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# A new species of *Perinereis* Kinberg, 1865 (Annelida: Nereididae) from the Western Mediterranean Sea revealed by morphological and molecular approaches

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## Abstract

This study uses morphological and molecular evidence to describe a new intertidal species of *Perinereis* Kinberg, 1865 (Annelida: Nereididae) from northeast Algeria. *Perinereis louizomarum* n. sp. belongs to Subgroup 1A, which is distinguished by a single bar-shaped paragnath on area VI of the proboscis and a dorsal ligule that is either slightly or not expanded in the posterior parapodia. The new species differs from *P. cultrifera* and similar congeners by having a bare area V and slightly enlarged posterior dorsal ligules. Furthermore, using newly generated and mined sequences from online databases, and mined sequences of *Perinereis* species from online, the analysis of cytochrome c oxidase subunit 1 (COI) mitochondrial gene sequences supported its status as new species. An identification key of the *Perinereis* species belonging to Subgroup 1A is also provided.

**Keywords:** Algeria; description; mtCOI; *Perinereis cultrifera*; Systematics.

## Introduction

In terms of environmental and socio-economic importance, *Perinereis* Kinberg, 1865 is possibly the most important nereidid genus. Species such as *P. aibuhitensis* (Grube, 1878), *P. cultrifera* (Grube, 1840), and *P. linea* (Treadwell, 1936) are utilized in fishing and aquaculture industries (Lee *et al*., 1992; Rouabah, 2003; Arias *et al.,* 2013), whereas some others, for instance, *P. gualpensis* (Jeldes, 1963) and *P. nuntia* (Savigny *in* Lamarck, 1818), are regarded as biomarkers in aquatic environmental contamination (Díaz-Jaramillo *et al*., 2010; Rhee *et al*., 2012). *Perinereis* species can also be found in a wide range of marine ecosystems, from shallow waters to abyssal zones (Wu *et al.,* 1985), although some dwell in estuarine or even semi-terrestrial environments (Glasby *et al*., 2009; Villalobos-Guerrero *et al*., 2021).

To facilitate practical identification, this heterogeneous and speciose nereidid genus has traditionally been divided into six informal morphological groups (1A, 1B, 2A, 2B, 3A, and 3B) (Hutchings *et al*., 1991). In particular, the *Perinereis* Subgroup 1A (hereafter SG-1A) was proposed for species that share the presence of a single bar-shaped paragnath on area VI and posterior chaetigers with dorsal ligules slightly or not expanded; twelve of the species are currently valid: *Perinereis arabica* (Mohammad, 1971) from Kuwait, *P. calmani* (Monro, 1926) from Australia and Macclesfield Bank (South China Sea), *P. cultrifera* (Grube, 1840) from the Tyrrhenian Sea (Italy), *P. dongalae* (Horst, 1924) from Indonesia, *P. falsovariegata* (Monro, 1933) from South Africa, *P. floridana* (Ehlers, 1868) from the Gulf of Mexico (USA), *P. helleri* (Grube, 1878) from the Philippines, *P. rullieri* (Pilato, 1974) from the Mediterranean Sea (Italy), *P. taorica* (Langerhans, 1881) from Madeira, *P. tenuisetis* (Fauvel, 1915) from the Mediterranean Sea (Italy), *P. villalobosi* (Rioja, 1947) from the Gulf of California, and also *P. capensis* (Kinberg, 1865) from South Africa, which was formerly overlooked by Hutchings *et al.* (1991).

*Perinereis cultrifera* is the most studied and culturally significant *Perinereis* SG-1A species in the Mediterranean Sea, particularly Algeria. It is widely exploited on the Northeast Algerian coast as bait in sport angling and recreational fishing for sea bream (*Dicentrarchus labrax*), sole (*Solea solea*), and other varieties of fishes, such as pageot (*Pagellus erythrinus*), small wolf (*Dicentrarchus *labrax*) and marbled (*Lithognathus mormyrus*) (Rouabah, 2003). However, Scaps *et al.* (2000) and Rouabah & Scaps (2003a) revealed that *P. cultrifera* might be a complex of species based on the morphological and biochemical divergence of specimens collected in Saint-Cloud, Annaba (Algeria) and from Saint-Aubin-sur-Mer, Normandy (English Channel). Similarly, Rouabah *et al.* (2009) also found that the spawning season, mode of reproduction, age of maturity, and biometric characteristics of specimens assigned to *P. cultrifera* differ according to the geographic location of the populations in question. Hence, it is likely that two (or more) species are involved under the same species name, *P. cultrifera*, in the Mediterranean Sea and the eastern North Atlantic.

Moreover, a total of eight species of *Perinereis* have been reported from the Mediterranean Sea: *Perinereis cultrifera* (Grube, 1840) from the Tyrrhenian Sea, Italy; *P. floridana* (Ehlers, 1868) from Florida, USA; *P. linea* (Treadwell, 1936) from Xiamen, China; *P. macropus* (Claparède, 1870) from the Tyrrhenian Sea, Italy; *P. marionii* (Audouin & Milne Edwards, 1833) from Bay of Biscay; *P. oliveirae* (Horst, 1889) from Portugal; *P. rullieri* (Pilato, 1974), and *P. tenuisetis* (Fauvel, 1915) from Sicily, Italy (Fauvel, 1915; Prevedelli *et al.,* 1990; Scaps *et al*., 2000; Rouabah & Scaps 2003a,b; Rouabah & Rouabah 2007; Rouhi *et al*., 2008; Arias *et al*., 2013; Meghlaoui *et al.,* 2015; Gasmi *et al*., 2016; Gillet, 2017; Bakalem *et al.,* 2020; Villalobos-Guerrero *et al*., 2021; Rezzag-Mahcene *et al*., 2022). Only four species (*P. cultrifera*, *P. macropus*, *P. rullieri*, and *P. tenuisetis*) were originally described from the Mediterranean Sea. Two other species have been considered alien in the Mediterranean Sea: *Perinereis linea* (Arias *et al*., 2013) and *P. nuntia* (Zenetos *et al.,* 2010); however, the presence of the former species in the region was recently questioned (Villalobos-Guerrero *et al*., 2021), whereas *P. nuntia* has been demonstrated as a complex of morphologically distinguishable species (Wilson & Glasby, 1993; Glasby & Hsieh, 2006), even between neighboring regions (Villalobos-Guerrero, 2019; Tosuji *et al*., 2019). The records of the other *Perinereis* species in the Mediterranean Sea need to be thoroughly assessed because it is likely that nominal species might be hidden in species complexes or correspond to new ones.

The combined use of morphology and DNA evidence is a powerful tool for disentangling nereidid species complexes or discovering previously unknown species (Glasby *et al*., 2013; Sampértegui *et al*., 2013; Kara *et al*., 2020; Tosuji *et al.,* 2019; Drennan *et al.*, 2021; Wang *et al*., 2021; Teixeira *et al*., 2022). In particular, the mitochondrial cytochrome c oxidase I (COI) gene has been used to explain the interspecific relationships between nereidid species and their phylogeographic relationships; hence, used to analyze population structure, evolutionary relatedness, and delimitation of species (e.g., Park & Kim, 2007; 2017; Villalobos-Guerrero & Carrera-Parra, 2015; Kara *et al*., 2018; Villalobos-Guerrero *et al*., 2021; Villalobos-Guerrero *et al.,* 2022).

The current study describes a new species from the Algerian coast that belongs to the *Perinereis* SG-1A genus. Morphological comparisons were made between *Perinereis* species from the Mediterranean Sea and those from SG-1A. In addition, partial sequences of the new species' COI region were amplified to analyze genetic distances and compared to molecular data from other *Perinereis* species.

## Material and Methods

### *Sampling sites*

Nereidid specimens were collected from three stations on the northeastern coast of Algeria: El-Kala, Annaba, and Skikda (Fig. 1; Table 1). At each station, three replicates were sampled monthly during 2019 using a quadrate (25 cm × 25 cm) at 0.5 m depth on algae attached on hard bottoms at the intertidal and shallow sublittoral. The algae were scraped and deposited in containers to search for nereidid individuals. Several specimens were

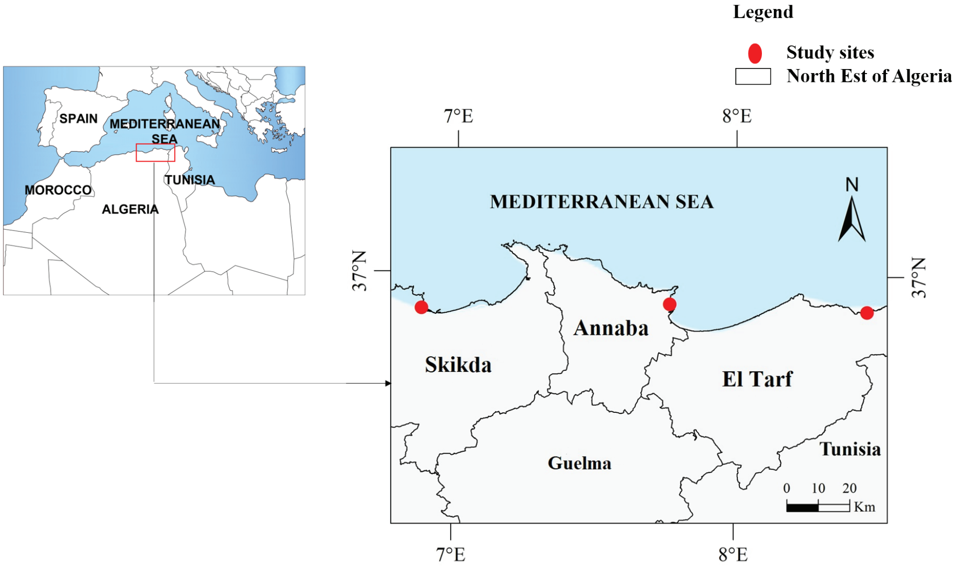

***Fig. 1:*** Map of the eastern coasts of Algeria showing the three sampling sites of this study.

**Table 1.** Location and characterization of sampling sites.

| Station | Locality | Coordinates | Biotopes |
|---|---|---|---|
| La Montagne | El-Kala | 36.89815, 8.450911 | Rocky shore, 0.5 m depth |
| Rezgui Rachid | Annaba | 37.53547, 8.289361 | Rocky and sandy shore, 0.5 m depth |
| Bikini | Skikda | 36.87191, 6.900911 | Rocky and sandy shore, 0.5 m depth |

found fragmented, with either the anterior or posterior end missing. Consequently, we also carried out a second method to prevent mechanical fragmentation. A small amount of powdered alum made of Kalunite was sprinkled on the rocky ground, which is natural potassium and does not contain aluminum hydroxide. No scientific studies to date have demonstrated natural alum stone toxicity. Before fixation, the specimens were forced to evert the proboscis by exerting pressure gently on the prostomium, which eventually facilitated the observation of the paragnaths through the microscope. The specimens were fixed individually in 1.5 ml microcentrifuge tubes with a 4% formalin solution and then preserved in 70% ethanol. After identification, the annelids intended for the genetic study were stored individually in 1.5 ml tubes containing ethanol at 90% and placed at a temperature of 4°C. Finally, all specimens examined in this study, including the type material, were deposited at the *Muséum National d'Histoire Naturelle* (MNHN), Paris.

### *Morphological observation*

Examination of the morphological features of the specimens, terminology, and standards to describe the species, and preparation and edition of figures are detailed elsewhere (Villalobos-Guerrero *et al.*, 2021 and references there cited). In addition, paired areas in the pharynx were indicated as 'a' for the left and 'b' for the right. Specimens were photographed using a digital camera (ZEISS AxioCam ICc1) and mounted on a stereomicroscope and compound microscope with a portable adaptor. Description of the species is based upon the morphology of the holotype unless otherwise stated, while variations are indicated separately for all the material. Anomalies found in one specimen are also described.

### *DNA extraction, PCR amplification and molecular analysis*

Partial sequences of mitochondrial cytochrome c oxidase subunit I (COI) region were amplified to analyze the genetic distances at the intraspecific level of *Perinereis* specimens collected in Algeria and to compare this data with other *Perinereis* species with sequences mined from BOLDSystems (BOLD) (Sujeevan & Hebert, 2007). DNA was extracted from tissue samples conserved in 90% ethanol using CTAB according to the protocol established by Winnepenninckx *et al.* (1993). The universal primer pair LoboF1 and LoboR1 (Lobo *et al.,* 2013) was used to amplify a fragment of the gene COI (Table 2). Polymerase Chain Reaction (PCR) amplifications were carried out using 12 µl of Taq DNA polymerase (Thermo Scientific), 14.88 µl of molecular biology grade water, 2.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.2 µm of each primer (10 µM) and 1 µl of template DNA to make up a total reaction volume of 25 µl. Thermal cycling conditions were settled as follows: initial denaturation at 94°C for 5 min, followed by 5 cycles of 94 °C for 30 s, 45°C for 1 min 30 s, and 72°C for 1 min, then by 45 cycles of 94°C for 30 s, 54°C for 1 min 30 seconds, and 72°C for 1 min and final extension time at 72°C for 5 min. Amplicons were Sanger sequenced at the GENEWIZ®, just the forward primer (LoboF1). Agarose gels with BET were used to control PCR product size. The products were then electrophoresed, one direction was sequenced for eight products, and only one was sequenced in two directions. All sequences generated were deposited in GenBank under accession numbers OP459427–OP459435, OP585368 (Table 3).

The obtained sequences were checked manually with the Bioedit sequence editor (Hall, 1999). After checking individual 1, no difference was observed between the sequencing of the two strands. Both sequences (forward and reverse) were used to build a consensual sequence, which was compared with the other eight individuals. Sequences were aligned using ClustalW implemented in MEGA-X (Kumar *et al.,* 2018). Nucleotide-sequence divergences of the partial sequences of the COI region were used to calculate the intraspecific and interspecific pairwise genetic distances using the Kimura two-parameter (KP2) model (Kimura, 1980). This newly obtained data for Algerian specimens of *Perinereis* was compared with mtCOI sequences of other species mined from BOLD: *Perinereis aibuhitensis, P. brevicirris, P. cultrifera, P.*

**Table 2.** Primer pair used to amplify COI sequences of *Perinereis* specimens in this study.

| Reference | Primer | Direction (5' – 3') |
|---|---|---|
| Lobo *et al.* (2013) | LoboF1 | (F) KBTCHACAAAYCAYAARGAYATHGG |
| | LoboR1 | (R) TAAACYTCWGGRTGWCCRAARAAYCA |

**Table 3.** Species of *Perinereis* and outgroups used in this study and locality of sequences with respective GenBank accession numbers.

| Species | GenBank COI | Location | n | Reference |
|---|---|---|---|---|
| *P. aibuhitensis* (Grube, 1878) | KC800611<br>KC800612<br>KC800613<br>KC800614 | China | 4 | Deng, unpubl. data |
| | KY129885 | China | 1 | Chen *et al.,* unpubl. data |
| | KF611806 | Korea | 1 | Kim *et al.* (2015) |
| *P. brevicirris* (Grube, 1866) | JX966314 | India | 1 | Sekar *et al.* (2016) |
| | JX503024<br>JX503025<br>JX503026 | South Korea | 3 | Kim *et al.*, unpubl. data |
| | KC800628<br>KC800630 | China | 2 | Deng, unpubl. data |
| *P. cultrifera* (Grube, 1840) | MN812983 | Arcacha–France<br>Portugal | 1 | Alves *et al*., 2020 |
| | KR916910<br>KR916911<br>KR916912 | Portugal | 3 | Lobo *et al*., 2016 |
| *P. euinii* Park & Kim, 2017 | MN256545<br>MN256546 | Unknown | 2 | Xing & Zhang, unpubl. data |
| | KC800624<br>KC800625<br>KC800627 | China | 3 | Deng, unpubl. data |
| | KY129883 | China | 1 | Chen*et al*., unpubl. data |
| *P. louizomarum* n. sp. | OP459427<br>OP459428<br>OP459429<br>OP459430<br>OP459431<br>OP459432<br>OP459433<br>OP459434<br>OP459435 | Algeria | 9 | This study |
| *P. suluana* (Horst, 1924) | JX420245<br>JX420246<br>JX420247<br>JX420251<br>JX420252<br>JX420253 | Australia | 6 | Glasby *et al*. (2013) |
| *P. vallata* (Grube & Kröyer *in* Grube, 1858) | JX676143<br>JX676159<br>JX676180 | India | 3 | Iyyapparajanarasimapallavan *et al.*, unpubl. data |
| | HQ705192<br>HQ705194<br>HQ705195 | Chile | 3 | Sampertegui *et al.* (2013) |
| *P. wilsoni* Glasby & Hsieh, 2006 | KC800623<br>KC800629<br>KC800631 | China | 3 | Deng, unpubl. data |
| | KY129887<br>KY129888<br>KY129889 | China | 3 | Chen *et al*., unpubl. data |
| *Nereis pelagica* (Linnaeus, 1758) | HQ023615 | Canada, Manitoba | 1 | Carr *et al.,* 2011 |
| *Myrianida rubropunctata* (Grube, 1860) | GQ856203 | Ferrol, Spain | 1 | Nygren & Pleijel (2010) |

*suluana, P. wilsoni,* and *P. vallata*, including *Nereis pelagica* (Linnaeus, 1758) and *Myrianida rubropunctata* (Grube, 1860) (Syllidae) as outgroups (Table 3). These species were chosen because the number of BOLD available sequences is equal to or greater than that obtained in this study for *Perinereis* Algerian individuals. Based on the K2P model, the same sequences were used to calculate intraspecific genetic distances.

## Results

### Molecular analyses

A total of 581 bp of the partial mtCOI sequences was analyzed from nine *P. louizomarum* n. sp. individuals. The intraspecific pairwise distances between the COI sequences were relatively low (Table 4), with a mean intraspecific distance of 0.02%. The nine sequences represent three haplotypes. Six annelids shared the most abundant sequence. Neither insertion nor deletion was detected between the nine individual sequences. The interspecific pairwise distances based on six *Perinereis* species showed mean values between 0.173 and 0.276; however, for *P. louizomarum* n. sp., they varied from 0.262 to 0.325 (Table 5). According to the Neighbour-Joining tree construction, all the *Perinereis* species formed distinct clusters, except *P. brevicirris*, separated into three clades. In particular, *P. louizomarum* n. sp. clustered in a genetically different group with a high bootstrap value (Fig. 2). The morphological and molecular evidence obtained in this study allows us to confirm that the latter is a new species.

### Morphology

A new *Perinereis* species was discovered among the rocky shores of Northeast Algeria. *Perinereis louizomarum* n. sp. has a single bar-shaped paragnath on area VI of the proboscis, and the proximal dorsal ligule is slightly expanded. The new species suits within the Subgroup 1A *sensu* Hutchings *et al.* (1991). *Perinereis louizomarum* n. sp. closely resembles the Mediterranean Sea species *P. cultrifera* and *P. rullieri*. However, morphological comparisons based on the descriptions, redescriptions, and illustrations of the type specimens (*fide* Grube, 1840; Pilato, 1974; Hutchings *et al.,* 1991; Park & Kim, 2017) reveal that *P. louizomarum* n. sp. can mainly be differentiated from both species by the counting of paragnaths on areas III and V, the form of distal dorsal ligules, and the location of dorsal cirri.

## SYSTEMATICS

Family NEREIDIDAE de Blainville, 1818

Genus *Perinereis* Kinberg, 1865

*Perinereis* Kinberg, 1865: 175; 1910: 52.

### Type species

*Perinereis novaehollandiae* Kinberg, 1865, by subsequent designation (*fide* Hartman, 1949). It is considered a junior synonym of *P. amblyodonta* Schmarda, 1861 (Ehlers, 1904; Hartman, 1959).

**Table 4.** Intraspecific pairwise distance of *Perinereis louizomarum* n. sp. calculated by Kimura-2-Parameters model.

| | Ind. 1 | Ind. 3 | Ind. 4 | Ind. 5 | Ind. 6 | Ind. 7 | Ind. 8 | Ind. 9 |
|---|---|---|---|---|---|---|---|---|
| *Ind. 3* | 0.0250 | | | | | | | |
| *Ind. 4* | 0.0163 | 0.0330 | | | | | | |
| *Ind. 5* | 0.0162 | 0.0473 | 0.0127 | | | | | |
| *Ind. 6* | 0.0179 | 0.0538 | 0.0079 | 0.0046 | | | | |
| *Ind. 7* | 0.0162 | 0.0554 | 0.0079 | 0.0108 | 0.0060 | | | |
| *Ind. 8* | 0.0163 | 0.0440 | 0.0047 | 0.0077 | 0.0031 | 0.0031 | | |
| *Ind. 9* | 0.0130 | 0.0406 | 0.0111 | 0.0093 | 0.0077 | 0.0093 | 0.0062 | |
| *Ind. 10* | 0.0365 | 0.0238 | 0.0393 | 0.0517 | 0.0626 | 0.0641 | 0.0451 | 0.0484 |

**Table 5.** Interspecific pairwise genetic distances of *Perinereis* species (K2P distances).

| Species | *P. aibuhitensis* | *P. brevicirris* | *P. cultrifera* | *P. suluana* | *P. vallata* | *P. wilsoni* |
|---|---|---|---|---|---|---|
| *P. brevicirris* | 0.241 | | | | | |
| *P. cultrifera* | 0.274 | 0.276 | | | | |
| *P. suluana* | 0.267 | 0.270 | 0.257 | | | |
| *P. vallata* | 0.222 | 0.222 | 0.251 | 0.254 | | |
| *P. wilsoni* | 0.235 | 0.173 | 0.264 | 0.237 | 0.255 | |
| *P. louizomarum* n. sp. | 0.325 | 0.285 | 0.268 | 0.282 | 0.262 | 0.282 |

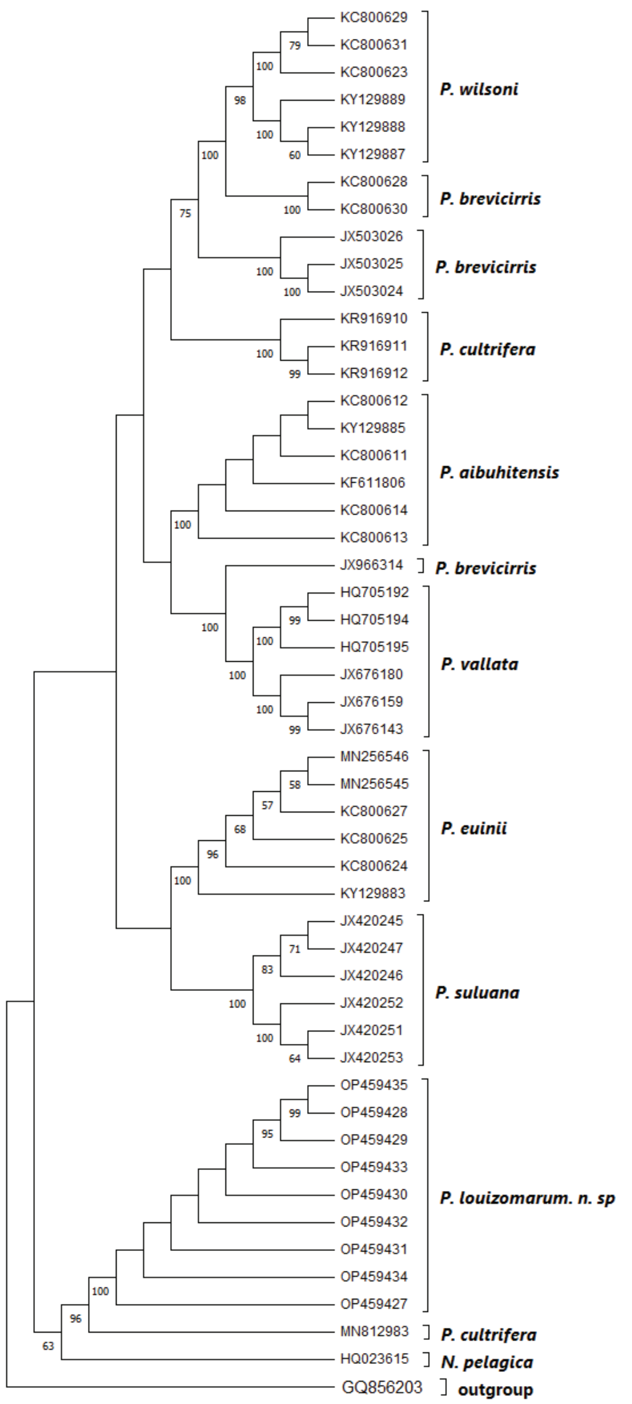

***Fig. 2:*** Neighbor-Joining tree generated with MEGA-X based on COI sequence of nine species of *Perinereis*. The gene divergence was represented using Kimura's two-parameter (K2P) model.

### *Perinereis louizomarum n. sp.*

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[Argelian name: *ver vert de roche*]

Figures 4, 5

***Type material.*** **Mediterranean Sea, Algeria.** Holotype: MNHN-IA-TYPE 2065, El-Kala (36.89815° N, 8.450911° W), Annaba, Skikda, 22 May 2019, coll. H. Rezzag Mahcene, 0.5 m, substratum covered by algae, rock, and sand, atokous female, in good condition.

***Paratypes.*** **Mediterranean Sea, Algeria.** Six specimens (MNHN-IA-TYPE 2066), El-Kala, Algeria (36.89815° N, 8.450911° W), 02 May 2019, coll. H. Rezzag Mahcene, 0.5 m, among rocks covered by algae, atokous. Two specimens, one of them contains only the proboscis (MNHN-IA-TYPE 2067), El-Kala, 24 March 2019, 0.5 m, among rocks covered by algae, atokous, coll. H. Rezzag Mahcene. One specimen (MNHN-IA-TYPE 2068), Skikda (36.87191° N, 6.900911° W), 25 January 2020, coll. H. Rezzag Mahcene, 0.5 m, among rocks covered by algae, atokous.

***Additional material.*** **Mediterranean Sea, Algeria.** One specimen (MNHN-IA-TYPE 2069), La Montagne Beach, El-Kala, Algeria (36.89815° N, 8.450911° W), 02 May 2019. Two specimens (MNHN-IA-TYPE 2070), Rezgui Rachid Beach, Annaba, Algeria (37.53547°N, 8.289361°W), 13 March 2019.

***Diagnosis***. *Perinereis* species belonging to subgroup 1A. Specimens with a crescent-shaped bar on area VI; areas VI–V–VI ridge pattern π-shaped; area V without paragnaths; areas VII–VIII with anterior band of paragnaths consisting of two rows; area III without laterally isolated paragnaths; posterodorsal tentacular cirri extending to chaetigers 4–6; distal dorsal ligule subequal in length throughout body; neuroacicular ligule markedly projected; falcigers with camerated shaft divided into four partitions.

***Description.*** Holotype atokous female, complete, in good condition, 56 mm LT, 12 mm L10, 3.2 mm W10, with 58 chaetigers. Body color brownish (Fig. 3A), with brown pigmentation on dorsum of prostomium (except mid-posteriorly; Fig. 3B), inner part of palpophores (Fig. 3A), proximal half of palpostyles and antennae (Fig. 3A, B), entire cirrophores of tentacular cirri (Fig. 3A, B), pygidium, pygidial cirri and on dorsum of all segments, more intense posteriorly.

Prostomium campanulate, as long as wide (Fig. 3B); anterior end broad, distally complete; anterolateral gap between antenna and palpophore narrow, three-quarters as wide as basal diameter of antennae Palpophores sub-conical, thick, as long as wide, two-thirds of entire prostomium (Fig. 3A); sub-distal transverse groove distinct, deeply embedded. Palpostyles ovoid, large, one-third as wide as diameter of palpophore (Fig. 3A).

Antennae tapered, thick, extending forwards slightly beyond palpophore and posteriorly to distal third of length of prostomium; antennae separated with gap as wide as three-quarters of basal diameter of antennae (Fig. 3A, B).

Paired eyes black, arranged in trapezoid form, gap between both pairs twice as wide as diameter of posterior pair of eyes (Fig. 3A, B); anterior pair of eyes oval, subequal to basal diameter of antennae, with gap between both eyes 5.5 times their diameter, with lens barely distinct, purplish, covering 40% of eye; posterior pair of eyes rounded, slightly narrower than basal diameter of antennae, with lens distinct, purplish, placed centrally in eyes and covering 40% of it.

Tentacular belt 3 times wider than long, 1.5 times as long as chaetiger 1, with even anterior margin, dorsum

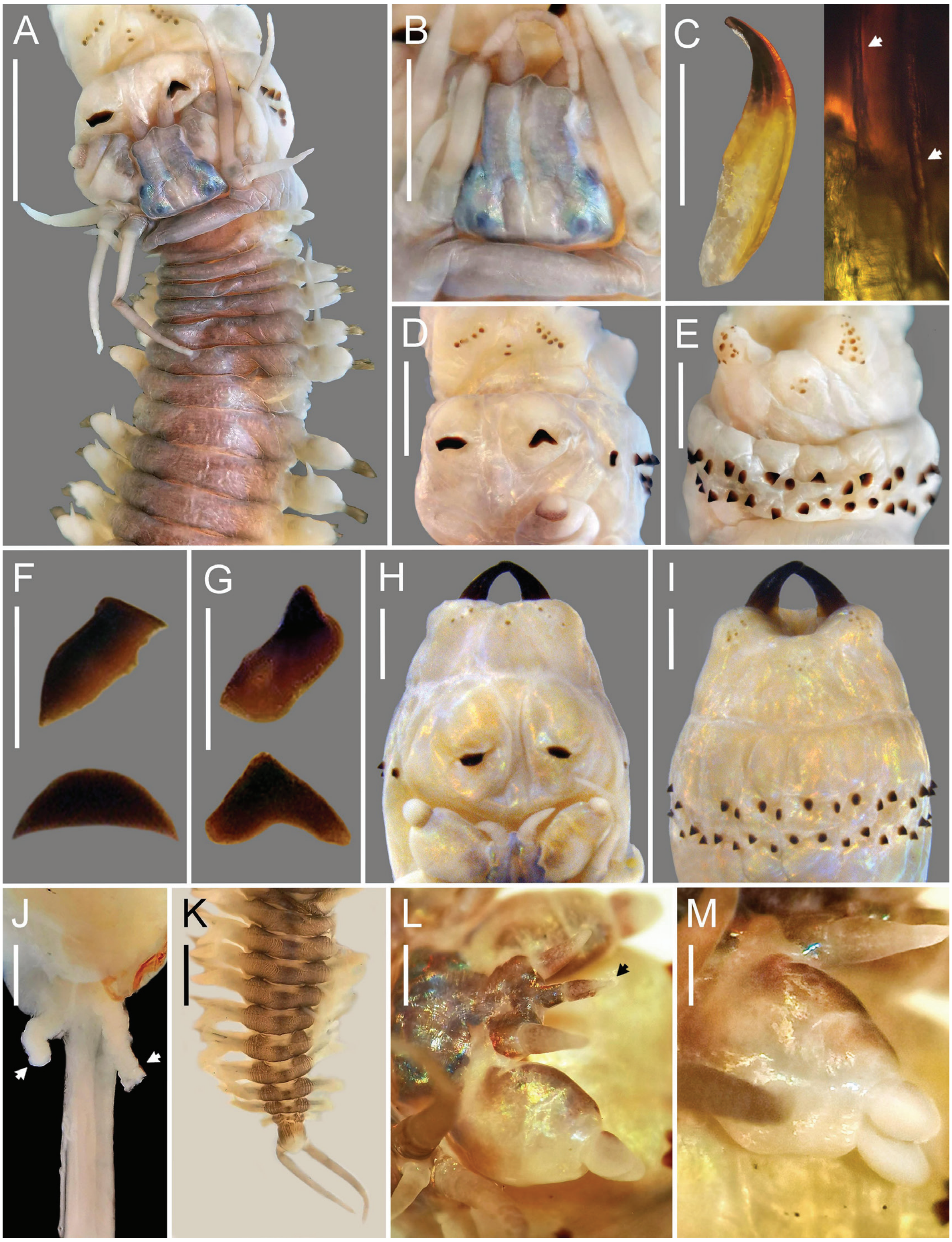

***Fig.*** 3: *Perinereis louizomarum* n. sp. (A–G, K) Holotype (MNHN-IA-TYPE 2065) from El-Kala, Algeria, atokous: (A) Anterior end in dorsal view; (B) prostomium in dorsal view; (C) right jaw in dorsal view, inset of inner canals indicated by arrows; (D) proboscis in dorsal view; (E) proboscis in ventral view; bar-shaped paragnath of (F) left side and (G) right side of area VI, above in front-lateral view and below in frontal view; (K) pygidium in dorsal view. (H, I) Paratype (MNHN-IA-TYPE 2066) from El-Kala, Algeria, atokous: proboscis in (H) dorsal and (I) ventral views. (J) Paratype (MNHN-IA-TYPE 2066) from El-Kala, Algeria, atokous female: paired oesophageal caeca in dorsal view (indicated by arrows). (L, M) Paratype (MNHN-IA-TYPE 2068) from Skikda, Algeria, atokous: (L) Prostomium in lateral view with third anomalous antenna (indicated by arrow); (M) duplicate right palpophore and palpostyle. Scale bars: A: 3.5 mm; B: 1.7 mm; C: 5 mm; D, E, H, I, J: 1.2 mm; F, G, K: 8 mm, L: 1 mm; M: 0.8 mm.

without marked transverse wrinkles.

Tentacular cirri slender, smooth (Fig. 3A); posterodorsal cirri extending posteriorly to chaetiger 2, 1.5 times as long as anterodorsal cirri; anterodorsal cirri extending posteriorly to chaetiger 1; posteroventral cirri extended over first third of prostomium; anteroventral cirri as long as two-thirds of posteroventral cirri and as long as palpophore; cirrophores of anteroventral cirri ring-shaped, remaining cirrophores cylindrical, posterodorsal cirrophores 1.5 times as long as anterodorsal cirrophores, anteroventral cirrophores broadest, posteroventral cirrophores narrowest.

Proboscis everted, with maxillary and oral rings cylindrical, wider than long. Jaws edenticulate, with distal half dark reddish, remaining yellow amber; 2 canals emerging from pulp cavity (Fig. 3C).

Proboscis with brownish, wear and small paragnaths on maxillary ring and dark-red and coarse paragnaths on oral ring; consisting of cones, except bars on area VI and areas VII–VIII (Fig. 3D–I). Area I: 2, longitudinal row of cones, distal one smaller. Areas IIa: 5 and IIb: 8, one or two irregular rows of uneven cones in oblique patch. Area III: 4, four slightly uneven cones in subcircular patch, without distinct isolated lateral groups. Areas IVa: 12 and IVb: 17, three irregular transverse rows of uneven cones in slightly curved patch. Area V: 0. Areas VIa: 1 and VIb: 1, crescent-shaped bars (Fig. 3F), right with concave base but asymmetrical and pointed (Fig. 3G). Areas VII–VIII: 38, two well-separated bands of coarse and uneven paragnaths, with anterior band consisting of two transverse nearly-aligned rows of cones and p-bars (Fig. 3E, I) (furrow rows with one p-bar, ridge rows with one cone), posterior band with two transverse rows of cones displaced from each other (paragnaths present only on ridge rows, with two cones on each region). Ridges of areas VI–V–VI with π-shaped pattern (Fig. 3D, H). Gap between areas VI and VIII broad, as wide as palpophore (Fig. 3D, H). Paired oesophageal caeca present (Fig. 3J).

Parapodia without glandular patches on dorsum of segments (Fig. 4K). Notopodia consisting of dorsal cirrus, dorsal ligule (distal and proximal), notopodial prechaetal lobe and median ligule in biramous parapodia (Fig. 4A–E).

Dorsal cirri conical, thick, extending beyond distal region of dorsal ligule in anteriormost and anterior parapodia (Fig. 4A, B), shorter than that in middle and posterior parapodia (Fig. 4C–E), then subequal in posteriormost parapodia; dorsal cirri 3–3.5 times as long as proximal region of dorsal ligule in anteriormost parapodia (Fig. 4A), 2–2.5 times as long as that in anterior parapodia (Fig. 4B), subequal to or slightly longer than that in following parapodia (Fig. 4C–E); attached basally to dorsal ligule in anteriormost parapodia (Fig. 4A), one-third in anterior parapodia (Fig. 4B), medially in following parapodia (Fig. 4C–E).

Proximal region of dorsal ligule even throughout body; shorter than distal region of dorsal ligule in anteriormost and anterior parapodia (Fig. 4A, B), subequal to that in following parapodia (Fig. 4C–E); one glandular patch sub-oval in anterior parapodia (Fig. 4B), becoming distinctly smaller towards posterior end.

Distal region of dorsal ligule extending markedly beyond end of notoaciculae throughout (Fig. 4B–E); thick, bluntly rounded and slightly longer than median ligule in anterior parapodia (Fig. 4B), longer than median ligule in following parapodia (Fig. 4C–E), bluntly triangular in middle parapodia (Fig. 4C), becoming digitiform towards posterior end (Fig. 4D, E); one massive glandular patch covering entirely distal dorsal ligule of anterior parapodia (Fig. 4B), much compact in middle and posterior parapodia.

Notopodial prechaetal lobe blunt, thick and short (Fig. 4B) in anterior parapodia, becoming narrower and reduced to notoacicular papilla from parapodia 14 (Fig. 4C–E).

Median ligule thick, bluntly rounded in anterior parapodia (Fig. 4B), bluntly triangular in middle parapodia (Fig. 4C), becoming digitiform towards posterior end (Fig. 4D, E).

Neuropodia consisting of neuroacicular ligule with inferior lobe, ventral ligule, and ventral cirrus (Fig. 4A–E); superior and postchaetal lobes absent throughout.

Neuroacicular ligule shorter than ventral ligule in anteriormost parapodia (Fig. 4A), distinctly longer than that in anterior and middle parapodia (Fig. 4B, C), becoming subequal to or slightly shorter than that towards posterior end (Fig. 4D, E); 1.5–1.7 times as wide as ventral ligule in anteriormost and anterior parapodia, as wide as that in following parapodia.

Inferior lobe as small, blunt process in anteriormost and anterior parapodia, becoming narrower and reduced from parapodia 18.

Ventral ligule well-developed throughout, thick; digitiform and shorter than median ligule throughout, except bluntly rounded in anterior parapodia (Fig. 4B).

Ventral cirri digitiform and thick in anterior-most parapodia (Fig. 4A), becoming conical in following ones; as long as two-thirds of ventral ligule throughout.

Aciculae black, with basal end uncoloured. Notoaciculae absent in first 2 parapodia (Fig. 4A). Neuroaciculae extending beyond distal end of notoaciculae throughout (Fig. 4B–E). Neuroaciculae longer than median ligule in anterior parapodia (Fig. 4B), distinctly shorter than that in following parapodia (Fig. 4C–E).

Notochaetae all homogomph spinigers throughout; 15–16 spinigers present in anterior parapodia, 5–7 spinigers in following parapodia.

Upper neurochaetae consisting of homogomph spinigers and heterogomph falcigers, both present throughout; 8 spinigers present in anteriormost parapodia, 12 spinigers in anterior parapodia, 3–4 spinigers in following parapodia; 3–4 falcigers present throughout.

Lower neurochaetae consisting of heterogomph spinigers and heterogomph falcigers, both present throughout; 1–2 spinigers present in all parapodia; 24–26 falcigers in anteriormost and anterior parapodia, 5–6 falcigers in middle parapodia, 4–5 falcigers in posterior parapodia.

Blade of both homogomph (Fig. 4F) and heterogomph spinigers (Fig. 4G) finely serrated towards toothed edge, evenly spaced. Blade of heterogomph falcigers short (*b/a* ratio 0.75–1.1), convex, terminal tooth blunt with inconspicuous tendon, serrations present in about one-third to two-fifths (0.31–0.4) of total blade length (Fig. 4H–K); supracicular blades of similar size (Fig. 4H, J), subacicular ones becoming slightly shorter and thicker downwards in parapodia (Fig. 4I, K). Shaft of falcigers camerated, with cavity divided sub-distally into four longitudinal partitions (Fig. 4J, K).

Pygidium well developed, as long as last 3 chaetigers; anal cirri thick, short, equalling length of last 6 chaetigers, with small cirrophores (Fig. 3K).

***Variation.*** Total length of complete specimens: 35–42

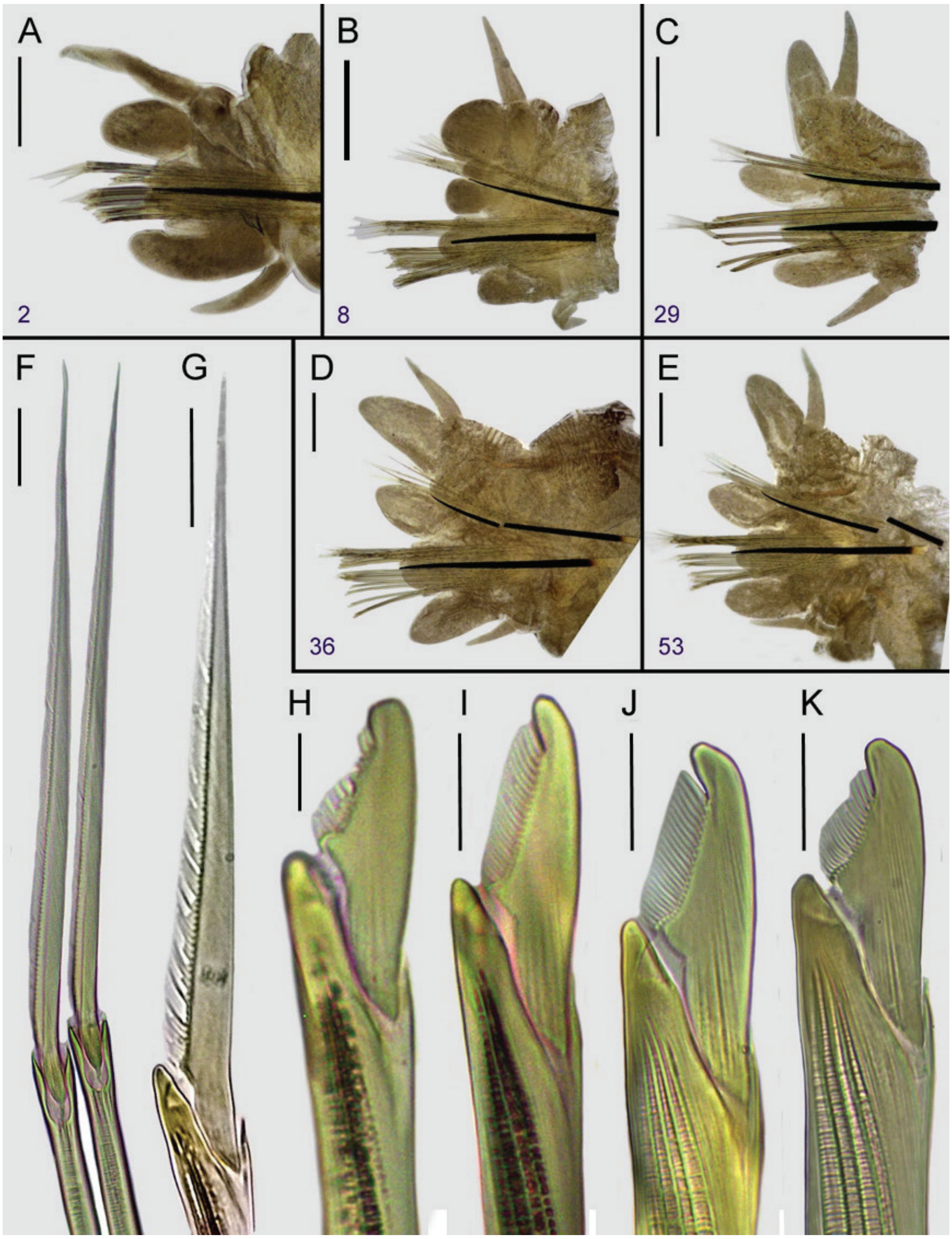

***Fig.*** 4: *Perinereis louizomarum* n. sp. (A–E) Holotype (MNHN N IA type N: 2065) from El-Kala, Algeria, atokous: (A) parapodium of chaetiger 2; (B) parapodium of chaetiger 8; (C) parapodium of chaetiger 29; (D) parapodium of chaetiger 36; (E) parapodium of chaetiger 53; (F) homogomph spinigers from notochaetae (chaetiger 53); (G) heterogomph spiniger from subacicular neurochaetae (chaetiger 53; (H) heterogomph falciger from supracicular neurochaetae (chaetiger 2); (I) heterogomph falciger from subacicular neurochaetae (chaetiger 2); (J) heterogomph falciger from subacicular neurochaetae (chaetiger 8); (K) heterogomph falciger from subacicular neurochaetae (chaetiger 53). Scale bars: A: 0.5 mm; B: 0.6 mm; C, D: 0.4mm; E: 0.3 mm; F: 30 µm; G: 40 µm; H: 10 µm; I-K: 20µm.

mm; L15: 9–12 mm, W15: 4–5 mm, 37–62 chaetigers. Anterodorsal tentacular cirri extending back to chaetiger 3–4. Posterodorsal tentacular cirri extending back to chaetiger 4–6. Jaws smooth, sometimes with 1–2 (rarely 3) thick and blunt denticles. Number and arrangement of paragnaths: area I with 1–2; area II with 3–8; area III with 1–5; area IV with 6–19; area V without paragnaths, rarely 1–2; area VI with crescent-shaped bar, sometimes with concave base and pointed tip; area VII–VIII with 26–38, in two bands, anterior row with p-bars present, less distinct in coarser paragnaths, posterior row with cones on ridge rows only, albeit occasionally seem also present on furrow rows. Anal cirri as long as last 4–5 chaetigers.

***Anatomical anomalies.*** One specimen (62 mm long, 3 mm wide, 62 chaetigers) with (1) three antennae of similar size, with the middle one thinner than laterals and bearing ceratophore (Fig. 2L); (2) two palpostyles on right palpophore (Fig. 3M); and (3) three tentacular cirri in the right side (Fig. 3M). Additionally, another specimen was found with area VII without paragnaths.

***Habitat.*** Rocky shores covered by algae at 0.5 m depth, including *Corallina* sp., *Dictyopteris* sp., *Ulva lactuca* Linnaeus, and *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier, and also found among the bivalve *Mytilus galloprovincialis* (Lamarck, 1819).

***Etymology.*** The species' name combines the first author's parents' names, Louiza and Omar, to honor their devotion, love, and the warm life they made for her. The name is a noun in the genitive (ICZN, 1999).

***Type locality.*** Annaba, Algeria, Mediterranean Sea.

***Taxonomic remarks.*** *Perinereis louizomarum* n. sp. is a member of the *Perinereis* species SG-1A. Among the other 13 species within this subgroup (after Hutchings *et al*., 1991), *P. louizomarum* n. sp. is similar to *P. cultrifera* from the Tyrrhenian Sea (Grube, 1840; Hutchings *et al.,* 1991; Park & Kim, 2017), *P. floridana* from the Gulf of Mexico (Ehlers, 1868; de León-González & Solís-Weiss, 1998) and *P. rullieri* from the Mediterranean Sea (Pilato, 1974) by having two well-distinguished bands of paragnaths on the areas VII–VIII, dorsal cirri of similar length to or slightly longer than distal dorsal ligule in anterior parapodia, and neuropodia without postchaetal lobe. Nevertheless, *P. louizomarum* n. sp. is distinguishable from these three species by the following features: (I) the lower number of denticles on jaws (normally 0, occasionally 2 or 3), in contrast to the higher number in *P. cultrifera* (4), *P. floridana* (9) and *P. rullieri* (4–5); (II) the absence of laterally isolated paragnaths on area III, in comparison to their presence in *P. cultrifera* and *P. rullieri* (rarely absent); (III) the lower number of paragnaths on areas III (1–5) and V (typically 0, rarely 1 or 2), in contrast to the higher number in *P. cultrifera* (area III: 5–12; V: 3, rarely 2, 4 or 5), *P. floridana* (area III: 16; V: 1, rarely 2) and *P. rullieri* (area III: 5–9, rarely higher; V: 3, rarely fewer or higher); (IV) the bluntly rounded distal dorsal ligule in anterior parapodia, in comparison to that conical or bluntly conical in *P. cultrifera*, *P. floridana* and *P. rullieri*; (V) the dorsal cirri located medially on dorsal ligule in posterior parapodia, in comparison to subdistally placed in *P. cultrifera* and *P. rullieri*; and (VI) the coarse paragnaths on areas VII–VIII, in comparison to those small and delicate in *P. cultrifera* and *P. rullieri*. Additionally, *P. louizomarum* n. sp. is different from *P. floridana* by having notopodial prechaetal lobes, at least in anterior parapodia, and neuropodia without superior lobe throughout the body, in contrast to their absence and presence in *P. floridana*, respectively.

Based on the biogeographic overlap, *P. tenuisetis* described from the Mediterranean Sea also warrants comparison with the new species. *Perinereis louizomarum* n. sp. can readably be distinguished from *P. tenuisetis* by the following diagnostic characteristics: in *P. louizomarum* n. sp., the aciculae are black, the dorsal cirri are placed medially on dorsal ligule in posterior parapodia, and neurochaetae have heterogomph spinigers and falcigers; in contrast, *P. tenuisetis* has yellow light aciculae, dorsal cirri placed basally on dorsal ligule in the same parapodia, and subacicular neurochaetae with homogomph spinigers and falcigers.

## *Key to species of Perinereis Kinberg, 1865 belonging to the Subgroup 1A*

This key includes all species now regarded as *Perinereis* Subgroup 1A *sensu* Hutchings *et al*. (1991). *Perinereis dongalae* originally described from Celebes, Indonesia by Horst (1924), is currently known only by the brief description and scarcely illustrated epitokous stage. Hylleberg *et al.* (1986) synonymized the species with *P. striolata* (Grube, 1878) without examination of the type material.

| | |
|---|---|
| 1. Area I with 7 or more paragnaths | 2 |
| — Area I with up to 4 paragnaths | 3 |
| 2. Area I with 16 paragnaths; area V with more than 8 paragnaths in two transverse rows | *P. arabica* Mohammad, 1971 (Kuwait) |
| — Area I with up to 8 paragnaths; area V with one paragnath | *P. dongalae* (Horst, 1924) (Celebes, Indonesia) |
| 3. Aciculae light yellow; dorsal cirri placed basally on dorsal ligule in posterior parapodia; neurochaetae with homogomph falcigers; subacicular neurochaetae with homogomph spinigers | *P. tenuisetis* (Fauvel, 1915) (Sicily, Italy) |
| — Aciculae dark brown or black; dorsal cirri placed medially or subdistally on dorsal ligule in posterior parapodia; neurochaetae with heterogomph falcigers; subacicular neurochaetae with heterogomph spinigers | 4 |
| 4. Areas VII–VIII with anterior band only, up to 13 paragnaths | 5 |
| — Areas VII–VIII divided into two well-separated bands (anterior and posterior), with more than 20 paragnaths | 6 |
| 5. Ridges of area VI distally and sub-medially coalesced (areas VI–V–VI ridge pattern λ-shaped); area III without laterally isolated paragnaths; area V without paragnaths; areas VII–VIII with 10 or more paragnaths | *P. calmani* (Monro, 1926) (Australia and Macclesfield Bank) |

| | |
|---|---|
| — Ridges of area VI distally separated from each other (areas VI–V–VI ridge pattern π-shaped); area III with laterally isolated paragnaths; area V with 1–3 paragnaths; areas VII–VIII with up to 6 paragnaths | *P. taorica* Langerhans, 1881 (Madeira) |
| 6. Area V without paragnaths | 7 |
| — Area V with paragnaths | 8 |
| 7. Neuropodial postchaetal lobe present throughout; dorsal cirri extending markedly beyond end of distal dorsal ligule (thrice ligule length) in posterior parapodia | *P. capensis* (Kinberg, 1865) (South Africa) |
| — Neuropodial postchaetal lobe absent throughout; dorsal cirri extending barely beyond end of distal dorsal ligule in posterior parapodia | *P. louizomarum* n. sp. (Algeria, Mediterranean Sea) *partim* |
| 8. Dorsal cirri extending markedly beyond end of distal dorsal ligule (more than twice ligule length) in posterior parapodia | 9 |
| — Dorsal cirri extending barely beyond end of distal dorsal ligule in posterior parapodia | 12 |
| 9. Area V with one paragnath | 10 |
| — Area V with 3 or more paragnaths (rarely 2) | 11 |
| 10. Proximal dorsal ligule not enlarged in posterior parapodia; heterogomph falcigers with serrations present in about half of total blade length | *P. falsovariegata* Monro, 1933 (South Africa) |
| — Proximal dorsal ligule enlarged in posterior parapodia; heterogomph falcigers with serrations present in about one-sixth of total blade length | *P. villalobosi* Rioja, 1947 (Gulf of California, Mexico) |
| 11. Areas VII–VIII with anterior band having two rows of paragnaths (one on furrows and one on ridges); area III with up to 9 paragnaths | *P. rullieri* Pilato, 1974 (Sicily, Italy) |
| — Areas VII–VIII with anterior band having only furrow row of paragnaths; area III with 12–26 paragnaths | *P. helleri* (Grube, 1878) (Philippines) |
| 12. Notopodial prechaetal lobe absent throughout; jaws with 9 denticles; area III with 16 paragnaths | *P. floridana* (Ehlers, 1868) (Gulf of Mexico, USA) |
| — Notopodial prechaetal lobe present in anterior parapodia; jaws with up to 5 denticles; area III with up to 12 paragnaths | 13 |
| 13. Distal dorsal ligule bluntly conical in anterior parapodia; dorsal cirri located subdistally on dorsal ligule in posterior parapodia; jaws with 4–5 denticles; area III with up to 5–12 paragnaths, with laterally isolated cones; areas VII–VIII with small and delicate paragnaths | *P. cultrifera* (Grube, 1840) (Italy, Tyrrhenian Sea) |
| — Distal dorsal ligule bluntly rounded in anterior parapodia; dorsal cirri located medially on dorsal ligule in posterior parapodia; jaws with up to 3 denticles; area III with up to 5 paragnaths, without laterally isolated cones; areas VII–VIII with coarse paragnaths | *P. louizomarum* n. sp. (Algeria, Mediterranean Sea) *partim* |

## Discussion

*Perinereis cultrifera* was first described from Italy and has since been documented along the coasts of several regions (Fauvel, 1923; Day, 1967; Wu *et al.,* 1985). The species' global distribution has been questioned several times, most recently by Park & Kim (2017). A thorough redescription is still required to delimit its morphology; however, the original description (Grube, 1840) and a few comments and illustrations provided on the type material (Park & Kim, 2017) provide primary features that can be used to evaluate specimens from other localities. This was true for the Algerian specimens examined here. *P. louizomarum* n. sp. is discovered after a thorough comparison with the relevant literature.

Gravier & Dantan (1928) indicated that the reproduction of *P. cultrifera* was mainly of an atokous type in the Mediterranean Sea by the very few epitokous specimens found during their samplings. However, Durchon (1955, 1957, 1965) proposed that these different modes of reproduction, heteronereid and atokal, represent two distinct groups of *P. cultrifera* on the shores of the North Atlantic and the Mediterranean Sea. For instance, the species in the English Channel developed a markedly epitokal transformation, whereas in the Mediterranean, particularly in Algeria, the reproduction was carried out in the atokous state.

Later, the observations made by Scaps, Rouabah, and other colleagues (Scaps *et al.,* 2000; Rouabah & Scaps, 2003a, b; Rouabah & Rouabah, 2007) in further localities from the littorals of France, United Kingdom, Argelia and Tunis, confirmed that a complex of species might be referred to *P. cultrifera* not only due to the mode of reproduction but also to differences in the spawning season, age at maturity, morphology and biochemical characteristics. The authors concluded that three different populations could be found: (I) the epitokous form of the English Channel and Atlantic, characterized by having an elevated and a higher number of chaetigers (up to 120); (II) the atokous form of Algeria, in which the number segment is below 80; and (III) the epitokous form from Tunis with biometric features between the two above-described forms. *Perinereis louizomarum* n. sp. belongs to the atokous form of '*P. cultrifera*' (Durchon, 1957) since no traces of epitokal modifications were found in our specimens, including a mature female, and the number of segments was below 70. Nevertheless, further surveys to collect the species should be carried out throughout the year to obtain individuals in different life stages since specimens representing both forms of reproduction have been documented to coexist in Algeria (Gravier & Dantan, 1928; Durchon, 1957).

Complementing morphological taxonomy with the molecular approach permits the direct comparison of species among a global reference library using fragments of specimens at any stage of the life cycle, therefore furnishing a universal master tool for the identification and discovery of cryptic forms (Costa & Carvalho, 2007). Several studies have examined variation in mitochondrial DNA sequences in a region of the COI gene as a dependable approach to distinguish polychaete species (e.g., Glover *et al.,* 2005; Bleidorn *et al.,* 2006; Rice *et al*., 2008; Pleijel *et al*., 2009; Barroso *et al.,* 2010; Nygren & Pleijel, 2011). For instance, species regarded as cosmopolitan were detected as species complexes of several cryptic species, such as the amphinomid *Eurythoe complanata* (Pallas, 1766) (Barroso *et al.,* 2010) or the phyllodocid *Eumida sanguinea* (Örsted, 1843), the latter comprising about ten species (Nygren & Pleijel, 2011).

In the present study, besides the morphological, reproductive, and biochemical differences settled in previous studies, *P. cultrifera* is confirmed as a species complex in the European seas but based on DNA sequence data. The mtCOI sequences available in GenBank for four specimens, called '*P. cultrifera*' from the South European Atlantic shelf (northern Portugal and northwest France) (Table 3), were compared with those retrieved here from the Western Mediterranean (Algeria). Interestingly, those sequences of *P. cultrifera* from the South European Atlantic shelf resulted in two distinct clades (Fig. 2), which suggests that two populations of '*P. cultrifera*' are present in the region. Marked genetic differences between these two populations with those specimens from Algeria were also found (Table 5). In addition to *P. cultrifera*, the sequences of the remaining six species also show distinct pairwise distances, which are relatively comparable with those documented to distinguish *Perinereis* species (Park & Kim, 2017; Villalobos-Guerrero *et al*., 2021), supporting the establishment of *P. louizomarum* n. sp. as a new species. Based on the mtCOI sequences of the South European Atlantic populations, one of them represents the "*P. cultrifera*" Type I indicated by Scaps *et al*. (2000) and Rouabah & Rouabah (2007), and at least one likely represents an undescribed species. However, the material of both populations needs to be examined to understand and delimit their morphology, including the reproductive patterns of epitokal characters, which are considered relevant in nereidids to distinguish among closely related species (Read, 2007; Conde-Vela & Salazar-Vallejo 2015; Villalobos-Guerrero & Bakken 2018; Villalobos-Guerrero & Idris 2021).

On the other hand, Lobo *et al.* (2016) reported that polychaetes are suitable candidates for surveilling benthic ecosystems. An innovative and alternative approach to develop a rapid evaluation of the ecological status of marine ecosystems is through genomic observations (Hajibabaei *et al.,* 2011; Avó *et al.,* 2017; Aylagas *et al.,* 2018). Studies on the molecular characterization of polychaete fauna along the Algerian waters are limited. Expanding the genetic studies of polychaetes in Algeria is recommended to see if environmental parameters impact them. Aylagas *et al*. (2014) suggested that the most abundant species encountered during monitoring programs should be sequenced with accurate taxonomic marking to assess ecological status reliably. Vijapure *et al.* (2019) reported that the polychaete DNA libraries could be expanded with sequences commonly found during ecological monitoring surveys.

Marine environments exposed to many anthropogenic pressures show a gradual and continuous loss of diversity (Halpern *et al*., 2008). Erpenbeck *et al.* (2016) proposed regular monitoring of the ecological quality of marine and estuarine waters. It is often achieved by establishing species inventories that can be used as a benchmark against which future disturbances of diversity loss can be measured. Therefore, appropriate management strategies can be applied in areas where species richness is declining or where the loss of diversity is expected to be imminent (Thomsen & Willerslev, 2015). Aylagas *et al.* (2018), Lejzerowicz *et al*. (2015), and Bevilacqua & Terlizzi (2016), reported that biomonitoring mainly relies on morphological taxonomy, which is often criticized as labor-intensive, time-consuming and costly. To avoid these problems, Bik *et al.* (2012) and Sigamani *et al.* (2016) have proposed the integration of genetics into biological assessment methods in addition to classical taxonomy.

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