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## Research Article

***Pseudopolydora kempii japonica* Imajima & Hartman, 1964 (Polychaeta: Spionidae): a controversial subspecies long overlooked in European waters**Lise Latry<sup>1</sup>, Guillemine Daffe<sup>2</sup>, Flore Daramy<sup>1</sup>, Jérôme Jourde<sup>3</sup> and Nicolas Lavesque<sup>1</sup><sup>1</sup>Univ. Bordeaux, CNRS, Bordeaux INP, EPOC, UMR 5805, F-33120 Arcachon, France<sup>2</sup>CNRS, Univ. de Bordeaux, Observatoire Aquitain des Sciences de l'Univers, UMS 2567 POREA, Pessac, France<sup>3</sup>LIENSs, Littoral, Environnement et Sociétés, CNRS, La Rochelle Université, UMR 7266, 2 rue Olympe de Gouges, F-17000, La Rochelle, FranceCorresponding author: Lise Latry ([lise.latry@u-bordeaux.fr](mailto:lise.latry@u-bordeaux.fr))**Citation:** Latry L, Daffe G, Daramy F, Jourde J, Lavesque N (2024)*Pseudopolydora kempii japonica* Imajima & Hartman, 1964 (Polychaeta: Spionidae): a controversial subspecies long overlooked in European waters. *BioInvasions Records* 13(2): 403–418, <https://doi.org/10.3391/bir.2024.13.2.09>**Received:** 10 August 2023**Accepted:** 8 March 2024**Published:** 20 May 2024**Handling editor:** Laura Garzoli**Thematic editor:** Andrew Davinack**Copyright:** © Latry et al.

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**Abstract**

Recently, using morphological and molecular analyses, several *Pseudopolydora* specimens (Polychaeta: Spionidae) from French coastal waters were identified as *Pseudopolydora kempii* ssp. *japonica* Imajima and Hartman, 1964. According to the samples examined, *P. kempii* ssp. *japonica* has been present in European waters since 2004. Previous misidentifications in France are likely due to its resemblance to the indigenous species *Pseudopolydora pulchra* (Carazzi, 1893), and to the status of *P. kempii* ssp. *japonica* which is still controversial. Material was collected from Arcachon Bay, Morbihan Bay, Aiguillon Bay, and in the Gironde estuary (Bay of Biscay, France). All these areas have extensive shellfish industries, especially the farming of the Japanese oyster *Magallana gigas* (Thunberg, 1793). The importation of these live oysters from Japan has often included other species including polychaete worms, indicating a major vector of exotic species.

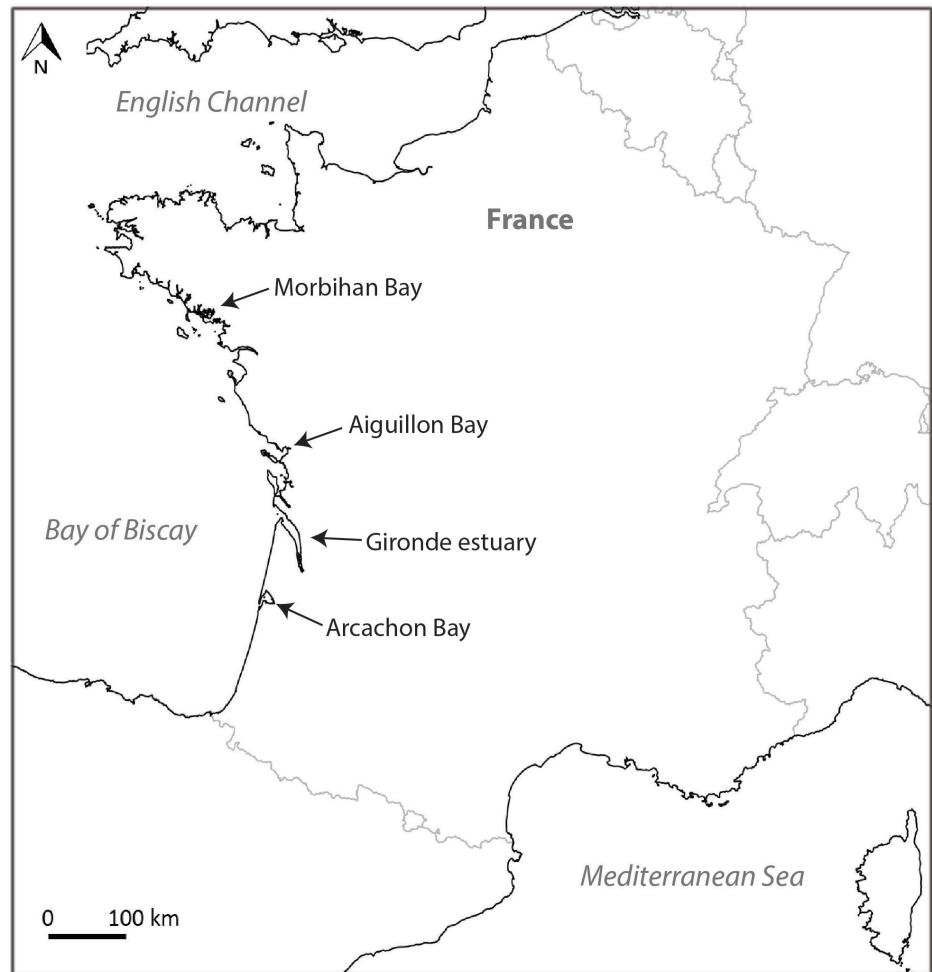
**Key words:** spionids, Bay of Biscay, non-indigenous species, misidentification, taxonomy, molecular analyses**Introduction**

Recent studies have shown the importance of both molecular data and morphological studies to detect introductions of non-indigenous species and monitoring their spread (Ojaveer et al. 2014; Massé et al. 2023). Among the benthic invertebrates introduced to European coasts, Spionidae (Annelida: Polychaeta) represents one of the dominant families (Massé et al. 2023). This family comprises 40 genera (Read and Fauchald 2023) including the *Pseudopolydora* Czerniavsky, 1881 genus, recently revised for European and adjacent waters (Radashevsky 2021). Among the 29 recognized *Pseudopolydora* species in the world (Read and Fauchald 2023), four were reported from European waters: *Pseudopolydora antennata* (Claparède, 1868) (type locality: Tyrrhenian Sea, Italy), *Pseudopolydora pulchra* (Carazzi, 1893) (type locality: Tyrrhenian Sea, Italy), *Pseudopolydora paucibranchiata* (Okuda, 1937) (type locality: Japan) and *Pseudopolydora nordica* Radashevsky, 2021 (type

locality: Norwegian Sea, Norway) (Radashevsky 2021). In France, *P. antennata*, *P. pulchra*, and *P. paucibranchiata* have been recorded from the English Channel to the Mediterranean Sea (Gil 2011; Radashevsky 2021; SNO BenthOBS – French marine stations and observatories network – database <https://benthobsb.sb-roscoff.fr/benthobsb/>) and *P. nordica* was recently reported from the Bay of Biscay (Gouillieux and Lavesque *pers. comm.*). Another species, not included in the European revision (Radashevsky 2021), was cited in the French SNO BenthOBS database: *Pseudopolydora kempi* (Southern, 1921) (type locality: Calcutta, India) and had previously been cited by Gil (2011) but its presence in European waters was considered doubtful by him, as most of the reports were unpublished except for two records from La Coruña in Spain (López-Jamar et al. 1986; Souprayen et al. 1991). Gil suggested that these identifications were based on using keys not relevant to the region and not represent valid records.

*Pseudopolydora kempi* was described by Southern (1921) from India. The description was based on five small posteriorly incomplete specimens. Later, Okuda (1937) described a variety of this species from Japan. Afterward, Imajima and Hartman (1964) designated this Japanese material as a subspecies and named it *Pseudopolydora kempi japonica* Imajima & Hartman, 1964. In 1969, Light described another subspecies, *Pseudopolydora kempi californica* (Light, 1969), from the Californian coast. Several authors (Blake and Woodwick 1975; Light 1978; Blake 1996) queried the validity of these subspecies, because of the inadequate original description of *P. kempi* and the lack of type material (Southern 1921). Radashevsky and Hsieh (2000) named their specimens collected in Taiwan as *Pseudopolydora cf. kempi japonica* as it closely fitted the descriptions of Okuda (1937) and Imajima and Hartman (1964). In their revision of *Pseudopolydora* species from Taiwan (2000), they provided a new description of the subspecies and a key. Sato-Okoshi (2000) also recorded her Japanese material as *Pseudopolydora cf. kempi*. In 2016, Abe et al. (2016) provided the first biomolecular analysis (18S, 28S) for Japanese specimens named as *P. cf. kempi*. Radashevsky et al. (2020) undertook a molecular study of the *Pseudopolydora* including *Pseudopolydora kempi japonica* from the Sea of Japan, using three genes (16S, 28S and H3). Finally, Abe and Sato-Okoshi (2021) compared the Japanese sequences of Abe et al. (2016), Radashevsky et al. (2020) and new data of *P. cf. kempi* (sequences 18S, 16S). They concluded that *P. cf. kempi* and *P. kempi japonica* belonged to the same species. All these previous authors pointed out that further investigations were needed to clarify the status of this taxon (Radashevsky and Hsieh 2000; Sato-Okoshi 2000; Abe et al. 2016; Abe and Sato-Okoshi 2021).

The aim of this study was to investigate the presence of *P. kempi japonica* in European waters using both morphological and molecular analyses.



**Figure 1.** Locations of *Pseudopolydora kempi japonica* Imajima & Hartman, 1964 in France.

## Materials and methods

### Sampling

Specimens were collected in four locations between the Morbihan Bay and the Arcachon Bay (Bay of Biscay, SW France) (Figure 1). Most of them were collected on intertidal mudflats with different kind of corers (depending of the study), but three specimens were sampled from a subtidal habitat, at depths of between 5 and 15 m, using a Van Veen grab (0.1 m<sup>2</sup>). All samples were sieved through a 1 mm mesh and fixed either in 4% formalin or in ethanol (70–99%). Once sorted, organisms were stored in 70% ethanol. A few parapodia were removed from some specimens and preserved in ethanol for molecular analysis. Specimens were examined under a Nikon SMZ25 stereomicroscope and a Nikon Eclipse Ci microscope, and photographed with a Nikon DS-Ri 2 camera. Measurements were made with the NIS-Elements Analysis software. A specimen from Arcachon Bay was prepared for SEM by critical point drying, coated with gold and examined and photographed with a Hitachi TM3030. Material is deposited at the Muséum National d’Histoire Naturelle, Paris (MNHN) and at the Arcachon Marine Station (SMA) (Table 2).

### *Molecular data and analyses*

Extraction of DNA was done with DNA blood and tissue (QIAGEN) following protocol supplied by the manufacturers. Approximately 330 bp of 28S, 300 bp of H3 and 630 bp of 18S genes were amplified, using the following primers: C1 and C2 for 28S (Le et al. 1993), H3af and H3ar for H3 (Colgan et al. 1998) and 18Sa2.0 and 9R for 18S (Giribet et al. 1996). PCR (Polymerase Chain Reaction) was performed with GoTaq® G2 Flexi DNA Polymerase Kit in 20 µL mixtures containing: 4 µL of 5X Green GoTaq® Flexi Reaction Buffer (final concentration of 1X), 1.2 µL of MgCl<sub>2</sub> (25 Mm) solution, 0.4 µL of PCR nucleotide mix (final concentration of 0.2 mM each dNTP), 0.2 µL of each primer (final concentration of 1 µM), 0.1 µL of Taq DNA Polymerase (5U/µl), 1 µL template DNA and 12.9 µL of nuclease-free water. The temperature profile was as follows 94 °C/5 min – (94 °C/15 s – 52 °C for 28S, 53 °C for H3, 49 °C 18S/30 s – 72 °C/60 s)\*35 cycles – 72 °C/7 min – 16 °C. PCR success was verified by electrophoresis in a 1% p/v agarose gel stained with ethidium bromide. Amplified products were sent to Eurofins Genomics Company to complete double strain sequencing, using same set of primers as used for PCR. Overlapping sequence (forward and reverse) fragments were merged into consensus sequences and aligned using Geneious Prime 2019.0.4.

Thirty-one sequences were downloaded from GenBank, with 29 sequences corresponding to *Pseudopolydora* species (15 of for 28S gene and 14 for H3 gene), whilst the remaining two were of *Polydorella dawydoffi*, the outgroup species (Table 1). In this study, four sequences of 28S, two of H3 and two sequences of 18S were also obtained for French specimens of *P. kempi japonica* and deposited in GenBank (Table 1).

All 28S and H3 sequences were aligned in Geneious Prime 2019.0.4 using the MAAFT plugin and default settings and we concatenated DNA data in Geneious Prime 2019.0.4 too. DNA data have been partitioned and the maximum likelihood analysis was performed in IQ-TREE 2.2.0 (Trifinopoulos et al. 2016) with the best fitting evolutionary model TIM2+G4 for 28S and TN+F+G4 for H3. IQ-TREE determine automatically the best-fit substitution model for our data. Bootstrap support was estimated using an ultrafast bootstrap algorithm (UFBoot) (Hoang et al. 2018) for 1000 replicates. FigTree v. 1.4.4 (Rambaut 2007) was used to visualise the majority-rule consensus tree displaying all nodes with a posterior probability > 0.8.

## **Results**

### *Material examined*

A total of 512 specimens were examined. Most of them were originally identified as *P. pulchra*. They were collected from 2004 to 2022 in the Gironde estuary (396 specimens in four stations), from 2017 to 2022 in Arcachon Bay (40 specimens in 14 stations), in 2017 in Morbihan Bay (one specimen), and in 2021 in Aiguillon Bay (75 specimens in one station) (Bay of Biscay, France) (Figure 1, Table 2).

**Table 1.** Terminal taxa used in the molecular part of the study (28S and H3 genes), with type localities, collection localities, GenBank accession numbers (Radashevsky et al. 2020, this study for French *P. japonica kempii*)

Species	Voucher	Type locality	Collection locality	28S GenBank accession number	18S GenBank accession number	H3 GenBank accession number
<i>Polydorella dawydoffi</i> Radashevsky, 1996	VIR 12089	China Sea	Vietnam, Nha Trang Bay	MG460975		MG460831
<i>Pseudopolydora achaeta</i> Radashevsky & Hsieh, 2000	VIR 13031	Taiwan	Taiwan, Tainan, Erhjen River	MG460978-79		MG460834-35
<i>Pseudopolydora bassarginensis</i> (Zachs, 1933)	VIR 11473	Japan	Russia, Sea of Japan, Vostok Bay	MG460970		MG460825
<i>P. bassarginensis</i> (Zachs, 1933)	VIR 20018	Japan	South Korea, East Sea, Jinhae Bay	MG461038		MG460883
<i>Pseudopolydora diopatra</i> Hsieh, 1992	VIR 13035	Taiwan	Taiwan, Hsinchu	MG460984		MG460837
<i>P. kempii japonica</i> Imajima & Hartman, 1964	VIR 11474	Japan	Russia, Sea of Japan, Vostok Bay	MG460973-74		MG460828-29
<i>P. kempii japonica</i> Imajima & Hartman, 1964	VIR 18015	Japan	South Korea, East Sea, Jinhae Bay	MG460996-97		MG460845-46
<i>P. kempii japonica</i> Imajima & Hartman, 1964	SMA-llkem-01	Japan	France, Arcachon Bay	PP706173	PP706118	PP750968
<i>P. kempii japonica</i> Imajima & Hartman, 1964	SMA-llkem-02	Japan	France, Arcachon Bay	PP706174	PP706119	PP750969
<i>P. kempii japonica</i> Imajima & Hartman, 1964	SMA-llkem-03	Japan	France, Gironde estuary		PP706120	
<i>P. kempii japonica</i> Imajima & Hartman, 1964	MNHN-IA-2017-2218	Japan	France, Arcachon Bay		PP706121	
<i>P. paucibranchiata</i> (Okuda, 1937)	VIR 18012	Japan	South Korea, East Sea, Jinhae Bay	MG460994		MG460843
<i>P. paucibranchiata</i> (Okuda, 1937)	VIR 18253	Japan	South Korea, East Sea, Jinhae Bay	MG461011		MG460860
<i>P. pulchra</i> (Carazzi, 1893)	VIR 18571	Tyrrhenian Sea, Italy	France, Brittany, Bay of Morlaix	MG461012		-
<i>P. pulchra</i> (Carazzi, 1893)	VIR 18598	Tyrrhenian Sea, Italy	France, Brittany, Roscoff	MG461013		MG460861

**Table 2.** Sampling date, coordinates and characteristics of the sampling sites.

Site	Location	N	Station	Date	Lat (N)	Long (W)	Sediment	Zone	Vouchers
1	Morbihan Bay	1	Réserve naturelle du marais de Séné	Nov 2017	2.704167	47.598056	Mud	intertidal	
2	Aiguillon Bay	75	Pointe de l'Aiguillon	Apr-Jun 2021	1.188611	46.272500	sandy mud	intertidal	
3		282	Saint-Christoly	2004-2022	0.821944	45.363056	Mud	intertidal	SMA-llkem-03
4	Gironde Estuary	35	Bonne Anse 2	Nov 2020	1.201944	45.688611	muddy sand – sandy mud; <i>Zostera</i> bed	intertidal	
5		78	Bonne Anse 3	Nov 2020	1.209444	45.680278	Mud	intertidal	
6		1	Talmont-sur Gironde	Nov 2020	0.915000	45.546944	Mud	intertidal	
7		7	Jacquets	Oct 2019	1.191389	44.722778	muddy fine sand; oyster reef	intertidal	MNHN-IA-2017-2219 (1 specimen)
8		2	Dispute-RS	Aug 2020	1.103056	44.709167	muddy fine sand; oyster reef	intertidal	MNHN-IA-2017-2221 (1 specimen)
9		2	Dispute-IM	Aug 2020	1.117222	44.706111	muddy fine sand; oyster reef	intertidal	
10		1	Afrique-C	Mar 2017	1.101111	44.679444	muddy fine sand	intertidal	
11		2	Afrique-B	Apr 2019	1.193056	44.685833	muddy fine sand	intertidal	MNHN-IA-2017-2220 (1 specimen)
12		3	Afrique-B	Jun 2020	1.193056	44.685833	muddy fine sand	intertidal	SMA-llkem-01-02
13	Arcachon Bay	2	Port of Arcachon	Sep 2020	1.150833	44.660833	muddy fine sand	subtidal	
14		3	Jalles	Jul 2019	1.190000	44.711389	muddy fine sand	intertidal	
15		5	Jalles	Sep 2020	1.190000	44.711389	muddy fine sand	intertidal	
16		3	Cassy	May 2019	1.060556	44.706667	muddy fine sand	intertidal	
17		3	Arès	Apr 2021	1.191389	44.722500	muddy fine sand	intertidal	SMA-llkem-04
18		1	Comprian	Sept 2021	1.103333	44.677500	fine sand	subtidal	
19		4	Ile aux Oiseaux - tonne / chenai	Oct 2022	1.178056	44.695278	mud – muddy fine sand	intertidal	MNHN-IA-2017-2218 (1 specimen)
20		2	Ile aux Oiseaux - Réousse	Oct 2022	1.198889	44.684444	sandy mud; eelgrass bed	intertidal	



### *Systematic*

Order Spionida sensu Rouse & Fauchald, 1997

Family Spionidae Grube, 1850

Genus *Pseudopolydora* Czerniavsky, 1881

Species *Pseudopolydora kempii* (Southern, 1921)

### **Subspecies *Pseudopolydora kempii japonica* Imajima and Hartman, 1964**

Figures 2, 3

### *Description*

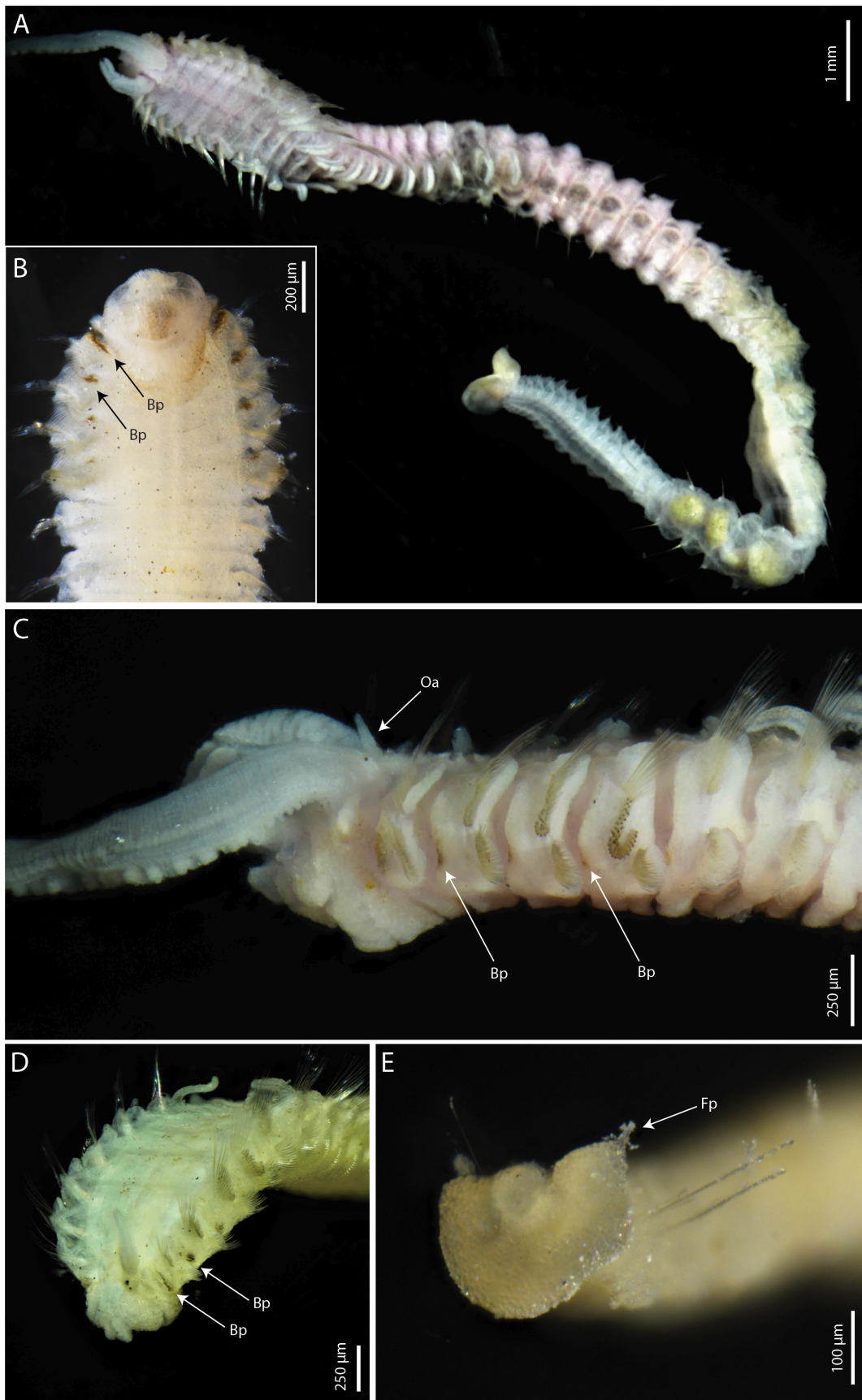
Largest entire (MNHN-IA-2017-2220) specimen 16.5 mm long for 55 segments and 1.4 mm wide at segment 5 (Figure 2A, C); smallest entire specimen 4.6 mm long for 33 segments and 0.6 mm wide at segment 5. Anterior region flattened and larger than rest of the body (Figure 2A). Prostomium anteriorly incised, forming two blunt lobes (Figure 2D). Two pairs of black eyes. Occipital antenna present (Figure 2C) and surrounded by notopodial lamellae of first segment. Palps reaching segment 15. Caruncle extended posteriorly to segment 3.

All specimens with black transverse bands on antero-lateral edges of anterior segments (Figure 2B–D). Some well-preserved specimens with two dorsal rows of black spots (Figure 2D), absent in most of specimens (presumed lost after fixation). Branchiae from segment 7 to about 20 (Figure 2A). Notochaetae from segment 2, absent on last segment. Notochaetal rows of segments 3–6 distinctly modified in shape and arrangement in comparison with other segments (Figure 2C). Segment 5 with the greatest modification with lower notochaetae arranged in J-shape double row (Figure 2C). Anterior row with broadly limbate chaetae and posterior row with about 14 blunt spines, gently curved terminally (Figure 3A, B). Neuropodial hooded hooks from segment 8; with about 20 per segment; with two teeth (Figure 3C). Main fang of hooks forming right angle to shaft; apical tooth closely applied to main fang (Figure 3C); shaft enlarged under the hood insertion (Figure 3C).

Pygidium large, thick flask-shaped with a wide dorsal gap crowned by two dorso-lateral lappets bearing a short filiform process (Figure 2E).

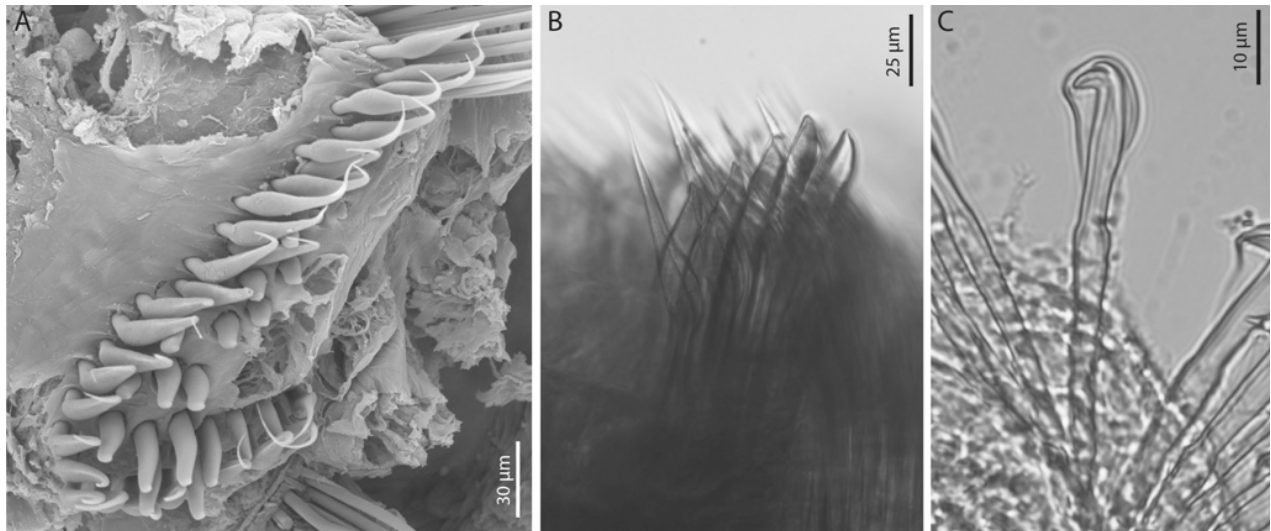
### *Taxonomic remarks*

Material examined during this study differs slightly from that described by Imajima and Hartman (1964), based on Okuda's description (1937). Japanese specimens were longer, with a longer caruncle extending to the anterior margin of segment 4 (instead of to segment 3 for French specimens), a larger number of branchiae (from segment 7 to segment 24–28, instead of to segment 21 in this study), more neuropodial hooded hooks per segment (to 23–30, instead of about 20 in the present study) and lacked colour patterns whereas French material all had black pigmentation. However, the shape of chaetae, especially in segment 5, and pygidium were very similar.



**Figure 2.** *Pseudopolydora kempi japonica* from Arcachon Bay: A. whole specimen (MNHN-IA-2017-2220); B. anterior end, ventral view (MNHN-IA-2017-2221); C. anterior end, lateral view (MNHN-IA-2017-2220); D. anterior end, dorso-lateral view (MNHN-IA-2017-2019); E. pygidium (MNHN-IA-2017-2221). Abbreviations: Bp, Black pigmentation; Fp, Filiform process; Oa, Occipital antenna.





**Figure 3.** *Pseudopolydora kempi japonica* from Arcachon Bay: A. segment 5 (SMA-Ilkem-04); B. limbate chaetae and spines of chaetiger 5 (MNHN-IA-2017-2220); C. hooded hook of segment 11 (MNHN-IA-2017-2220).

Japanese specimens of Myohara (1979) resembled the French material with similar body and palps sizes and the same number of branchiae, but this author didn't mention filiform processes on the pygidium.

Japanese specimens studied by Abe et al. (2016) were similar to French material in being of similar size, with a caruncle extending to chaetiger 3, a similar number of branchiae (from segment 7 to 17–25), and a similar pigmentation for fixed individuals. However, the pygidium differs by the shape of the dorsal lappets which are short erect process for Abe et al. 2016, without the filiform appendages present on French specimens.

Specimens from Taiwan (Radashevsky and Hsieh 2000) were similar to French material in being of similar size, branchiae (from segment 7 to 20), about 22 neuropodial hooded hooks per segment, and with same pigmentation. As previously, authors described a pygidium with wide dorsal gap and dorso-lateral lappets, but without any filiform process.

The Californian specimens observed by Blake and Woodwick (1975), which agreed with description given by Okuda (1937) and questioned the validity of these subspecies *californica*, matched with our specimens in having well developed occipital antenna and the same pygidium. According to their analysis, dorsal elevations or projections on the pygidium appear during the growth of specimens. This analysis could explain the morphological differences between pygidium described in the different descriptions mentioned above.

#### *Type locality*

Japan (Imajima and Hartman 1964)

#### *Distribution*

*Pseudopolydora kempi japonica* has been recorded from Japan (Imajima and Hartman 1964; Abe et al. 2016, 2019; Radashevsky et al. 2020; Abe and Sato-Okoshi 2021), Taiwan (Radashevsky and Hsieh 2000) and France (this study).

Previous records of *P. kempii* cryptic species and their status need to be clarified of records from India (Southern 1921), Korea (Okuda 1937), Mozambique (Day 1955, 1967), USA (Blake and Woodwick 1975; Light 1978; Hiebert 2015), Canada (Banse 1972), Australia (Blake and Kudenov 1978), Russia (Radashevsky 1993; Buzhinskaja 2013) and in China (Zhou et al. 2010).

### *Habitat*

Japan: sand flat, tidal flat and mudflat (Abe et al. 2016; Abe and Sato-Okoshi 2021).

Taiwan: soft bottoms of brackish-water environments (Radashevsky and Hsieh 2000).

Present study: muddy sands to fine muds in mesohaline to euryhaline waters. Oyster reefs and *Nanozostera noltei* (Hornemann) Tomlinson and Posluszny 2001 eelgrass beds (Table 2). Mostly in intertidal zone (Table 2).

Radashevsky and Hsieh (2000) described specimens with muddy tubes, which were not found during our study.

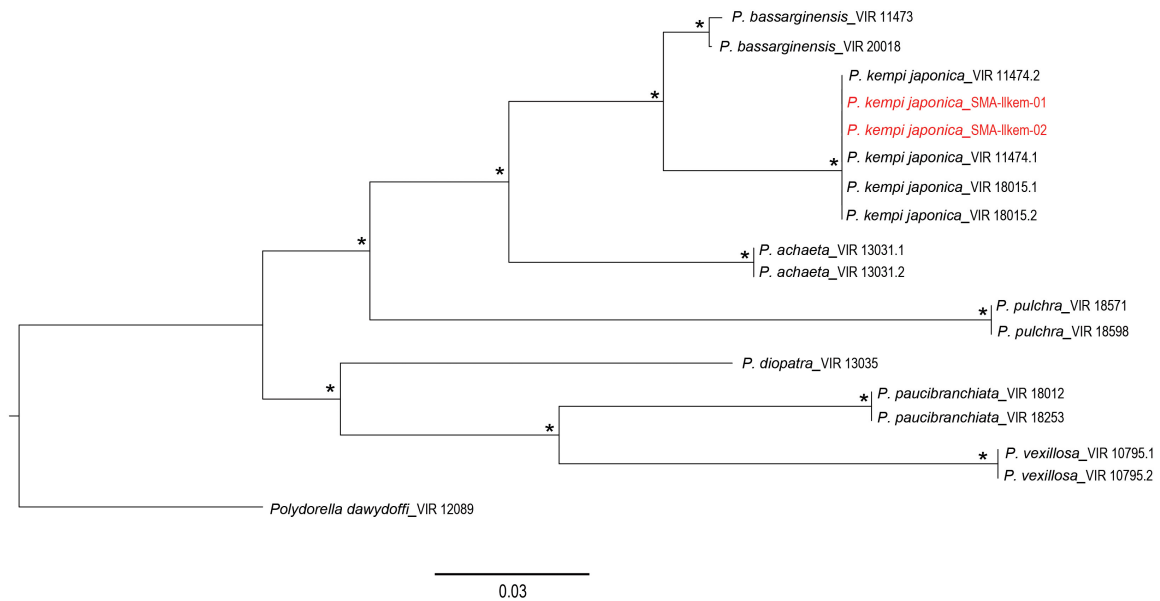
*Pseudopolydora kempii japonica* is typically found on intertidal mudflat in confined environments such as estuaries, bays, ports and lagoons, often in brackish waters (Okuda 1937; Radashevsky and Hsieh 2000; Kondoh et al. 2017; Abe et al. 2019; this study).

### *Biology*

*Pseudopolydora kempii japonica* has an adelphophagic and lecithotrophic larval development in which larvae feed on nurse eggs in brood capsules deposited into a sand/mud tube constructed by adult females and growth for 14 days before hatching (Kondoh et al. 2017). Then, they have a short-term pelagic life as they can drift and settle on sediment within a day (Kondoh et al. 2017). Metamorphosis of the pelagic larva into a benthic juvenile lead to the loss or modification of larval organs and to the development of other organs suitable for endogean and sedentary life in a sand/mud tube (Blake and Woodwick 1975; Myohara 1979). It may have rapid crawling activity and be able to hide in sediment or crevices when removed from its habitat (Sato-Okoshi 2000).

### *Genetic data*

H3, 18S and 28S genes were successfully sequenced for several specimens and published at NCBI GenBank (Table 1, Figure 4). Based on the majority rule consensus tree of the Bayesian inference analysis combining 28S and Histone 3 sequences, French specimens are similar to specimens from South Korea and Russia, confirming the identity of this species, which clearly differs from the other ones with available sequences on Genbank.



**Figure 4.** Majority rule consensus tree of the Bayesian inference analysis of the combined 28S and Histone 3 sequences of *Pseudopolydora* spp. rooted with sequences of *Polydorella dawydoffi* (Table 1). Asterisks indicate posterior probability > 80%. Text in red indicates specimens used in this study.

*Identification key to Pseudopolydora species from European Waters, based on Radashevsky (2021) and Gil (2011) keys*

- 1a. Prostomium anteriorly entire (rounded). Occipital antenna present or absent..... 2
- 1b. Prostomium anteriorly incised (bilobed). Occipital antenna present..... 3
- 2a(1a). Occipital antenna present. Palps with branching yellow chromatophores in living polychaetes, absent in fixed worms.....  
..... *P. paucibranchiata* (Okuda, 1937)
- 2b(1a). Occipital antenna absent. Palps without pigment .....  
.....*P. nordica* (Radashevsky, 2021)
- 3a(1b). Prostomium forming two relatively long lobes. Anterior part of the body without dark pigmentation. Caruncle extends back to the end of segment 4.....*P. antennata* (Claparède, 1868)
- 3b(1b). Prostomium forming two blunt lobes. Anterior part of the body with dark pigmentation. Caruncle not extending beyond segment 3 ..... 4
- 4a(3b). Intense black pigment diffused on prostomium, peristomium, palps and on the dorsal and lateral parts of the anterior segments; juveniles up to about 30-segment stage without pigmentation. Caruncle extends back to end of segment 1. Notochaetae of the 5<sup>th</sup> segment arranged in a U to a flared J-shape double row. Pygidium large disc with dorsal gap, without appendage .....*P. pulchra* (Carazzi, 1893)
- 4b(3b). Dark pigmentation only on lateral edges of anterior segments (two dorsal rows of black spots on well preserved specimens). Caruncle extends back to segment 3. Notochaetae of the 5<sup>th</sup> segment arranged in a sharpened J-shape double row. Pygidium large disc with dorsal gap surrounding by 2 dorso-lateral digitiform lappets .....  
..... *P. kempi japonica* Imajima & Hartman, 1964

## Discussion

### *Possible vectors for the introduction of the species*

The non-indigenous *P. kempi* ssp. *japonica* is reported for the first time from European waters. While the first occurrence was from Gironde estuary in 2004, it seems likely it was introduced much earlier (Lavesque et al. 2020a, b). In the 1970's, after the widespread mortality of the farmed species Portuguese cupped oysters *Magallana angulata* (Lamarck, 1819), the Japanese oyster *Magallana gigas* (Thunberg, 1793), also known as the Pacific cupped oyster, was massively imported into Arcachon Bay, Pertuis Charentais (Aiguillon Bay) and the Gironde estuary. About 1200 t of spat of Pacific cupped oysters collected from Sendai Bay (Japan) were introduced in Arcachon Bay in the early 1970's, including exotic polychaete worms (Lavesque et al. 2020b). *Pseudopolydora kempi japonica* is not known to be a commensal species of the oysters, but can be associated with them as they are found in same kind of confined environments (Okuda 1937; Radashevsky and Hsieh 2000; Kondoh et al. 2017; Abe et al. 2019; Lavesque et al. 2020b; this study). The Japanese oyster was also imported to several other oyster farms along the northern (e.g. Normandy, Brittany) and the Mediterranean coasts of France, which transfer oysters between them on an ongoing basis for decades. These transfers, coupled with the opportunistic behaviour of *P. kempi japonica*, suggest that the species might have a wider geographical range along French coasts than we have recorded. A recent study by Sato-Okoshi et al. (2022) on polydorids associated with *M. gigas* in Normandy (France) did not find *P. kempi japonica* but it is unclear if this is related to water temperatures or other factors. As the oyster *M. gigas* has been imported to other European countries and elsewhere (Ruesink et al. 2005), *P. kempi japonica* may occur elsewhere outside of its native range.

In addition to aquaculture, another potential vector of introduction could be the ballast waters (Carlton and Geller 1993; Carlton 1996). However, *P. kempi japonica* has a short-term pelagic life of one day before settling (Kondoh et al. 2017), which raises the question about the survival rate of post-larvae landing at the bottom of the ballasts until their opening. In any case, this way of introduction cannot concern Arcachon Bay which is a semi-enclosed lagoon where large ships cannot enter (Lavesque et al. 2013). Moreover, due to this short planktonic life, it appears unlikely that the introduction in Arcachon Bay comes from adjacent areas as it is geographically too far from other environments favorable to the establishment of the species. The closest favorable area is the Gironde estuary, 100 km north of Arcachon Bay. The maximum speed of tidal currents between these two localities is 0.5 m/s (Lazure and Desmare 2012), or 43 km in 24 hours. Following Light (1978), the Japanese oyster was also the most likely vector of introduction of Californian populations of *P. kempi*.

For now, *P. kempi japonica* has not shown any impact on the ecosystems where it was observed. The population in the Gironde estuary seems to decrease over time and the number of specimens collected, as for the other French sites, remains too low to detect any invasive or harmful nature of the species.

### *Identification problems linked to a species-complex which remains to be clarified*

In France, this spionid species has probably been confused with the indigenous species *Pseudopolydora pulchra*. The two species are similar in the shape of their prostomium which is bilobed, and in the presence of dark pigmentation on the anterior part of the body.

The French specimens, as the very similar Taiwanese specimens (Radashevsky and Hsieh 2000), showed some morphological differences with the Japanese original description (Okuda 1937; Imajima and Hartman 1964). However, molecular analysis enabled to confirm the conspecificity of French specimens and the Japanese material analyzed by Radashevsky et al. (2020) and by Abe and Sato-Okoshi (2021). Our study supports the hypothesis that Taiwanese specimens (Radashevsky and Hsieh 2000) belong to the same species, since French specimens well correspond to their morphological description (Radashevsky and Hsieh 2000).

Finally, this study takes part in the issue concerning the validity of the different *P. kempi* subspecies (Blake and Woodwick 1975; Light 1978; Blake 1996; Radashevsky and Hsieh 2000; Sato-Okoshi 2000; Abe et al. 2016; Abe and Sato-Okoshi 2021). First of all, the lack of material used for the original description of the species *P. kempi* (only five small specimens without pygidium) is irrelevant in terms of taxonomic comparisons. Okuda (1937) already put forward significant morphological differences such as the length of the caruncle prolongation (absent for Indian specimens (Southern 1921) vs reaching segment 4 for Japanese ones (Okuda 1937)). Based on morphological criteria only, this Japanese taxon could have been described as a new species. In addition, the geographical remoteness between India and Japan is an important fact to consider. Further biomolecular works should be conducted to compare the different populations belonging to the *P. kempi* complex from the western Pacific and Europe (Imajima and Hartman 1964; Radashevsky and Hsieh 2000; Abe et al. 2016; Abe and Sato-Okoshi 2021; this study), from the eastern Pacific (Light 1969; Blake and Woodwick 1975), and from India (Southern 1921).

### **Authors' contribution**

LL: research conceptualization; investigation and data collection; original draft, review and edition. GD: data analysis and interpretation. FD: data analysis and interpretation. JJ: investigation and data collection; review and edition. NL: research conceptualization; investigation and data collection; review and editing.



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