and new species were recently described, even from shallow environments where polychaete fauna is better known (Sardá *et al.*, 2009; Çinar *et al.*, 2011; Çinar & Dağli, 2013). Moreover, within Paraonidae, the taxonomy of several genera is unclear and the whole family needs a taxonomic revision based on the re-examination of existing type material and the study of new material, covering also poorly known areas, using both morphological and molecular data.

The genus *Cirrophorus* Ehlers, 1908 in the Mediterranean Sea has been generally considered to be represented by two species, *Cirrophorus branchiatus* Ehlers, 1908 and *Cirrophorus furcatus* (Hartman, 1957). Some authors list an additional species, *Cirrophorus lyriformis* Annenkova, 1934 (Çinar *et al.*, 2014), a species synonymized with *C. branchiatus* by Strelzov (1973) even if most of its Mediterranean records were later attributed to *C. furcatus* (see Katzmann & Laubier, 1975, and references

therein). On the other hand, the identification of the Mediterranean *C. furcatus* has been more problematic, and several different species, or at least morphotypes, seem to be involved. The identity of Mediterranean individuals of the shallow water *Cirrophorus* nr *furcatus*, commonly found in brackish-water habitats and, in a more general way, organically-enriched environments, was questioned previously (Castelli *et al.*, 1995), as the Mediterranean individuals show morphological and ecological differences with respect to those from California, the type locality of the species (Hartman, 1957).

Previous works on polychaetes have widely demonstrated that the combined use of morphological and molecular data is a powerful approach to disentangle complex systematic problems and make attempts to clarify evolutionary processes (e.g. Cadman & Nelson-Smith, 1990; Wu *et al.*, 1991; Maltagliati *et al.*, 2001, 2004, 2005; Nygren & Pleijel, 2011). Within the frame of a systematic revision of the Mediterranean Paraonidae, and on the basis of morphological and molecular data, in this work we identify two divergent morphotypes that were historically identified as *Cirrophorus furcatus*. One of these morphotypes is described here as a new species, whereas the other represents a putative new species, but is not described due to the scarcity of the material available. Moreover, the synonymy between the genera *Cirrophorus* and *Paradoneis* Hartman, 1965, and the use of the generic names *Paraonides* Cerruti, 1909 and *Paraonella* Strelzov, 1973 are discussed based on the results from molecular data and the critical analysis of the literature.

Corresponding author:
J. Langeneck
Email: jlangeneck@biologia.unipi.it

871

MATERIALS AND METHODS

Samples of *Cirrophorus* nr *furcatus* for morphological study were obtained from the collection of the University of Pisa. Specimens were fixed with 4% neutralized formaldehyde in seawater and subsequently preserved in 70% ethanol. Measurements and counts were performed with a Primo Star Zeiss light microscope equipped with an ocular micrometer; drawings were made from images taken with a digital camera, and refined with GIMP 2.8.18 (software downloadable and documentation available at http://www.gimp.org), following the guidelines in Montesanto (2015). Type specimens were deposited in the Museo di Storia Naturale of the University of Pisa (Italy) (MSNP); non-type material was deposited in the Natural History Museum of Los Angeles, USA (LACM – AHF) and in the collections of the Stazione Zoologica Anton Dohrn of Naples (Italy) (SZN – LAM). Live Paraonidae for molecular analyses were collected at several localities in the Mediterranean Sea and Atlantic Ocean (Table 1), fixed directly in 96% or 70% ethanol and preserved at 4 °C until DNA extraction. We included material of the species of *Cirrophorus* and *Paradoneis* Hartman, 1965 available to us, including respectively the type species *C. branchiatus* Ehlers, 1908 and *Paraonis* (*Paraonides*) *lyra* Southern, 1914. The type species of *Aricidea* Webster, 1879 (*Aricidea fragilis* Webster, 1879), *Levinsenia* Mesnil, 1897 (*Aonides gracilis* Tauber, 1879) and *Paraonis* Grube, 1873 (*Aonides fulgens* Levinsen, 1884) were also included. DNA extraction was carried out using the GenElute™ Mammalian Genomic DNA Miniprep Kit distributed by Sigma-Aldrich, following the manufacturer's instructions. For phylogenetic reconstruction we amplified the genes for 16S rRNA and COI (mitochondrial) and 18S rRNA (nuclear). 16S rDNA amplification was obtained using the primer pair 16SarL (5′-CGCCTGTTT AACAAAAACAT-3′) and H3080 (5′-CCGGTCTGAACT CAGATCACGT-3′) (Palumbi *et al.*, 1991), whereas for COI amplification we used the universal primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer *et al.*, 1994) and the annelid-specific primers POLYLCO (5′-GAYTATWTTCAACAAATCATAAAGATATTGG-3′) and POLYHCO (5′-TAMACTTCWGGGTGACCAAARAA TCA-3′) (Carr *et al.*, 2011). 18S rDNA amplification was obtained using the primers F9 (5′-CTGGTTGATCCTG CCAG- 3′) (Medlin *et al.*, 1988) and R1513 (5′-TGATCCTTCYGCAGGTTC-3′) (Petroni *et al.*, 2002). Polymerase chain reaction (PCR) amplifications were carried out in 20 µl solutions using 1.5 mM of MgCl$_2$, 0.2 mM of each dNTP, 0.1 µM of each primer, 1 U of DreamTaq DNA polymerase (Thermo Scientific), and ~2.5 ng of template DNA. For 16S rDNA and COI the PCR profile was set as follows: initial denaturing step at 94 °C for 3 min; 34 cycles of denaturing at 94 °C for 45 s, annealing at 54 °C for 1 min, and extending at 72 °C for 1 min, and a final extending step at 72 °C for 7 min. A negative control was included in each reaction. For 18S rDNA, PCRs were carried out in 45 µl using a protocol with low ramp speed, and annealing temperature set at 50 °C (Lorenz, 2012). PCR products were precipitated with sodium acetate and absolute ethanol and sent to Macrogen Europe for sequencing.

Sequences from each gene were aligned with ClustalX 2.1 (Larkin *et al.*, 2007), and alignments were edited in BIOEDIT version 7.2.5 (Hall, 1999). The program jModelTest 2.1.6 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012), based on the hierarchical likelihood ratio test, was used to assess the best model of evolution for the sequences under the Akaike Information Criterion (AIC) (Akaike, 1974). For molecular comparison and phylogenetic reconstruction, we used additional sequences downloaded from GenBank for *Cirrophorus furcatus* (accession numbers AY532349.1 and AY532330.1); moreover, we used *Ophelina acuminata* Örsted, 1843 as outgroup (accession numbers AY340471.1, AY340439.1 and HQ024164.1). The choice of the outgroup was based on Bleidorn's (2005) remarks, who identified Opheliidae as a likely sister taxon of Paraonidae.

A Bayesian consensus phylogenetic tree based on the three concatenated markers was constructed using MrBayes 3.2 (Ronquist *et al.*, 2011), which allowed phylogenetic inference by treating each gene with its own substitution model. Four replicate runs were carried out with a total of three Markov chains per run for 2×10^6 generations. The chain was sampled every 100 generations to obtain 20,000 sampled trees. The first 5000 sampled trees (25%) were discarded as burn-in phase, with the remaining 15,000 trees used to estimate the Bayesian posterior probability (PP) of tree nodes. The convergence of Bayesian analyses was checked through the standard deviation of split frequencies, that should reach a value < 0.01 at the end of the analysis (Ronquist *et al.*, 2011).

RESULTS

SYSTEMATICS
Class Annelida
SCOLECIDA Rouse & Fauchald, 1997
Family PARAONIDAE Cerruti, 1909

Genus *Cirrophorus* Ehlers, 1908
Cirrophorus nikebianchii sp. nov.
(Figure 1A – F)

Cirrophorus furcatus [*non* (Hartman, 1957)]: Castelli, 1985: 277; Castelli & Lardicci, 1985: 765 – 766; Castelli, 1987: 327; Rossi & Lardicci, 1995: 33; Como *et al.*, 2004: 165; Chessa *et al.*, 2007: 14 – 15; Schirosi *et al.*, 2010: 238 – 239. *Paradoneis lyra* [*non* (Southern, 1914)]: Bonvicini Pagliai & Cognetti, 1982: 55; Maggiore & Keppel, 2007: 193 – 194; Simboura *et al.*, 2007: 57.

TYPE MATERIAL
Holotype: Sea of Sardinia, Italy, north-west Sardinia, Calich Pond (40°35.8′N 8°17.3′E), silty sand, brackish-water coastal pond, 1 m (March 1995) (MSNP P/3800).

Paratype: Locality and collection as holotype (MSNP P/3801).

COMPARATIVE MATERIAL EXAMINED
Balearic Sea, Spain: Ebro River Delta, Alfacs Bay (40°36.8′N 0°36.5′E), semi-closed inlet with freshwater inflow, 4 m: 3 individuals (September 1992) [1]; **Sea of Sardinia, Italy**: north-west Sardinia, Calich Pond (40°35.8′N 8°17.3′E), silty sand, brackish-water coastal pond, 1 m: 4 individuals (March 1988); 26 individuals (March 1995) [2]; **Sardinian Channel, Italy**: south-west Sardinia, Gulf of Cagliari (39°5.9′N 9°2.6′E), silty sand in fully marine environment, 20 m: 1 individual (1982) [3]; **Tyrrhenian Sea, Italy**: north-east Sardinia, Golfo Aranci, Gulf of Olbia (40°59.9′N 9°37.1′E), silty sand, under fish cages in fully marine environment, 5 m: 1 individual (January 1997) [4];

Table 1. Paraonidae included in the molecular analysis.

Species	Locality	Latitude	Longitude	Depth	Date	N
Aricidea fragilis Webster, 1879	Gulf of Manfredonia (Ad)	41°35.2′N	15°54.1′E	8 m	09/2014	1
Cirrophorus branchiatus Ehlers, 1908	Tuscan Archipelago (T)	43°30.8′N	9°55.3′E	110 m	11/2014	1
Cirrophorus nikebianchii n. sp.	Livorno port (T)	43°34.4′N	10°18.9′E	3 m	04/2016	1
Cirrophorus nikebianchii n. sp.	Porto Pozzo (T)	41°11.5′N	9°16.7′E	0.5 m	07/2015	2
Cirrophorus nikebianchii n. sp.	Venice Lagoon (Ad)	45°29.2′N	12°29.4′E	1 m	03/2015	4
Cirrophorus sp. B	Livorno port (T)	43°34.4′N	10°18.9′E	3 m	04/2016	1
Levinsenia gracilis (Tauber, 1879)	Loch Creran (At)	56°32.7′N	5°16.3′W	19 m	09/2003	1
Paradoneis armata Glémarec, 1966 I	Bay of Biscay (At)	43°18.2′N	2°9.0′W	30 m	04/2015	1
Paradoneis armata Glémarec, 1966 II	Elba Island (T)	42°45.3′N	10°18.2′E	7 m	06/2014	1
Paradoneis ilvana Castelli, 1985 I	Pianosa Island (T)	42°35.0′N	10°5.9′E	0.8 m	04/2015	1
Paradoneis ilvana Castelli, 1985 II	Capraia Island (T)	43°1.0′N	9°50.3′E	20 m	07/2015	1
Paradoneis lyra (Southern, 1914) I	Loch Creran (At)	56°32.9′N	5°16.3′W	25 m	09/2003	1
Paradoneis lyra (Southern, 1914) II	Tuscan Archipelago (T)	43°30.8′N	9°55.3′E	110 m	02/2016	1
Paraonis fulgens (Levinsen, 1884)	Newton-by-the-Sea (At)	55°30.8′N	1°36.9′W	0.2 m	08/2003	1

Ad, Adriatic Sea; At, Atlantic Ocean; T, Tyrrhenian Sea.

north Sardinia, Porto Pozzo (41°11.5′N 9°16.7′E), gravel, semi-closed inlet with brackish-water inflow, 0,8 m: 4 individuals (July 1987); 22 individuals (July 2015) [5]; eastern Sardinia, Tortolì Pond (39°56.9′N 9°41.2′E), gravel, brackish-water coastal pond, 1 m: 5 individuals (May 2016) [6]; Capraia Island (43°2.4′N 9°50.8′E), silt, under fish cages in fully marine environment, 33,5 m: 43 individuals (July 2003) [7]; Portoferraio Bay, Elba Island (42°48.7′N 10°18.5′E), silt, organically enriched environment, 8 m: 2 individuals (November 1982); 5 individuals (May 1990) [8]; Gulf of Follonica (42°55.1′N 10°44.3′E), fine sand in fully marine environment, 8 m: 8 individuals (December 1987) [9]; Livorno port (43°34.4′N 10°18.9′E), silt, organically enriched environment with brackish-water inflow, 3 m: 15 individuals (April 2016) [10]; **Adriatic Sea, Italy**: Southern Adriatic Sea, Acquatina Pond (40°26.7′N 18°14.3′E),

silt, brackish-water coastal pond 2 m: 68 individuals (November 2014) [11]; Southern Adriatic Sea, Varano Lagoon (41°52.4′N 15°42.3′E), silty shell grit, brackish-water lagoon, 3 m: 4 individuals (November 2014); 7 individuals (October 2015) [12]; Northern Adriatic Sea, Venice Lagoon (45°29.2′N 12°29.4′E), silt, brackish-water lagoon system, 0,8 m: 66 individuals (March 2015) [13]; **Aegean Sea, Greece**: Agiasma Lagoon (40°52.9′N 24°37.1′E), brackish-water lagoon system, 2 m: 25 individuals (date unknown) [14].

Cirrophorus sp. B (see below in the Remarks section): Livorno port (43°34.4′N 10°18.9′E), silt, organically enriched environment with brackish-water inflow, 3 m: 1 individual (April 2016) [10].

DESCRIPTION

Holotype complete, ∼12 mm long, 0.45 mm wide for 126 chaetigers (Figure 1A). Prostomium roughly trapezoidal, slightly longer than wider, antenna short, blister-like, with median insertion, ∼1/6 of the prostomium length; in juveniles prostomial antenna extremely reduced and can be difficult to observe. Eyes absent, nuchal organs small, comma-shaped, difficult to examine in preserved individuals, more conspicuous in living specimens. Three pre-branchial chaetigers, with post-chaetal notopodial lobes gradually increasing in length towards the branchial region; Notopodial lobes are short, basally swollen, skittle shaped in anterior branchial region, then gradually increase in the posterior part, becoming distinctly longer, and tapered in the post-branchial region. Seventy-five pairs of branchiae from chaetiger 4; branchiae pointed, approximately as long as body width in anterior part, ∼6–7 times length of notopodial lobes, then becoming gradually shorter, and in posterior branchial region almost tubercular, shorter than notopodial lobes (Figure 1B). In the additional material examined their number ranges from 24 to 72 pairs, more frequently 40–65; number of branchiae pairs coarsely correlated with size of specimen (Figure 2). Pygidium rounded, with three thread-like, elongated anal cirri, approximately twice the length of last notopodial lobes.

Parapodia biramous, composed of several thick, slightly curved capillaries (Figure 1C); 1–4 (usually 2–3) notopodial lyrate chaetae (Figure 1E) from chaetiger 3. In additional material, some individuals with lyrate chaetae from chaetiger 2. Lyrate chaetae with branches of sub-equal thickness, one
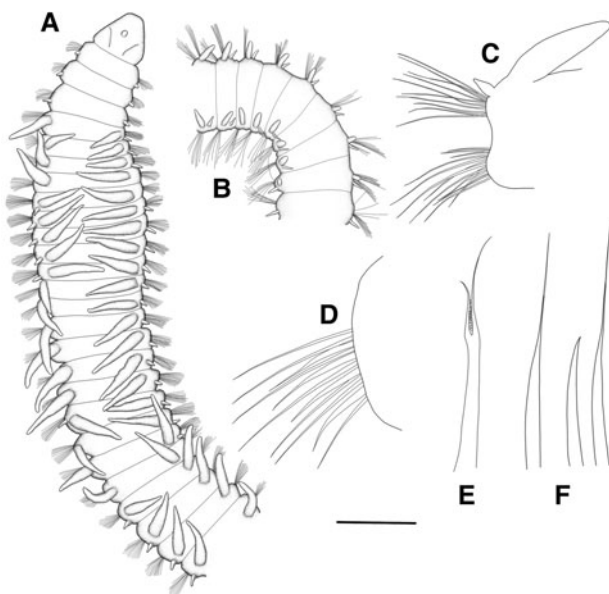

Fig. 1. *Cirrophorus nikebianchii* sp. nov., holotype (MSNP: P/3800): (A) dorsal view of the anterior region; (B) end of the branchial region (71th to 79th chaetiger); (C) left parapodium in the anterior region (6th chaetiger); (D) ventral part of a parapodium in the post-branchial region (110th chaetiger); (E) lyrate notopodial chaeta; (F) thickened neuropodial chaetae from the post-branchial region. Scale bar: 0.3 mm (A, B), 0.1 mm (C), 50 μm (D), 12 μm (E), 30 μm (F).
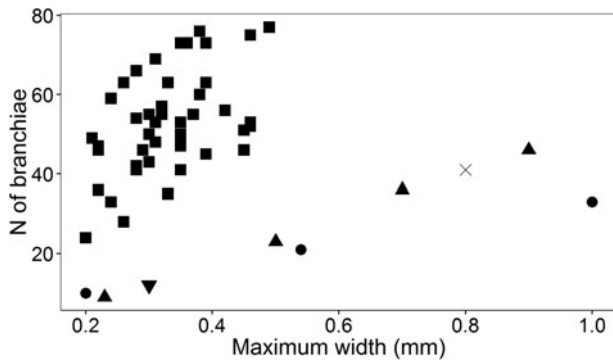
Fig. 2. Relationship between maximum width and number of pairs of branchiae in five *Cirrophorus* species with lyrate notopodial chaetae. Legend: ■: *Cirrophorus nikebianchii* sp. nov. (present study); ▲: *Cirrophorus americanus* Strelzov, 1973 (from Strelzov, 1973); ●: *Cirrophorus furcatus* (Hartman, 1957) (from Strelzov, 1973); X: *Cirrophorus miyakoensis* Imajima, 1973 (from Imajima, 1973); ▼: *Cirrophorus* sp. B (present study).

approximately twice as long as other. Internal edge of each branch with several short, slender spines. Modified neurochaetae from chaetiger 96, gradually increasing in number from 1–2 to 6–7, intermixed with capillaries (Figure 1D). Modified chaetae are thickened capillaries, slightly curved, with or without tapered tips (Figure 1E). Starting point of modified neurochaetae at chaetiger 44–96, not always easy to identify.

Preserved individuals brownish to reddish; live colour bright orange, posterior part of the body often golden-green.

DISTRIBUTION

Mediterranean Sea. Collected in the Balearic Sea, Sea of Sardinia, Sardinian Channel, Tyrrhenian Sea, Adriatic Sea and Aegean Sea (Figure 3).

ECOLOGY

Present in organically enriched environments, from 0.5–3 m depth in brackish-water lagoons and coastal ponds, from 3.5 to ~30 m depth in marine environments. In brackish-water environments it is often associated with *Zostera* and *Nanozostera* meadows, whereas in marine environments its presence is typically related to anthropogenic organic enrichment, such as sewage pollution and fish farms. *Cirrophorus nikebianchii* sp. nov. shows remarkably high densities and a patchy distribution, typical of an opportunistic species occurring in enriched and polluted environments.

ETYMOLOGY

It is an honour to dedicate the new species to Carlo Nike Bianchi, in recognition of his important work on Mediterranean polychaetes.

REMARKS

Currently, the genus *Cirrophorus* Ehlers, 1908 comprises seven valid species; the majority of them are poorly known and need to be re-described. *Cirrophorus branchiatus* Ehlers, 1908, which includes *Cirrophorus lyriformis* (Annenkova, 1934) as a synonym (Strelzov, 1973), and *Cirrophorus aciculatus* (Hartman, 1957) can be easily distinguished due to the presence of thick notopodial acicular chaetae and branchiae starting at chaetiger 5. The remaining species are characterized by the presence of a median antenna in adults (stated to be absent in juveniles or small specimens of *Cirrophorus americanus* Strelzov, 1973, and *Cirrophorus brevicirratus* Strelzov, 1973), three pre-branchial chaetigers and lyrate notopodial chaetae (Table 2). *Cirrophorus nikebianchii* sp. nov. clearly differs from *C. brevicirratus* Strelzov, 1973 in
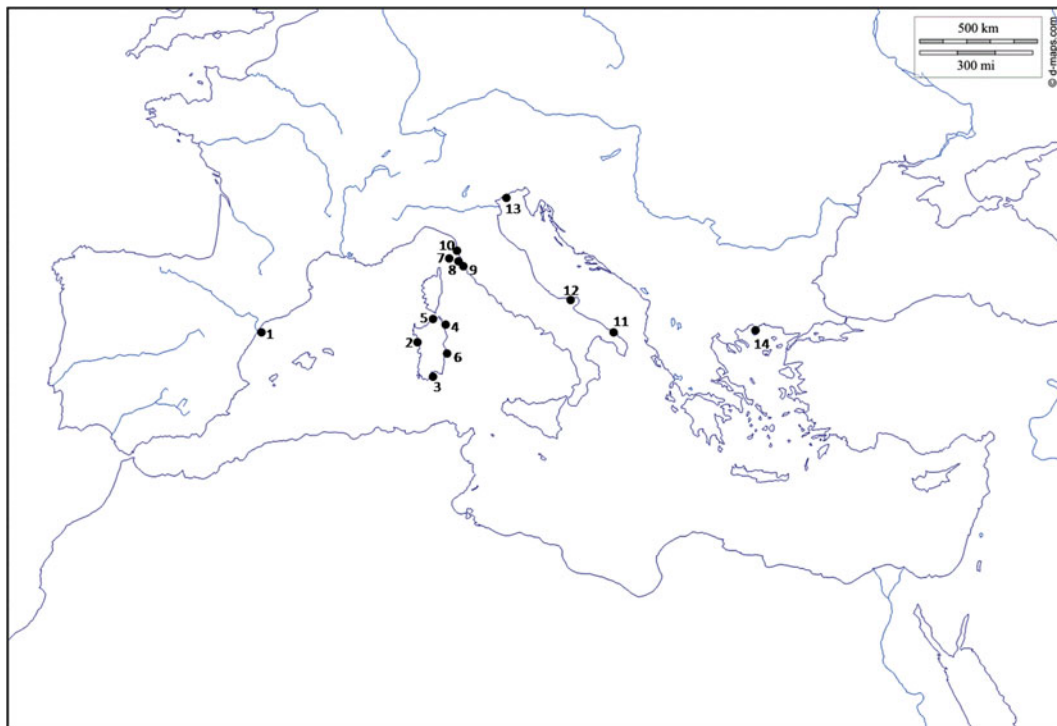

Fig. 3. Known distribution of *Cirrophorus nikebianchii* sp. nov. Localities: 1, Ebro River Delta; 2, Calich Pond (Type locality); 3, Gulf of Cagliari; 4, Gulf of Olbia, 5, Porto Pozzo; 6, Tortolì Pond; 7, Capraia Island; 8, Elba Island; 9, Gulf of Follonica; 10, Livorno port; 11, Acquatina Pond; 12, Varano Lagoon; 13, Venice Lagoon; 14, Agiasma Lagoon. See Systematics for locality details.

Table 2. Comparison among species of *Cirrophorus* Ehlers, 1908 with lyrate chaetae, highlighting the main differences in the most important taxonomic characters.

Species	Antenna (shape)	Antenna : prostomium (length)	Branchiae (pairs)	Notopodial lobes of prebranchial, branchial and postbranchial segments	Starting point of modified notochaetae (chaetiger)	Modified neurochaetae	References
Cirrophorus americanus Strelzov, 1973[a]	Absent of cirriform	1 : 3	9–46	Lobes gradually elongating in pre-branchial chaetigers; lobes long and slender in the branchial and postbranchial regions	2–3	±	Strelzov (1973)
Cirrophorus brevicirratus Strelzov, 1973	Absent or blister-like	<1 : 10	9–15	Very short in the prebranchial region, gradually elongating towards the pygidium	6	−	Strelzov (1973)
Cirrophorus furcatus (Hartman, 1957)	Cirriform	1 : 4	25–33	Relatively long and slender in the prebranchial region, decreasing in size in the branchial region, very short in the postbranchial region	3	−	Hartman (1957); Blake (1996)
Cirrophorus longifurcatus (Hartmann-Schröder, 1965)	Cirriform, elongate	2 : 3	10–22	Very short in the prebranchial region, slender and increasing in length in the branchial region, still elongate but shorter in the postbranchial region	8	−	Hartmann-Schröder (1965)
Cirrophorus miyakoensis Imajima, 1973	Cirriform	1 : 3	39–42	Relatively long and slender in the prebranchial region, decreasing in size in the postbranchial region	2	−	Imajima (1973)
Cirrophorus nikebianchii sp. nov.	Blister-like	1 : 6 or less	24–76 (usually 40–65)	Very short in the prebranchial region, gradually increasing in length in the branchial region, long and slender in the postbranchial region	2–3	+	Present study
Cirrophorus sp. B[b]	Blister-like or cirriform	1 : 6 to 1 : 3	11–27	Short in the prebranchial region, longer and slender in the branchial region, decreasing again in the postbranchial region	2–3	−	Laubier & Ramos (1974); Katzmann & Laubier (1975); present study

[a]Based on the debatable assumption that all material described by Strelzov (1973) should be referred to the same species.
[b]Corresponding to *Cirrophorus* cf. *lyriformis* sensu Laubier & Ramos (1974) and *Cirrophorus furcatus* sensu Katzmann & Laubier (1975).

the shape of the prostomium (triangular and elongated in *C. brevicirratus*, trapezoidal in *C. nikebianchii*), the segment of appearance of lyrate chaetae (chaetiger 6 in *C. brevicirratus*, chaetiger 2–3 in *C. nikebianchii*) and the number of branchiae (up to 14–15 pairs in *C. brevicirratus*, against usual 40–65 pairs in the new species). *Cirrophorus americanus* Strelzov, 1973, *Cirrophorus furcatus* (Hartman, 1957), *Cirrophorus longifurcatus* (Hartmann-Schröder, 1965) and *Cirrophorus miyakoensis* Imajima, 1973 are closer to *Cirrophorus nikebianchii* sp. nov. as regards the shape of the prostomium, but they show some differences, in particular in the shape and size of the antenna. The description of *C. americanus* is ambiguous and could refer to two different species, as the holotype shows the presence of thickened neuropodial chaetae in the posterior chaetigers and lacks the median antenna, whereas the remaining examined material has a well-developed median antenna and lacks modified neuropodial chaetae. Provisionally accepting that the holotype and the remaining material are conspecific, *C. nikebianchii* sp. nov. differs from *C. americanus* in the size of the median antenna (short, blister-like in *C. nikebianchii*, *vs* cirriform and ∼1/3 of the prostomium length in *C. americanus*) and in the higher number of branchiae (up to 46 pairs in *C. americanus*, against 40–65 pairs in the new species). Moreover, in *C. americanus* notopodial lobes in the pre-branchial region are of the same size, while in *C. nikebianchii* sp. nov. they gradually increase in size. As with the new species, *C. furcatus* has lyrate chaetae from the 3rd chaetiger, notopodial lobes of increasing size in the pre-branchial region and a short median antenna; nevertheless, this species shows a remarkably larger size (up to 1 mm), a distinctly lower number of branchiae (up to 33 pairs), a slender and longer cirriform antenna, and neuropodial thickened chaetae are absent. In addition, in *C. furcatus* the size of the notopodial lobes decreases towards the pygidium (Blake, 1996), whereas it increases in *C. nikebianchii* sp. nov. *Cirrophorus longifurcatus*, a species known from Chile, and rarely reported after the original description, has a long prostomial antenna, almost reaching the 2nd chaetiger (Hartmann-Schröder, 1965), whereas it is very short, blister-like in the new species, and there is a large difference in length between the notopodial lobes in the pre-branchial and branchial region, compared with similar sized in *C. nikebianchii* sp. nov. Lastly, *C. miyakoensis* is similar to *C. nikebianchii* sp. nov., but the median antenna and notopodial lobes are distinctly slender, cirriform, and the size of the notopodial lobes decreases towards the pygidium (whereas it increases in *C. nikebianchii* sp. nov.). The Japanese species also shows a lower number of branchiae (up to 42 pairs for 0.8 mm maximum width). Another Mediterranean morphotype differs from *C. nikebianchii* sp. nov. mainly in the number of branchiae (usually 15–22 pairs) and in the pattern of notopodial lobes, that become very short in the posterior part of the body (whereas in *C. nikebianchii* they remain long and slender). This morphotype has been collected until now only in a marine organically enriched environment and morphologically corresponds to *Cirrophorus lyriformis sensu* Laubier & Ramos (1974) and to *Cirrophorus furcatus sensu* Katzmann & Laubier (1975). Since molecular data did not support its assignment to *Cirrophorus nikebianchii* sp. nov., and given the scarce material available, we provisionally consider it as a putative new species, *Cirrophorus* sp. B.

Within the genus *Cirrophorus*, *C. nikebianchii* sp. nov. can be easily identified based on the extremely small size of the median antenna, the relatively small body size (maximum width = 0.5 mm) and the high number of branchiae (up to more than 70 in large individuals, and an average number of 40–65). The ratio between the number of branchiae and maximum width of the animal is similar for *C. americanus*, *C. furcatus*, *C. miyakoensis* and *Cirrophorus* sp. B, but it is clearly different in *C. nikebianchii* sp. nov. (Figure 2). Finally, the occurrence in brackish-water environments at very shallow depths seems to be a peculiarity of this species.

Two species of *Paradoneis*, namely *Paradoneis eliasoni* Mackie, 1991 and *Paradoneis strelzovi* de Léon-González & Díaz-Castañeda, 2011, resemble *C. nikebianchii* sp. nov. in possessing both notopodial lyrate and neuropodial thickened chaetae. However, in both species there are only single (rather than multiple) thickened chaetae in each posterior neuropodium. In addition, both completely lack a median antenna, and have much fewer branchiae (7–12 and 6–7 pairs) than the new species. *Paradoneis eliasoni* and *C. nikebianchii* sp. nov., both have long anal cirri, but the former has tuberculate (rather than slender projecting) pre-branchial notopodial lobes. *Paradoneis strelzovi* differs markedly from the new species in having very short anal cirri.

MOLECULAR PHYLOGENETIC ANALYSIS

We obtained sequences of 471 bp for 16S rDNA (GenBank accession numbers: KX901418 to KX901433), 670 bp for COI (GenBank accession numbers: KX901434 to KX901446), and 1790 bp for 18S rDNA (GenBank accession numbers: KX901405 to KX901417). The best fitting nucleotide substitution models were GTR + G for 16S rDNA, and GTR + I + G for COI and 18S rDNA. The tree showed the presence of a well supported clade (clade I: posterior probability, PP = 1) with two separated lineages that corresponded morphologically to *Paradoneis ilvana*, a third one relative to *Paradoneis armata* (PP = 1), and a fourth with *Cirrophorus* sp. B (Figure 4). Another well supported clade (clade II: PP = 1) included *Paradoneis lyra*, *C. furcatus* and *C. nikebianchii* sp. nov. (Figure 4). *Paradoneis lyra* individuals, in their turn, grouped in a strongly supported clade (PP = 1) as well as *C. furcatus* and *C. nikebianchii* (PP = 1). Although *C. furcatus* and *C. nikebianchii* are very close and represent a well supported clade, the divergence between the two lineages is comparable to that observed between different species, thus supporting the distinction at species level. In a third clade *Paraonis fulgens*, and *Aricidea fragilis* grouped with high statistical support (clade III: PP = 1). *Levinsenia gracilis* represents the sister group of all remaining Paraonidae with high statistical support (PP = 1). The position of *C. branchiatus*, and the relationships among the three clades, are however not resolved in our reconstruction (Figure 4).

DISCUSSION

According to our results, the doubts on the identity of the Mediterranean individuals identified as *Cirrophorus* nr *furcatus* (Castelli *et al.*, 1995) were well founded, since both morphological and molecular data supported the distinction between *Cirrophorus nikebianchii* sp. nov. and *Cirrophorus furcatus*. It is noteworthy, however, that not all Mediterranean reports of *C. furcatus* can be referred to *C. nikebianchii*; in particular, the descriptions of individuals referred to *C. furcatus* (or to *C. lyriformis*) in fully marine
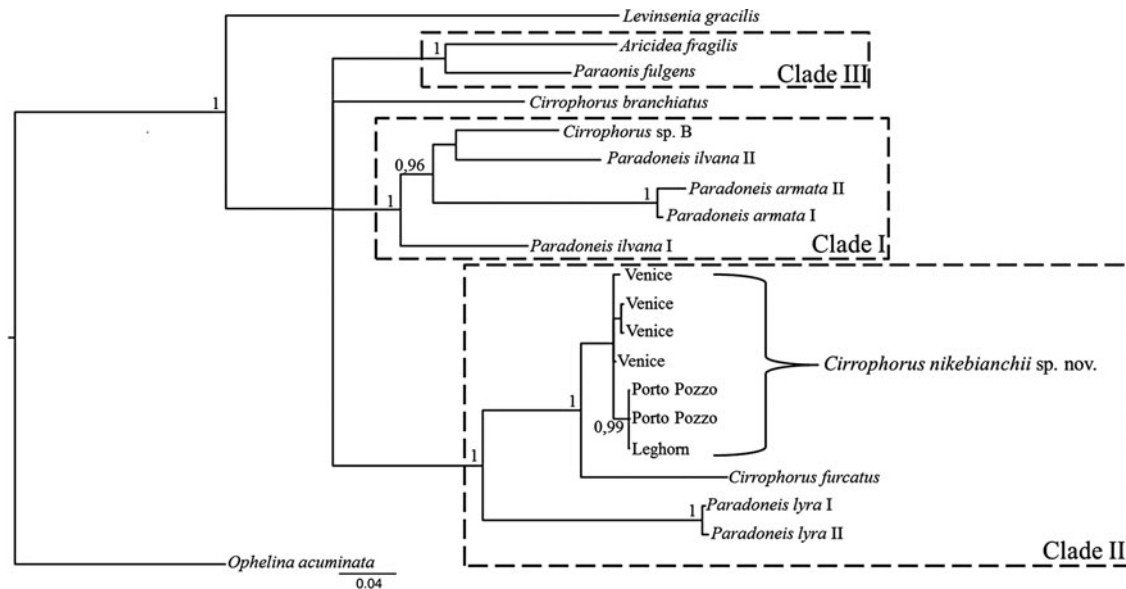
Fig. 4. Bayesian tree obtained from the concatenated 16S rDNA, 18S rDNA and COI sequences. Node values are Bayesian posterior probabilities; only statistically significant values are reported.

environments (Laubier, 1966; Laubier & Ramos, 1974; Katzmann & Laubier, 1975) are morphologically different from *C. nikebianchii*, mainly as regards the number of pairs of branchiae (11–27 *vs* 24–75, respectively) and the length of the antenna. These individuals appear more similar to *Cirrophorus* sp. B. Unfortunately, the material available for this last putative species is scarce and not sufficient for a formal species description. On the other hand, the extremely small size of the median antenna led several authors to misidentify *C. nikebianchii* sp. nov. as *Paradoneis lyra*. However, *P. lyra* lacks thickened neuropodial chaetae (Mackie, 1991). Such chaetae are present in *P. eliasoni* from northern European waters, but they are single, not multiple, and this species has not been found in the Mediterranean as yet. Shallow-water individuals, and in particular brackish-water records of *P. lyra* in the Mediterranean Sea probably can all be referred to *C. nikebianchii*. *Paradoneis lyra* is probably less widespread in the Mediterranean than commonly stated, and its distribution could be restricted to circalittoral and epibathyal bottoms.

The distinction between *Cirrophorus* Ehlers, 1908 and *Paradoneis* Hartman, 1965, based on the presence of a median antenna in the former genus and absence in the latter, was questioned by Strelzov (1968, 1973), who observed that in some species, such as *Cirrophorus americanus* and *Cirrophorus brevicirratus*, the antenna is present only in large adults, and absent in juveniles. The proposal of a synonymy between *Cirrophorus* and *Paradoneis*, however, was rejected by most of the subsequent authors (e.g. Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Mackie, 1991; Blake, 1996, 2016; Aguirrezabalaga & Gil, 2009). Recently, Reuscher (2013) carried out a morphological cladistic analysis of Paraonidae and identified a clade including all species of *Paradoneis* and *Cirrophorus*. This cladistic analysis highlighted that the median antenna characteristic of *Cirrophorus* was acquired and lost several times in the evolutionary history of the clade and, therefore, it could not be considered a useful taxonomic character. In Reuscher's (2013) morphologically based phylogenetic reconstruction,

the species with notopodial spines, *Paradoneis spinifera* (Hobson, 1972) and *Paradoneis drachi* Laubier & Ramos, 1974, were the sister group of the remaining *Cirrophorus/Paradoneis* clade, and Reuscher (2013) assigned these two species to a new genus yet to be described. Species without notopodial modified chaetae and median antenna were assigned to the genus *Paraonides* Cerruti, 1909 and are basal to the whole *Cirrophorus/Paradoneis* clade. Molecular data from our work did not support this reconstruction, and the tree obtained showed a very different topology, though the 'Paradoneis-Cirrophorus' clades in each analysis contained taxa with and without median antennae. Doubts about the reliability of the median antenna as a defining character led Strelzov (1973) to synonymize *Paradoneis* with *Cirrophorus*, and Reuscher (2013) came to the same conclusion following his analyses. In our analysis, species assigned to the genera *Cirrophorus* and *Paradoneis* were distributed in two different clades with high statistical support: the first clade includes *C. furcatus*, *C. nikebianchii* and *P. lyra* and the second clade includes *Paradoneis armata* Glémarec, 1966, two strongly divergent lineages that were morphologically assigned to *Paradoneis ilvana* Castelli, 1985, and *Cirrophorus* sp. B, this last probably representing an undescribed species. *Cirrophorus branchiatus* Ehlers, 1908 does not belong to either of the clades. Therefore the *Cirrophorus/Paradoneis* group was paraphyletic and the relationship between all the clades in our tree was unresolved.

Incongruence between molecular and morphological data has been observed in a number of polychaete studies (e.g. Bleidorn, 2005; Zanol *et al.*, 2014). Even though the results of the molecular phylogenetic reconstruction applied to the *Cirrophorus/Paradoneis* group were quite striking and statistically well-supported, we prefer to adopt a conservative approach, awaiting for a more complete phylogenetic reconstruction involving more taxa, and preferably based on both molecular and morphological data. However, some nomenclatural notes on the genera *Cirrophorus*, *Paradoneis* and *Paraonides* are in our opinion useful in anticipation of a necessary taxonomic revision of the family Paraonidae.

The genus *Cirrophorus* Ehlers, 1908 was created for *C. branchiatus*, and subsequently considered as a subgenus of *Aricidea* Webster, 1879, which also had a median antenna (Hartman, 1957). This taxonomic arrangement was questioned by Day (1963) and Laubier (1966) and lastly rejected by Strelzov (1968), who stressed the similarity between *Cirrophorus* and *Paradoneis*. On the other hand, *Paradoneis* Hartman, 1965 was created for species lacking median antenna and with notopodial modified chaetae, with *Paraonis* (*Paraonides*) *lyra* Southern, 1914 as type species. The genus *Paraonides* Cerruti, 1909, created as subgenus of *Paraonis* Cerruti, 1909 with *Paraonis* (*Paraonides*) *neapolitana* Cerruti, 1909 as type species, was initially used for species with notopodial modified chaetae (Southern, 1914; Fauvel, 1927), but its diagnosis was subsequently emended (Hartman, 1965) and this genus was used to define species without modified chaetae. The question of the correct use of *Paraonides* has been long debated, and the main problem regarding this issue is the uncertain identity of the type species. In fact, *P. neapolitana* was described for the Gulf of Naples as a species without median antenna, with three pre-branchial chaetigers, nine pairs of branchiae and modified notopodial chaetae from chaetiger 12 (Cerruti, 1909). The notopodial modified chaetae were apparently shorter than the capillaries, somewhat thicker and leaf-shaped, even though this kind of modified chaetae has never been reported in later descriptions of Paraonidae. Strelzov (1973) demonstrated that leaf-shaped chaetae could be an artefact of the fixation of lyrate chaetae in Canada balsam, since balsam and chaetae have a very similar refraction index that could prevent a clear distinction of the two branches of the lyrate chaeta. For this reason, Strelzov (1973) considered *Paraonides* a synonym of *Cirrophorus* and *Paradoneis*. Since the type material is lost (Castelli, 1987), Strelzov (1973) re-described *Cirrophorus neapolitanus* on the basis of North and South Atlantic, and Black Sea individuals; an act considered highly questionable without the examination of topotypic material (Katzmann & Laubier, 1975). The rejection of this re-description maintained *Paraonides* as diagnosed by Hartman & Fauchald (1971), even with the possibility that Strelzov's (1973) remarks on the peculiar chaetae of *P. neapolitana* were correct. Topotypic material in good condition would be necessary to properly clarify the identity of *P. neapolitana*. It may be noted, however, that this species has never been re-described in any taxonomic work from the Mediterranean (Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Castelli, 1985; Çinar *et al.*, 2011; Çinar & Dağli, 2013). In recent years this species has been cited only in species checklists of soft bottom ecology works (Gambi *et al.*, 1998; Simonini *et al.*, 2007; De Biasi & Pacciardi, 2008). Thus, it is likely that *P. neapolitana* is actually a species with lyrate chaetae, and that the genus *Paraonides* is not suitable for species lacking of modified notopodial chaetae. Because of the unclear identity of the type species of *Paraonides*, we suggest to precautionarily use *Paraonella* Strelzov, 1973 for species lacking both notopodial and neuropodial modified chaetae and prostomial antenna, although we recognize that this group may turn out to be artificial, since the loss of modified chaetae could have happened several times in the evolutionary history of Paraonidae.

The paraphyletic condition of the *Cirrophorus/Paradoneis* group makes taxonomic revisions challenging. In our analysis, the type species of *Cirrophorus* was not included in any of the two highly supported clades and its relationships with the other groups within Paraonidae were unclear. Moreover, the uncertainty about the identity of *Paraonides neapolitana* does not allow us to settle the question of its relationships or possible synonymy with *Paradoneis*. As observed in other polychaete families, morphological traits of the family Paraonidae may be misleading with regard to the actual evolutionary history of the group, and their evolutionary meaning should be, therefore, critically evaluated. An important contribution to Paraonidae systematics can be provided by molecular tools alongside critical morphological investigations in order to obtain a more sound classification of this taxonomically complex family.

REFERENCES

Aguirrezabalaga F. and Gil J. (2009) Paraonidae (Polychaeta) from the Capbreton Canyon (Bay of Biscay, NE Atlantic) with the description of eight new species. *Scientia Marina* 73, 631–666.

Akaike H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19, 716–723.

Annenkova N.P. (1934) Paraoniden der Meeren des Fernen Osten der USSR. *Doklady Akademii Nauk SSSR* 3, 656–658.

Blake J.A. (1996) Family Paraonidae Cerruti, 1909. In Blake J.A., Hilbig B. and Scott P.H. (eds) *Taxonomic atlas of the benthic fauna of the Santa Maria Basin and Western Santa Barbara Channel. 6 – The Annelida Part 3. Polychaeta: Orbiniidae to Cossuridae.* Santa Barbara, CA: Santa Barbara Museum of Natural History, pp. 27–70.

Blake J.A. (2016) Paraonidae Cerruti, 1909. In Westheide W. and Purschke G. (eds) *Handbook of zoology online, Annelida: polychaetes edition.* Berlin: De Gruyter, pp. 6–52.

Bleidorn C. (2005) Phylogenetic relationships and evolution of Orbiniidae (Annelida, Polychaeta) based on molecular data. *Zoological Journal of the Linnean Society* 144, 59–73.

Bonvicini Pagliai A.M. and Cognetti G. (1982) Ecology of polychaetes in the Orbetello lagoon (Tuscany). *Bollettino di Zoologia* 49, 51–60.

Cadman P.S. and Nelson-Smith A. (1990) Genetic evidence for two species of lugworm (*Arenicola*) in South Wales. *Marine Ecology Progress Series* 64, 107–112.

Carr C.M., Hardy S.M., Brown T.M., Macdonald T.A. and Hebert P.D.N. (2011) A tri-oceanic perspective: DNA barcoding reveals

geographic structure and cryptic diversity in Canadian polychaetes. *PLoS ONE* 6, e22232.

Castelli A. (1985) Paraonidae (Annelida, Polychaeta) des fonds meubles infralittoraux des côtes toscanes. *Cahiers de Biologie Marine* 26, 267–279.

Castelli A. (1987) Censimento dei polcheti dei mari italiani: Paraonidae Cerruti, 1909. *Atti della Società Toscana di Scienze Naturali, Memorie, Serie B* 94, 319–340.

Castelli A., Abbiati M., Badalamenti F., Bianchi C.N., Cantone G., Gambi M.C., Giangrande A., Gravina M.F., Lanera P., Lardicci C., Somaschini A. and Sordino P. (1995) Annelida Polychaeta, Pogonophora, Echiura, Sipuncula. In Minelli A., Ruffo S. and La Posta S. (eds) *Checklist delle specie della fauna italiana*. Bologna: Calderini, Volume 19, 45 pp.

Castelli A. and Lardicci C. (1985) Note preliminari allo studio dei Polcheti della Baia di Portoferraio (Isola d'Elba). *Oebalia* 11, 763–766.

Cerruti A. (1909) Contributo all'anatomia, biologia e sistematica delle Paraonidae (Levinsenidae), con particolare riguardo alle specie del golfo di Napoli. *Mitteilungen aus der zoologischen Station zu Neapel* 19, 459–512.

Chessa L.A., Scardi M., Serra S., Pais A., Lanera P., Plastina N., Valiante L.M. and Vinci D. (2007) Small-scale perturbation on soft bottom macrozoobenthos after mechanical cleaning operations in a Central-Western Mediterranean lagoon. *Transitional Waters Bulletin* 2, 9–19.

Çinar M.E. and Dağli E. (2013) Polychaetes (Annelida: Polychaeta) from the Aegean and Levantine coasts of Turkey, with descriptions of two new species. *Journal of Natural History* 47, 911–947.

Çinar M.E., Dağli E. and Açik S. (2011) Annelids (Polychaeta and Oligochaeta) from the Sea of Marmara, with descriptions of five new species. *Journal of Natural History* 45, 2105–2143.

Çinar M.E., Dağli E. and Kurt Şahin G. (2014) Checklist of the Annelida from the coasts of Turkey. *Turkish Journal of Zoology* 38, 734–764.

Como S., Rossi F. and Lardicci C. (2004) Response of deposit-feeders to exclusion of epibenthic predators in a Mediterranean intertidal flat. *Journal of Marine Biology and Ecology* 303, 157–171.

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

Day J.H. (1963) Polychaete Fauna of South Africa: Part 7. Species from depths between 1000 and 3330 meters west of Cape Town. *Annals of the South African Museum* 46, 353–371.

De Biasi A.M. and Pacciardi L. (2008) Macrobenthic communities in a fishery exclusion zone and in a trawled area of the middle Adriatic Sea (Italy). *Ciencias Marinas* 34, 433–444.

de Léon-González J.A. and Díaz-Castañeda V. (2011) A new species of *Paradoneis* (Polychaeta: Paraonidae) from the western coast of Baja California, Mexico. *Proceedings of the Biological Society of Washington* 124, 40–44.

Ehlers E. (1908) Die bodensässigen Anneliden aus den Sammlungen der deutschen Tiefsee-Expedition. *Wissenschaftliche Ergebnisse der deutschen Tiefsee – Expedition auf dem Dampfer 'Valdivia' 1898–1899*, 16, 1–168.

Fauvel P. (1927) *Polychètes sédentaires*, Faune de France 16 edition. Paris: Lechevalier, 494 pp.

Folmer O., Black M., Hoeh W., Lutz R. and Vrijenhoek R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3, 294–299.

Gambi M.C., Conti G. and Bremec C.S. (1998) Polychaete distribution, diversity and seasonality related to seagrass cover in shallow soft bottoms of the Tyrrhenian Sea (Italy). *Scientia Marina* 62, 1–17.

Glémarec M. (1966) Paraonidae de Bretagne: description de *Paradoneis armata* nov. sp. *Vie et Milieu, Ser. A* 17, 1045–1052.

Grube A.E. (1873) Über ein paar neue Anneliden aus der Familie der Spiodeen. *Jahres-Bericht der Schlesichen Gesellschaft für vaterländische Cultur* 50, 57–58.

Guindon S. and Gascuel O. (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52, 696–704.

Hall T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series* 41, 95–98.

Hartman O. (1957) Orbiniidae, Apistobranchidae, Paraonidae and Longosomidae. *Allan Hancock Pacific Expeditions* 15, 211–393.

Hartman O. (1965) Deep-water benthic polychaetous annelids off New England to Bermuda and other North Atlantic areas. *Occasional Papers of the Allan Hancock Foundation* 28, 1–378.

Hartman O. and Fauchald K. (1971) Deep-water benthic polychaetous annelids off New England to Bermuda and other North Atlantic Areas. Part II. *Allan Hancock Monographs in Marine Biology* 6, 1–327.

Hartmann-Schröder G. (1965) Die Polychaeten des Sublitorals. In Hartmann-Schröder G. and Hartmann G. (eds) *Zur Kenntnis des Sublitorals der chilenischen Küste unter besonderer Berücksichtigung der Polychaeten und Ostracoden. (Mit bemerkungen über den Einfluss sauerstoffarmer Strömungen auf die Besiedlung von marinen Sedimenten.).* Hamburg: Mitteilungen aus dem Hamburgischen zoologischen Museum und Institut, Volume 62, pp. 59–305.

Hobson K.D. (1972) Two new species and two new records of the family Paraonidae (Annelida, Polychaeta) from the northeastern Pacific Ocean. *Proceedings of the Biological Society of Washington* 85, 549–556.

Imajima M. (1973) Paraonidae (Polychaeta) from Japan. *Bulletin of the National Scientific Museum of Tokyo* 16, 253–292.

Katzmann W. and Laubier L. (1975) Paraonidae (Polychètes Sédentaires) de l'Adriatique. *Annales der Naturhistorischen Museum in Wien* 79, 567–588.

Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J. and Higgins D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23, 2947–2948.

Laubier L. (1966) Sur la présence du genre *Cirrophorus* (Polychètes, Paraonidae) en Méditerranée. *Bulletin de la Société Zoologique de France* 90, 469–477.

Laubier L. (1967) Sur quelques *Aricidea* (Polychètes, Paraonidae) de Banyuls-sur-Mer. *Vie et Milieu, Ser. A.* 18, 99–132.

Laubier L. and Ramos J. (1974) Paraonidae (Polychètes Sédentaires) de Méditerranée. *Bulletin du Muséum National d'Histoire Naturelle Ser. 3* 168(Zool. 113), 1097–1148.

Levinsen G.M.R. (1884) Systematisk-geografisk Oversigt over de nordiske Annulata, Gephyrea, Chaetognathi og Balanoglossi. *Videnskabelige Meddelelser fra den naturhistoriske Forening i Kjöbenhavn* 45, 92–350.

Lorenz T.C. (2012) Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. *Journal of Visualized Experiments* 63, e3998.

Mackie A.S.Y. (1991) *Paradoneis eliasoni* sp. nov. (Polychaeta: Paraonidae) from northern European waters, with a redescription of *Paradoneis lyra* (Southern, 1914). In Petersen M.E. and Kirkegaard J.B. (eds) *Systematics, biology and morphology of world Polychaeta.*

Proceedings of the 2nd International Polychaete Conference, Copenhagen, 1986. Ophelia Suppl. 5, 147–155.

Maggiore F. and Keppel E. (2007) Biodiversity and distribution of polychaetes and molluscs along the Dese estuary (Lagoon of Venice, Italy). *Hydrobiologia* 588, 189–203.

Maltagliati F., Camilli L., Lardicci C. and Castelli A. (2001) Evidence for morphological and genetic divergence in *Perinereis cultrifera* (Polychaeta: Nereididae) from two habitat types at Elba Island. *Journal of the Marine Biological Association of the United Kingdom* 81, 411–414.

Maltagliati F., Casu M. and Castelli A. (2004) Morphological and genetic evidence supports the existence of two species in the genus *Ophelia* (Annelida, Polychaeta) from the Western Mediterranean. *Biological Journal of the Linnean Society* 83, 101–113.

Maltagliati F., Casu M., Lai T., Iraci Sareri D., Casu D., Curini-Galletti M., Cantone G. and Castelli A. (2005) Taxonomic distinction of *Ophelia barquii* and *O. bicornis* (Annelida, Polychaeta) in the Mediterranean as revealed by ISSR markers and the number of nephridiopores. *Journal of the Marine Biological Association of the United Kingdom* 85, 835–841.

Medlin L., Elwood H.J., Stickel S. and Sogin M.L. (1988) The characterization of enzymatically amplified 16Slike rRNA-coding regions. *Gene* 71, 491–499.

Mesnil F. (1897) Études de morphologie externe chez les Annélides. II. Remarques complémentaires sur les Spionidiens. La famille nouvelle des Disomidiens. La place des *Aonides* (sensu Tauber, Levinsen). *Bulletin scientifique de la France et de la Belgique* 30, 83–100, pl. 3.

Montesanto G. (2015) A fast GNU method to draw accurate scientific illustrations for taxonomy. *Zookeys* 515, 191–206.

Nygren A. and Pleijel F. (2011) From one to ten in a single stroke – resolving the European *Eumida sanguinea* (Phyllodocidae, Annelida) species complex. *Molecular Phylogenetics and Evolution* 58, 132–141.

Örsted A.S. (1843) *Annulatorum danicorum conspectus. Volume Fasc. 1 Maricolae (Quaestio ab universitate Hafniensis ad solvendum proposita et proemio ornate)*. Hafniae (Copenhagen): Librariae Wahlianae, 52 pp.

Palumbi S., Martin A., Romano S., McMillan W.O., Stice L. and Grabowski G. (1991) *The simple fool's guide to PCR, version 2.0*. Honolulu: Department of Zoology and Kewalo Marine Laboratory, University of Hawaii.

Petroni G., Dini F., Verni F. and Rosati G. (2002) A molecular approach to the tangled intrageneric relationships underlying phylogeny in *Euplotes* (Ciliophora, Spirotrichea). *Molecular Phylogenetics and Evolution* 22, 118–130.

Reuscher M.G. (2013) Cladistic analysis of the family Paraonidae (Annelida: Polychaeta) and the genera *Cirrophorus* and Paradoneis based on morphological characters. In Reuscher M.G. (ed.) *Biodiversity, ecology, and natural history of polychaetous annelids from the Gulf of Mexico. PhD thesis*. Corpus Christi University, Corpus Christi, TX, pp. 192–237.

Ronquist F., Teslenko M., van der Mark P., Ayres D., Darling A., Höhna S., Larget B., Liu L., Suchard M.A. and Huelsenbeck J.P. (2011) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61, 539–542.

Rossi F. and Lardicci C. (1995) Evoluzione delle comunità zoobentoniche di fondo molle della Laguna di Orbetello: variazioni nella composizione specifica. *Atti della Società Toscana di Scienze Naturali, Memorie, Ser. B* 102, 31–36.

Rouse G.W. and Fauchald K. (1997) Cladistics and polychaetes. *Zoologica Scripta* 26, 139–204.

Sardá R., Gil J., Taboada S. and Gili J.M. (2009) Polychaete species captured in sediment traps moored in northwestern Mediterranean submarine canyons. *Zoological Journal of the Linnean Society* 155, 1–21.

Schirosi R., Musco L. and Giangrande A. (2010) Benthic assemblage of Acquatina Lake (South Adriatic Sea): present state and long-term faunistic changes. *Scientia Marina* 74, 235–246.

Simboura N., Reizopoulou S., Arvanitidis C. and Basset A. (2007) New information on the biogeographical and ecological distribution of *Pionosyllis anophthalma* Capaccioni & San Martín, 1989 (Polychaeta, Syllidae). *Transitional Waters Bulletin* 4, 53–58.

Simonini R., Ansaloni I., Bonini P., Grandi V., Graziosi F., Iotti M., Massamba N'Siala G., Mauri M., Montanari G., Preti M., De Nigris N. and Prevedelli D. (2007) Recolonization and recovery dynamics of the macrozoobenthos after sand extraction in relict sand bottoms of the Northern Adriatic Sea. *Marine Environmental Research* 64, 574–589.

Southern R. (1914) Clare Island Survey. Archiannelida and Polychaeta. *Proceedings of the Royal Irish Academy* 31, 1–160.

Strelzov V.E. (1968) *Polychaete worms of the family Paraonidae (Polychaeta, Sedentaria) from the Barents Sea*. Trudy *Murmanskogo biologicheskogo instituta*, Volume 17, 74–95. [In Russian]

Strelzov V.E. (1973) *Polychaete worms of the family Paraonidae Cerruti, 1909 (Polychaeta, Sedentaria)*. Moscow: Akademia Nauk, 170 pp. [In Russian]

Tauber P. (1879) *Annulata Danica. En kritisk revision af de i Danmark fundne Annulata Chaetognatha, Gephyrea, Balanoglossi, Discophoreae, Oligochaeta, Gymnocopa og Polychaeta*. Copenhagen: Reitzel, 143 pp.

Webster H.E. (1879) The Annelida Chaetopoda of the Virginian Coast. *Transactions of the Albany Institute* 9, 202–269.

Wu B., Qian P.-Y. and Zhang S. (1991) Morphology, reproduction, ecology and allozyme electrophoresis of three *Capitella* sibling species in Qingdao (Polychaeta: Capitellidae). In Petersen M.E. and Kirkegaard J.B. (eds) *Systematics, biology and morphology of world Polychaeta. Proceedings of the 2nd International Polychaete Conference, Copenhagen, 1986. Ophelia* Suppl. 5, 391–400.

and

Zanol J., Halanych K.M. and Fauchald K. (2014) Reconciling taxonomy and phylogeny in the bristleworm family Eunicidae (polychaete, Annelida). *Zoologica Scripta* 43, 79–100.

Correspondence should be addressed to:
J. Langeneck
Department of Biology, University of Pisa, via Derna 1, 56126 Pisa, Italy
email: jlangeneck@biologia.unipi.it