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A new species from the Mediterranean Sea and North-Eastern Atlantic Ocean: *Knoutsodonta pictoni* n. sp. (Gastropoda Heterobranchia Nudibranchia)

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ABSTRACT *Knoutsodonta pictoni* n. sp. (Gastropoda Heterobranchia Nudibranchia) is described here based on morphological and molecular analyses of specimens from Mediterranean Sea and North Atlantic Ocean. Ecological notes on the egg development and new species distribution range are also presented. COI DNA barcoding was used to molecularly identify this species and to assess one sequence present in Genbank but for which identification was not provided. Furthermore, the intraspecific genetic divergence was explored for specimens belonging to different populations.

KEY WORDS DNA-Barcoding; new species; Nudibranchs; Onchidorididae; *Knoutsodonta*.

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INTRODUCTION

At the beginning of 2015 the genera placed within the family Onchidorididae (Gastropoda Heterobranchia Nudibranchia) were: *Acanthodoris* Gray, 1850, *Adalaria* Bergh, 1878, *Calycidoris* Abraham, 1876, *Corambe* Bergh, 1869, *Diaphorodoris* Iredale et O'Donoghue, 1923, *Onchidoris* Blainville, 1816 and *Onchimira* Martynov, Korshunova, Sanamyan et Sanamyan, 2009. Later on, Hallas & Gosliner (2015) based on the results of molecular and morphological analyses reestablished the families Corambidae, with the genus *Corambe*, and Calycidorididae including the genera *Calycidoris* and *Diaphorodoris*. The remaining genera were maintained as genus as in the case of the *Acanthodoris*, or divided into two different genera as happened for *Onchidoris* and *Adalaria*. In particular, the species of *Onchidoris* and *Adalaria* with a rachidian tooth were grouped into the genus *Onchidoris* whereas species with no rachidian tooth were placed in *Knoutsodonta* Hallas et Gosliner, 2015. In 2015, almost concurrently, specimens of the *Onchidoris*-like group were photographed and collected in Sardinia (Italy), in Catalunya (Spain) and in Ireland. Some photographed individuals, displayed in social media, showed a strong similarity with specimens from Mediterranean Sea and North-Eastern Atlantic Ocean.

In Trainito & Doneddu (2015) the Sardinian findings were reported as *Onchidoris* sp. and in the discussion the possibility was expressed that they should be assigned to a new species, considering that their external morphology did not match any of the described species of the family Onchidorididae. One specimen was found at East Wall Loch Nevis, Scotland on 22nd August 2015 and afterwards published by J. Anderson on the web as *Knoutsodonta* sp. A. (http://www.nudibranch.org/Scottish%20Nudibranchs/html/knoutsodonta-spA-01.html).

A similar specimen was photographed in 2011 by G. Brown at Loch Sween, Scotland and published by M. Faasse on the Facebook group page of NE Atlantic Nudibranchs. Subsequently, in Ballesteros et al. (2016), one photograph of an individual was reported showing the same external features of the samples found in 2015. In the discussion, it was described as *Knoutsodonta* sp. A, based on a preliminary analysis of the radula that lacked the rachidian tooth, and the dataset of observations was increased, dating back to 1992. In 2016 other individuals with the same external morphology were photographed and collected in Northern Adriatic Sea (Sistiana, Trieste, Italy). One individual with the characteristic egg coils, was found at Cape Noli (Liguria, Italy) and preliminarily identified as *Onchidoris pusilla* (Alder et Hancock, 1845) (Betti et al., 2017), but the external morphology revealed this to be more similar to the specimens reported in Trainito & Doneddu (2015). Furthermore, other specimens with the same phenotype have been reported as *Onchidoris pusilla* from Ensenada de los Berengueles (Granada, Spain), Estartit, Blanes and Tossa de Mar (Catalunya, Spain) (GROC, http://www.opistobranquis.org/en/guia/10).

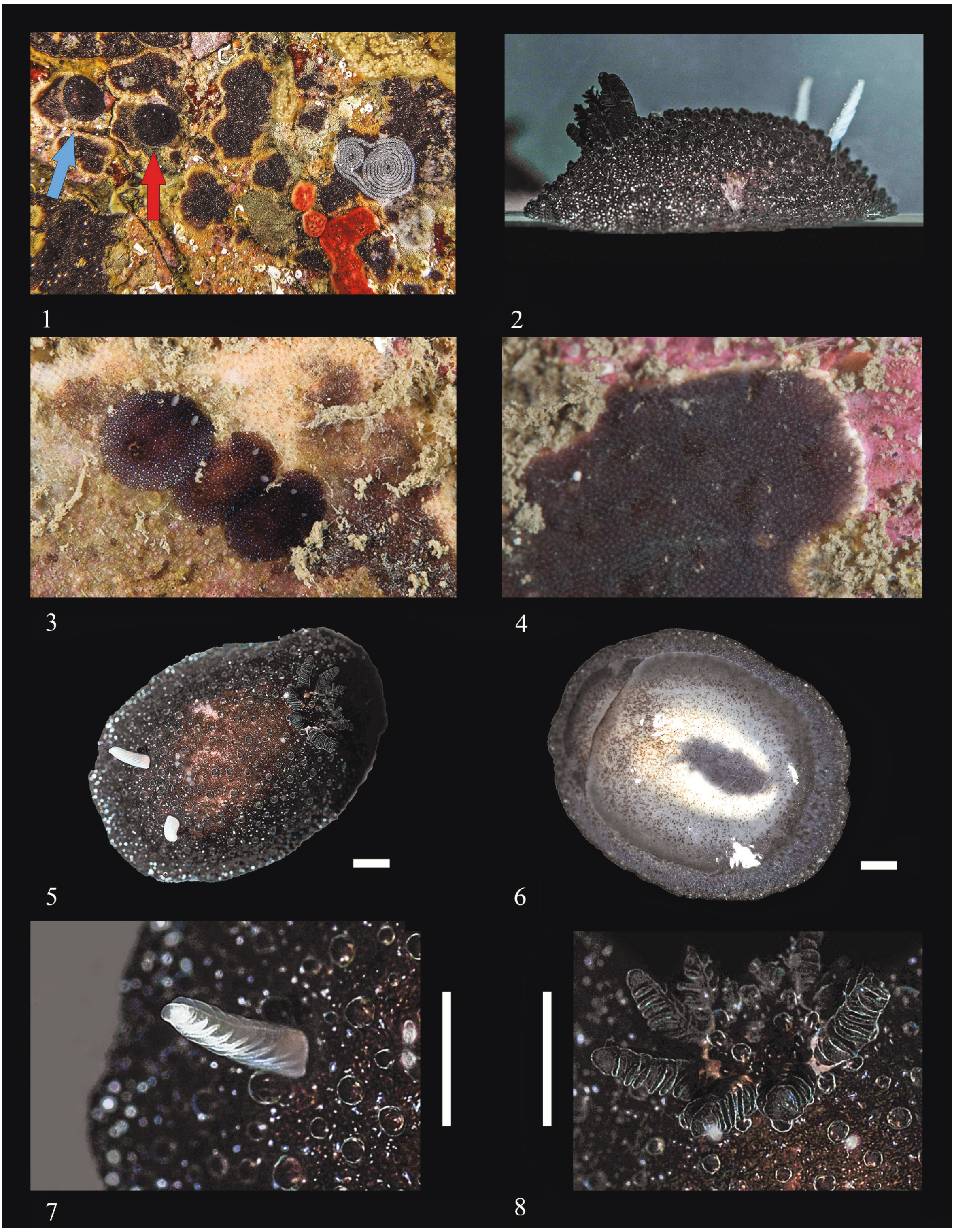
In this paper we describe this species as new to science, through morphological and molecular analyses on individuals collected from Central Tyrrhenian (North-Eastern Sardinia) and Adriatic (Trieste) Seas and from the North-eastern Atlantic Ocean (Ireland), discussing their taxonomic position with a focus on other Mediterranean species of the genus.

MATERIAL AND METHODS

Specimens of the new species were collected by scuba diving from different localities (Table 1). Individuals, egg spawns and the bryozoans on which

Species	Voucher	Locality	COI accession number	References
Knoutsodonta brasiliensis	BNHS-Opistho-336	--	KC255226	Bhave et al., unpublished
Knoutsodonta depressa	CASIZ186769A	Huelva, Spain	KP340409	Hallas & Gosliner, 2015
Knoutsodonta jannae	CASIZ175578	Pillar Point: San Mateo Co., California	KP340392	Hallas & Gosliner, 2015
Knoutsodonta oblonga	MN3010A	Mewstone, Skomer	KP340410	Hallas & Gosliner, 2015
Knoutsodonta pictoni	BAU02982	South of Inishgalloon, Purteen, Keel, Achill Island, Ireland	LT840347	Present study
Knoutsodonta pictoni	BAU02983	South of Inishgalloon, Purteen, Keel, Achill Island, Ireland	LT840348	Present study
Knoutsodonta pictoni	BAU02985	Sistiana, Trieste, Italy	LT840346	Present study
Knoutsodonta pictoni	MNHN-IM-2000-33333	Porto San Paolo, Sardinia, Italy	LT840344	Present study
Knoutsodonta pictoni	MNHN-IM-2000-33334	Porto San Paolo, Sardinia, Italy	LT840345	Present study
Knoutsodonta sp. A	CASIZ 208194	Scotland	KP340411	Hallas & Gosliner, 2015
Onchidoris bilamellata	MT09252	North Sea	KR084801	Barco et al., 2016
Onchidoris bilamellata	CASIZ_101555	Peninsula Point, Marin Co., California	KP340408	Hallas & Gosliner, 2015
Onchidoris bilamellata	10NBMOL-10020	Passamaquoddy Bay, St. Andrews, New Brunswick, Canada	KF644026	Layton et al., 2014
Onchidoris bilamellata	10NBMOL-10019	Passamaquoddy Bay, St. Andrews, New Brunswick, Canada	KF643873	Layton et al., 2014
Onchidoris bilamellata	10BCMOL-00203	Rocky beach, Wizard Islet, Bamfield, British Columbia, Canada	KF643475	Layton et al., 2014
Onchidoris bilamellata	10NBMOL-10018	Passamaquoddy Bay, St. Andrews, New Brunswick, Canada	KF643245	Layton et al., 2014
Onchidoris evincta	CASIZ_187758B	Puget Sound, Kitsap Co., Washington	KP340391	Hallas & Gosliner, 2015
Onchidoris evincta	CASIZ_186817	Puget Sound, Kitsap Co., Washington	KP340389	Hallas & Gosliner, 2015
Onchidoris loveni	NTNU_65511B	Mausund, Norway	KP340395	Hallas & Gosliner, 2015
Onchidoris loveni	NTNU_65511A	Mausund, Norway	KP340394	Hallas & Gosliner, 2015
Onchidoris loveni	NTNU_66880A	Gløssvika, Norway	KP340393	Hallas & Gosliner, 2015
Onchidoris muricata		Clachan Seil, Scotland	AY345033	Grande et al., 2004
Onchidoris muricata	MT07703	North Sea	KR084489	Barco et al., 2016
Onchidoris muricata	CASIZ_184185A	New Castle Portsmouth Bay: Rockingham Co., New Hampshire	KM219681	Hallas & Gosliner, 2015
Onchidoris muricata	CASIZ_181312	Asilomar, Monterey Co., California	KM219680	Hallas & Gosliner, 2015
Onchidoris muricata	10BCMOL-00318	Juskalta Narrows, Haida Gwaii, British Columbia, Canada	KF643468	Layton et al., 2014
Onchidoris muricata		Kristineberg, Bohuslan, Sweden	AJ223271	Thollesson M., 2000
Onchidoris proxima	CASIZ_183931A	Passamaquody Bay Eastport: Washington Co., Maine	KM219677	Hallas & Gosliner, 2015
Onchidoris proxima	CASIZ_183921A	Passamaquody Bay Eastport: Washington Co., Maine	KM219676	Hallas & Gosliner, 2015
Diaphorodoris luteocincta	BAU2754	Gulen, Norway	LT615386	Furfaro et al., 2016
Diaphorodoris luteocincta	BAU2755	Gulen, Norway	LT615387	Furfaro et al., 2016
Diaphorodoris luteocincta	BAU2756	Gulen, Norway	LT615388	Furfaro et al., 2016

Table 1. List of the species names, vouchers, collection localities, COI GenBank accession numbers and references of the species of *Knoutsodonta* and *Onchidoris* genera and the out-group.



Figures 1–4. In situ photographs of the living specimensof *Knoutsodonta pictoni* n. sp. Fig. 1: holotype (MNHN-IM-2000-33333) (red arrow) and paratype (MNHN-IM-2000-33333) (light blue arrow) and the eggs (on the right side). Fig. 2: lateral view of the *K. pictoni* n. sp. Sardinian specimen (MNHN-IM-2000-33333). Fig. 3: three adults specimens from Ireland. Fig. 4: the encrusting bryozoan *Reptadeonella violacea* on which the new nudibranch species feed on. Figures 5–8. Holotype of the *Knoutsodonta pictoni* n. sp. (MNHN-IM-2000-33333). Fig. 5: dorsal view of the living adult. Fig. 6: ventral view. Fig. 7: a particular of the shape of the rhinophores. Fig. 8: a particular of the gills. Scale bar = 1 mm.

they were feeding were documented in situ with high definition photographs. Sardinian specimens were kept in an aquarium where both egg deposition and development were documented up to the veliger stage. The holotype and the paratype of the new species were preserved in Ethanol 95% and deposited at the Muséum national d'histoire naturelle (MNHN). All other collected individuals were stored in the malacological collection at the Department of Biology and Biotechnologies "Charles Darwin" ("Sapienza" University of Rome, Italy) (Table 1).

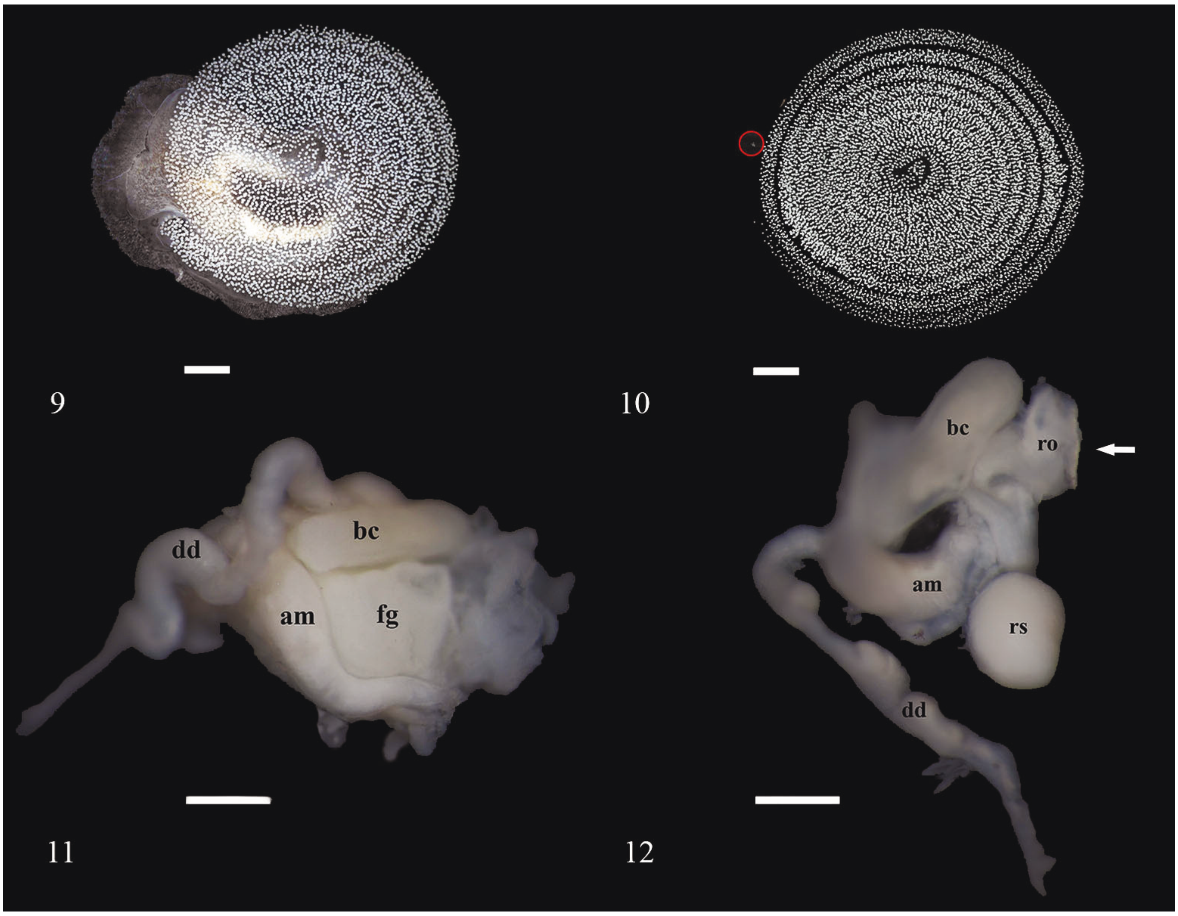
Anatomy of the reproductive system was studied under a dissecting microscope from at least two individuals. The buccal mass was placed in a 10% NaOH solution to isolate the radula, which was dehydrated to 100% ethanol, critical point-dried, gold coated, and examined by a Dualbeam SEM. The reproductive system was observed under a dissecting optical microscope and photographed at different stages of dissection.

Morphological analyses of the radula structure were performed using both SEM and optical microscope techniques.

Molecular identity was tested by using a partial sequence of the molecular marker mostly used for DNA barcoding of nudibranchs, the mitochondrial cytochrome c oxidase subunit I (COI) (see Table 1 for full list of samples, localities, and voucher references). A piece of tissue was cut from the foot for DNA extraction. Total genomic DNA was extracted using a standard proteinase K phenol/chloroform method with ethanol precipitation, as reported in Oliverio & Mariottini (2001). Partial sequences of COI were amplified by polymerase chain reaction (PCR) using the primers LCO1490(5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994) (PCR profile: 5 min denaturation step at 94°C; 35 cycles of 94°C/30 s, 48°C/60 s, 72°C/60 s; 7 min final extension at 72°C). Amplicons were sequenced by European Division of Macrogen Inc. (Amsterdam, The Nederland), using the same PCR primers. Sequences from each DNA strain were assembled and edited with Staden Package 2.0.0b9 (Staden et al., 2000). BLASTN (Altschul et al., 1990) search was conducted to exclude contamination. Sequences obtained were aligned together with those already present in GenBank using Muscle algorithm implemented in MEGA 6.0 (Tamura et al., 2013). We employed the Automatic Barcode Gap Discovery (ABGD, available at http://wwwabi.snv.jussieu.fr/public/abgd/), a distance-based method designed to detect the so called "barcode gap" in the distribution of pairwise distances calculated in a sequence alignment (Puillandre et al., 2012a, b) to test species delimitation in the newly produced COI dataset and produce primary species hypotheses based on DNA distances. Alignment of the COI sequences was submitted and processed in ABGD using the Kimura two parameters (K2p) nucleotide substitution model and the following settings: a prior for the maximum value of intraspecific divergence between 0.001 and 0.1, 30 recursive steps within the primary partitions defined by the first estimated gap, and a gap width of 0.1. The uncorrected pairwise genetic distances (p-distance) among COI sequences of the putative new species were also calculated with MEGA 6.0, and the maximum intraspecific distance estimated. Phylogenetic analyses on the COI dataset, were then performed basing on Bayesian Inference (BI) and Maximum Likelihood (ML) methods to test whether the primary species hypotheses of ABGD proved also monophyletic. In particular, BI was performed using MrBayes 3.2.6 (Ronquist et al., 2012) with four Markov-chains of five million generations each, sampled every 1000 generations. Consensus trees were calculated on trees sampled after a burnin of 25%. ML searches were performed using MEGA 6.0 with a starting tree topology generated by a Neighbour Joining algorithm (Zwickl, 2006). Nodal support was assessed by means of 1000 bootstrap replicates. The model of evolution was selected in JModel Test 0.1 (Posada, 2008) according to the Bayesian Information Criterion (BIC). *Diaphorodoris luteocincta* (Voucher BAU2754) was used as out-group since it shows a basal placement within Onchidoridoidea according to Hallas & Gosliner (2015).

RESULTS AND DISCUSSION

Ten individuals were observed in situ (Figs. 1–4) and, within these, six were collected, from different localities (Table 1), and studied in an aquarium (Figs. 5–8) before anatomical analysis. The egg development was documented until the veliger stage (Figs. 9, 10).



Figures 9–12. *Knoutsodonta pictoni* n. sp. Fig. 9: in aquarium eggs deposition. Fig. 10: eggs at different developmental stages. One veliger is indicated with a red circle. Figs. 11, 12: reproductive system: bc = bursa copulatrix, ro = reproductive opening, dd = deferent duct, am = ampulla, fg = female gland, rs = receptaculum seminis. Scale bar = 1 mm.

Diagnostic anatomical features were compiled from at least two specimens (Figs. 11, 12; Figs. 13–18).

DNA-barcoding. The COI sequences were deposited at the European Nucleotide Archive (http://www.ebi.ac.uk/ena) and the information on voucher, accession numbers and collection localities are listed in Table 1. The BLAST search found a high similarity (99%) between the new species sequences and one sequence present in GenBank (Accession number: KP340411, voucher: CASIZ 208194) identified as *Knoutsodonta* sp. A. The pairwise genetic distances calculated on the COI sequences revealed 2.0% of maximum intraspecific divergence of the putative new species (Table 2). A final COI dataset, excluding the out-group, consisted of 29 sequences from ten different species belonging to *Knoutsodonta* and *Onchidoris*. The COI final alignment consisted of 609 nucleotide positions with 198 polymorphic sites.

Results from the ABGD analysis returned 11 Preliminary Species Hypothesis (PSH) with *O. bilamellata* sequences split in two different PSH. All the recursive steps in the ABGD analysis resulted in the same sequence repartition, with all the sequences of the putative new species grouped in a single PSH (Fig. 19) including *Knoutsodonta* sp. A (KP340411).

The phylogenetic analyses resulted in monophyletic clades (Fig. 20) that were congruent with the PSH obtained with the ABGD analysis. The phylogenetic inference confirmed that all the specimens belonging to the putative new species grouped in one monophyletic clade together with the GenBank sequence of *Knoutsodonta* sp. A (KP340411) with high support values (BI=1, ML=100). The

COI resulting topology showed also that the sequences ascribed to *O. bilamellata* split in two divergent clades congruently with the ABGD species delimitation results. In particular, the specimen of '*O. bilamellata*' with COI accession number KR084801, was the sister to the new species. with high statistical support (BI=1, ML=99).

TAXONOMY

Familia ONCHIDORIDIDAE Gray, 1827
Genus *Knoutsodonta* Hallas et Gosliner, 2015

Knoutsodonta pictoni n. sp. (Figs. 1–8)

TYPE MATERIAL. Holotype. Voucher MNHN-IM-2000-33333, 11 mm in length alive, Tavolara-Punta Coda Cavallo Marine Protected Area (MPA), Porto San Paolo, North Eastern Sardinia, Central Tyrrhenian Sea, Mediterranean Sea, Italy, (40.881635°N, 9.637065°E), 31.III.2015 (Figs. 1, 2, 5–8).

Paratype. Voucher MNHN-IM-2000-33334, one specimen, dissected, 10 mm in length alive, same data as the holotype (Fig. 1).

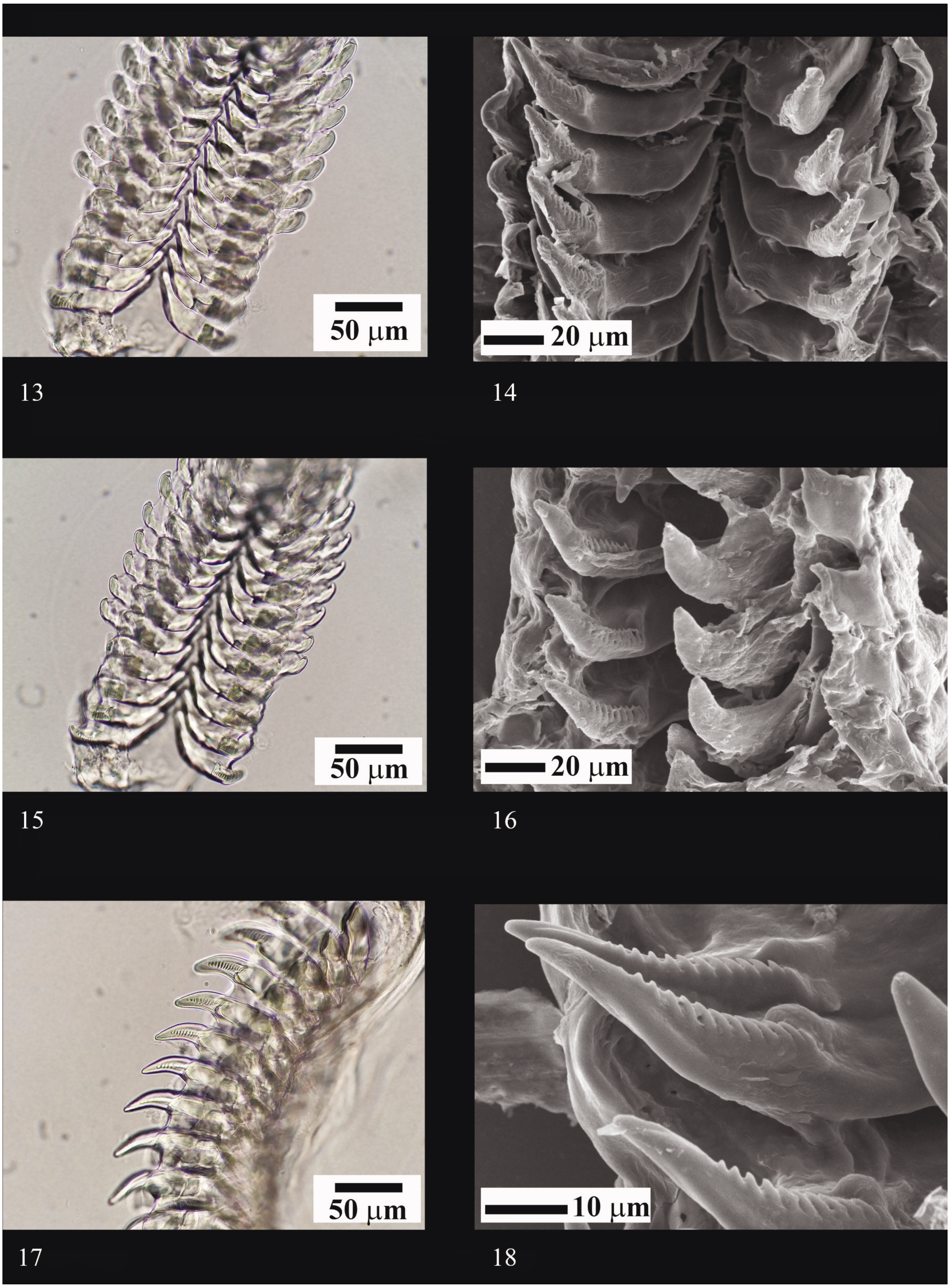
OTHER EXAMINED MATERIAL. BAU02982, one specimen, dissected, 8 mm in length alive, South of Inishgalloon, Purteen, Keel, Achill Island, Ireland, Atlantic Ocean, (53.9556°N, 10.1023°W), 05 April 2015 (Fig. 3). BAU02985, one specimen, 9 mm in length alive, 6 m depth, Sistiana, Trieste, North Adriatic Sea, Mediterranean Sea, Italy, (45.7728726°N,13.6292581°E), 31 December 2016. BAU02983, one specimen, 6 mm in length alive, South of Inishgalloon, Purteen, Keel, Achill Island, Ireland, Atlantic Ocean, (53.9556°N, 10.1023°W), 05 April 2015 (Fig. 3). BAU02984, one specimen, 6 mm in length alive, South of Inishgalloon, Purteen, Keel, Achill Island, Ireland, Atlantic Ocean, (53.9556°N, 10.1023°W), 05 April 2015 (Fig. 3).

DESCRIPTION OF HOLOTYPE. External morphology: body elliptical, depressed, equally rounded both in anterior and posterior edges. Body colour uniform dark brown, almost black with small bluish white speckles scattered along the mantle (Figs. 1, 5). Body colour of ventral part light grey with diffuse spots dark brown as mantle (Figs. 5, 6). Long and slender rhinophores, totally white, retractable, lamellate (10 lamellae) (Fig. 7). Foot not projected beyond notum: anterior margin indented forming two large lobes, posterior part forms a rounded tail (Fig. 6). Mantle structure very hard, with strong reticulation formed by translucent spicules. Almost globular tubercles, evenly spaced, covering mantle and surrounding anus opening and rhinophores sheaths. Mouth surrounded by large oral veil with just outlined lateral lobes (Fig. 6). Non-retractile gills with 9 bipinnate branchial leaves, larger anteriorly than posteriorly, forming an almost complete semicircle around the anus: one tubercle may be present just behind the anus (Fig. 8). Several narrow tubercles of variable height inside the gill circlet.

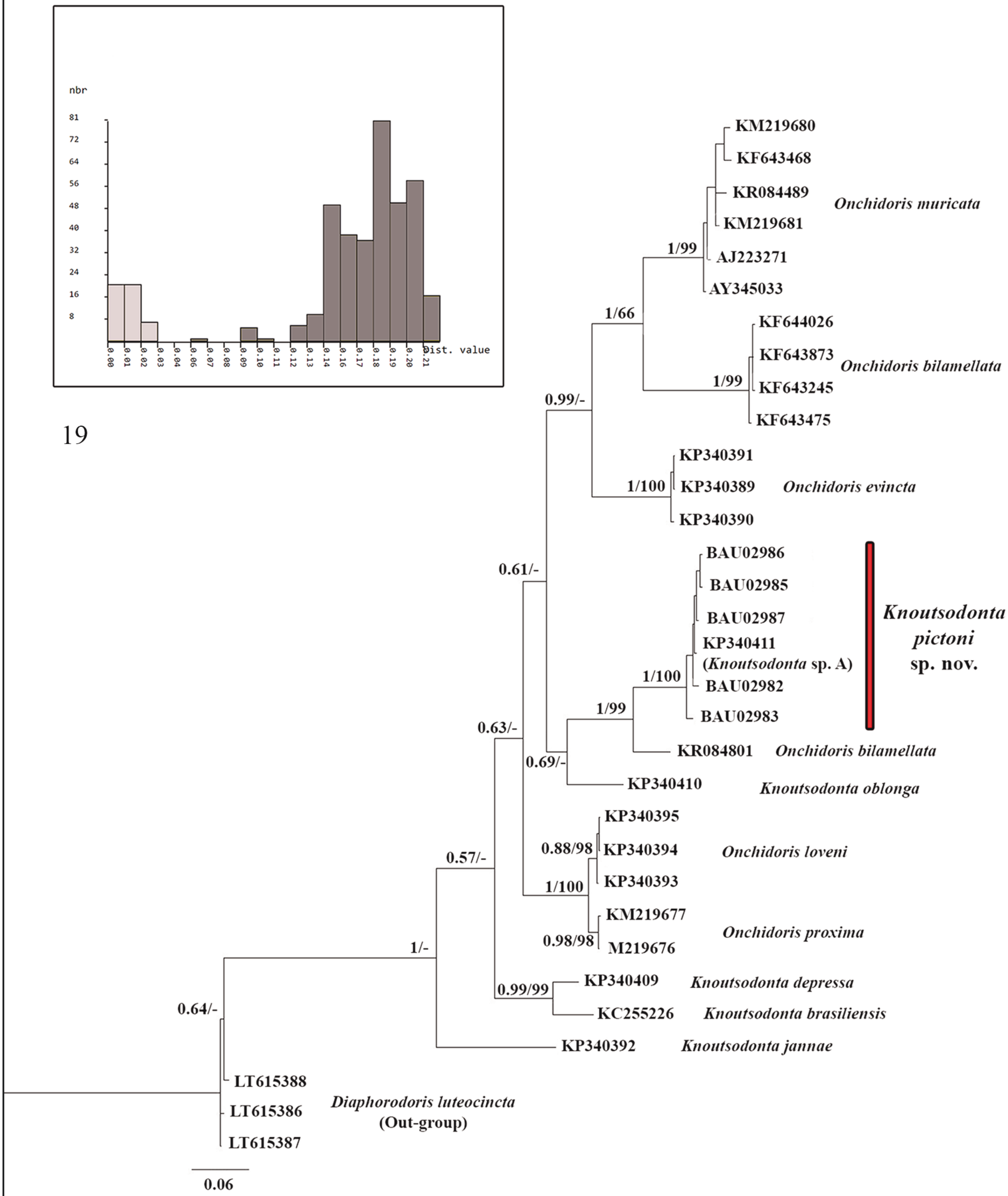
VARIABILITY. Body length ranging from 9 mm to 12 mm (10 specimens examined). Rhinophores with 9–11 lamellae. Gills with 9 or 10 bipinnate branchial leaves.

Paratype internal anatomy: Rachidian tooth absent. Radular formula 25–28 x 1.1.0.1.1. Radular teeth almost colourless (Figs. 13, 14). First lateral teeth with long, wide base and strong, almost straight beak-shaped cusp, denticulate with 10–13 small denticles along the internal surface (Figs. 15–18). Second lateral teeth in shape of rectangular plates, with downward directed cusp on lower outside corner (Figs. 15, 16). Swollen tube of ampulla connected through a short duct to bursa copulatrix and seminal male duct. Bursa copulatrix leads to distal part of female duct, in connection with seminal receptaculum ending into vagina and female opening. Relatively long loop of prostatic part of vas deferens adjacent to bursa copulatrix (Figs. 11, 12). Prostate smooth, not granulated, first narrow, then rapidly wide into a long swollen penial sheath with several folds of ejaculatory duct. (Figs. 11, 12). Bean-shaped bursa copulatrix, slightly yellowish (Figs. 11, 12), enters distal part of vagina through a relatively long stalk. At its base, a duct leads to ovoid seminal receptacle (Figs. 11, 12). Vagina wide and long (Figs. 11, 12).

ETYMOLOGY. The new species is named after Bernard Picton ((National Museums Northern Ireland, 153 Bangor Road, Cultra, Holywood, BT18 0PE, UK)) who kindly presented us specimens from Ireland and whose prominent work on North Atlantic nudibranchs is a mandatory reference for researchers and nudibranch enthusiasts all over the world.



Figures 13–18. Radula of the *Knoutsodonta pictoni* n. sp. Figs. 13, 15, 17: Optical microscope images. Figs. 14, 16, 18: SEM images at different magnification levels.



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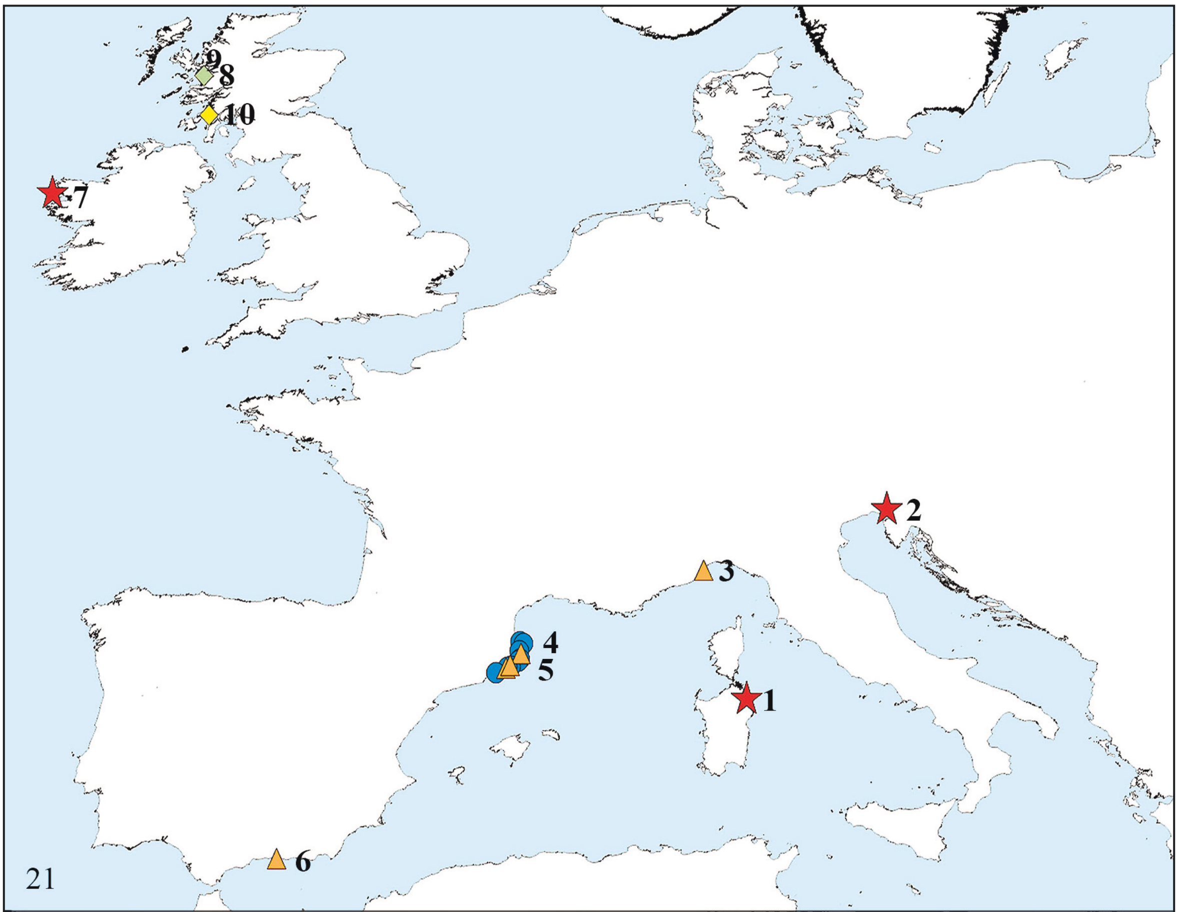
Figures 19, 20. DNA Barcoding analyses. Fig. 19: the ABGD histogram of the COI barcoding region shows the distribution of the pairwise estimated genetic distances (K2p) in intraspecific (left, light grey) and interspecific (right, dark grey) comparisons. Fig. 20: Bayesian resulting tree of the COI dataset. Numbers at nodes are Bayesian posterior probability and ML bootstrap support, respectively.



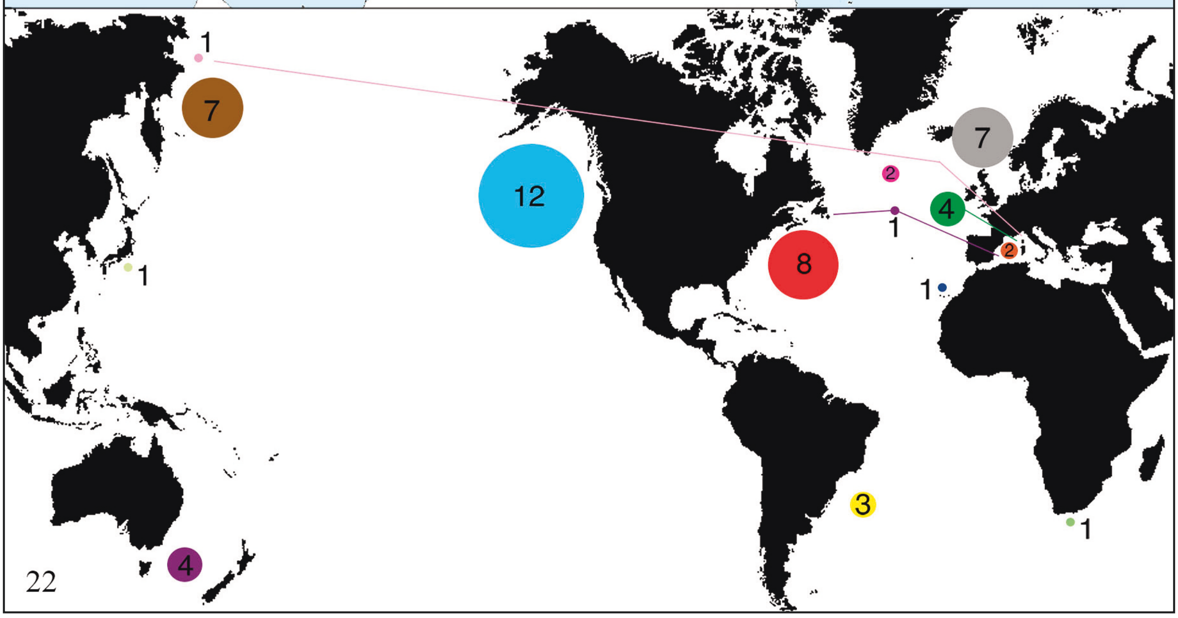


Figure 21. Distribution of *Knoutsodonta pictoni* n. sp. The black numbers correspond to the sites indicated in Table 3. Specimens examined in the present paper, red star; Atlantic specimen identified as *Knoutsodonta* sp. A (KP340411), green lozenge; Atlantic specimen identified as *Onchidoris* sp., yellow lozenge; Mediterranean specimens identified as *Onchidoris pusilla*, orange triangle; Mediterranean specimens identified as *Knoutsodonta* sp. A, blue circle. Figure 22. Distribution of the species belonging to the family Onchidorididae. The numbers correspond to the number of species with the same distribution, the size of the circles is correlated to the number of species.

	K. pictoni BAU02986	*K. pictoni* BAU02987	*K. pictoni* BAU02985	*K. pictoni* BAU02982	*K. pictoni* BAU02983	*K.* sp. A KP340411
K. pictoni BAU02986	0.000					
K. pictoni BAU02987	0.007	0.000				
K. pictoni BAU02985	0.003	0.007	0.000			
K. pictoni BAU02982	0.015	0.011	0.015	0.000		
K. pictoni BAU02983	0.020	0.016	0.020	0.018	0.000	
K. sp. A KP340411	0.008	0.005	0.008	0.010	0.015	0.000

Table 2. Pairwise distances (p-distance) between specimens belonging to *Knoutsodonta pictoni* n. sp.

***Knoutsodonta pictoni* n. sp.**		
Map sites	**Locality**	**Reference source**
1	MPA Tavolara, Sardegna, Italy	Present paper
2	Sistiana, Trieste, Italy	Present paper
3	Cape Noli, Liguria, Italy	as *Onchidoris pusilla* in Betti et al. 2017. J.Molluscan Stud., 83:325–332 https://doi.org/10.1093/mollus/eyx019
4	Catalunya, Spain	as *Knoutsodonta* sp. A. in Ballesteros M., Madrenas E. & Pontes M., 2016. Actualización del catálogo de los moluscos opistobranquios (Gastropoda: Heterobranchia) de las costas catalanas. Spira,6: 1–28.
5	Catalunya, Spain	as *Onchidoris pusilla* in GROC, 2017. http://www.opistobranquis.org/en/guia/100.
6	Granada, Spain	as *Onchidoris pusilla* in GROC, 2017. http://www.opistobranquis.org/en/guia/100.
7	South of Inishgalloon, Purteen, Keel, Achill Island, Ireland	Present paper
8	Scotland	as *Knoutsodonta* sp. A , in Hallas J.M. & Gosliner T.M., 2015. Family matters: the first molecular phylogeny of the Onchidorididae Gray, 1827 (Mollusca, Gastropoda, Nudibranchia). Molecular phylogenetics and evolution, 88: 16–2
9	Scotland, East Wall Loch Nevis	as *Knoutsodonta* sp. A. in http://www.nudibranch.org/Scottish%20Nudibranchs/html/knoutsodonta-spA-01.html.
10	Scotland, Loch Sween	as *Onchidoris* sp. in NE Atlantic Nudibranch group page posted by M. Faasse on 03/02/2012

Table 3. List of the map sites, localities and reference sources of the records of *Knoutsodonta pictoni* n. sp. Mediterranean Sea, green lines; North Atlantic Ocean, blue lines.

Species attributed to the genus *Knoutsodonta*					
Species	**Body**	**Rhinophores**	**Gills**	**Radular formula**	**Distribution**
***Knoutsodonta pictoni* n. sp.**	Uniform dark brown, almost black with small bluish white speckles scattered along the mantle	White	Dark	25-28 x 1.1.0.1.1	NE Atl. - Med
***Knoutsodonta albonigra* (Pruvot-Fol, 1951)** = *Onchidoris albonigra*	Translucent white, almost entirely covered by a black texture in relief: at the center of the mantle can be seen the red mass of viscera	White	Whitish	30x1.1.0.1.1	NE Atl. - Med
***Knoutsodonta bouvieri* (Vayssieère, 1919)** = *Onchidoris bouvieri*	Pale translucent pink with scattered red brown patches distributed in three longitudinal bands	Yellowish pink	Yellowish pink	40x1.1.0.1.1	Med. endemic?
***Knoutsodonta brasiliensis* (Alvim, Padula et Pimenta, 2011)** = *Onchidoris brasiliensis*	Greyish white or yellowish orange, both translucent with a pattern of scattered dark brown (sometimes orange) tiny spots, tending towards the mid-line	Translucent white	Translucent white	16-17x1.1.0.1.1	SE Atlantic
***Knoutsodonta cervinoi* (Ortea et Urgorri, 1979)** = *Onchidoris cervinoi*	Orange, centrally intense, fading at the borders. Some specimens' borders almost whitish	Orange with dark brown leaflets	Dark brown circled by a light area	?x1.0.1	NE Atlantic
***Knoutsodonta depressa* (Alder et Hancock, 1842)** = *Onchidoris depressa*	Pale or translucent brown with scattered orange or purple-brown speckles	Translucent	Translucent	33-34x1.1.0.1.1	NE Atl. - Med
***Knoutsodonta inconspicua* (Alder et Hancock, 1851)** = *Onchidoris inconspicua*	White or pale brown, often tinged with a purple hue, covered by small specks of brown pigment	Pale yellow	White	29x1.1.0.1.1	NE Atlantic
***Knoutsodonta jannae* (Millen, 1987)** = *Adalaria jannae*	Pale yellow or creamy white or orange or lemon yellow	Darker than body	Darker than the body	28-39x4-6.1.0.1.4-6	NE Pacific
***Knoutsodonta iannaella* (Martynov, Sanamyan et et Korshunova, 2015)** = *Adalaria jannaella*	Translucent, centrally whitish with a brown patch, central tubercles yellowish, lateral whitish	Yellowish	White	20x2-4.1.0.1.4-2	NE Pacific
***Knoutsodonta maugeansis* (Burn, 1958)** = *Onchidoris maugeansis*	Pale pattern with shades or colours of yellow and/or orange	Yellowish	Yellowish	22 rows	SW Pacific
***Knoutsodonta neapolitana* (Delle Chiaie, 1841)** = *Onchidoris neapolitana*	Yellowish or pale brown covered with dense and intensive reddish or purple brown; at the notal edge pigment becomes linear	Dark	Dark	24x1.1.0.1.1	Med endemic
***Knoutsodonta oblonga* (Alder et Hancock,1845)** = *Onchidoris oblonga*	Grey with a few darker blotches on the back	Yellowish	Yellowish white	28x1.1.0.1.1	NE Atlantic
***Knoutsodonta pusilla* (Alder et Hancock,1845)** = *Onchidoris pusilla*	Dense dark brown pigment spots responsible for dark appearance	Transparent	Transparent	21-29x1.1.0.1.1	NE Atlantic
***Knoutsodonta reticulata* (Ortea,1979)** = *Onchidoris reticulata*	Whitish intensely pigmented in green at center, fading at borders; whitish longitudinal bands forming with bands running from tubercles a net surrounding brown green patches	Large brown stains	Pigmented dirt cream	51-54x1.1.0.1.1	NE Atlantic
***Knoutsodonta sparsa* (Alder et Hancock,1846)** = *Onchidoris sparsa*	Pale brown mantle with regularly spaced, darker blotches, forming patches	Blotched with olive-brown	Colourless	32-36x1.1.0.1.1	NE Atl. - Med
***Knoutsodonta tridactila* (Ortea et Ballesteros, 1982)** = *Onchidoris tridactila*	Whitish with orange or dark reddish spots; in between rhinophores and gills up to six irregular lines formed by this pigment	Yellow or light pink	Yellow	12x1-1-0-1-1	NE Atlantic

Table 4. Comparison between external morphology, radular formula and distribution of *Knoutsodonta pictoni* n. sp. with those of the other 14 *Knoutsodonta* species (Mediterranean species in grey lines).

DISTRIBUTION. The species is distributed in Western and Central Mediterranean and in North East Atlantic Ocean. In addition to the localities listed in Table 1 this species has been also recorded under the name of *Knoutsodonta* sp. in different localities along the coast of Catalunya (Ballesteros et al., 2016); it is also recorded in the coast of Granada, in Estartit, in Tossa de Mar and Blanes (Spain) and in Cape Noli (Italy) as *Onchidoris pusilla* (Alder et Hancock, 1845) (GROC, www.opistobranquis.org, and Betti et al., 2017). The species distribution is summarized in figure 21 and Table 3.

REMARKS. At present, there are 15 Onchidorididae species that can be ascribed to the genus *Knoutsodonta*, based on their radular formula (Hallas & Gosliner, 2015) and among them, five have a Mediterranean distribution (Table 4).

The main morphological features that diagnose *K. pictoni* n. sp. are the dark brown background body colour, the white rhinophores and the dark gills. None of the known Onchidorididae displays these three combined external morphological characters (Table 4). The confusion that has occurred with *O. pusilla* can be easily resolved mainly because this species has translucent white gills and secondly for the body colour whose apparent darkness is due to the presence, on the mantle, of dense dark brown pigmented spots. A comparison of the main morphological features between correlated species of the genus *Knoutsodonta* is reported in Table 4.

All the specimens collected were found feeding and spawning on the encrusting bryozoan *Reptadeonella violacea* (Johnston, 1847) (Gymnolaemata: Chelostomatida: Adeonidae) (Fig. 4), a species of warm temperate waters, distributed in the Mediterranean, and from the North East Atlantic to West Africa waters. The species is also present in West Atlantic (North America and Gulf of Mexico) and along the Pacific Coast of North America (Hayward & McKinney, 2002). When the nudibranchs are upon their bryozoan prey they are very cryptic, and in fact they can be detected only for the presence of a discoloured area of the bryozoan where they have fed (or are feeding) or for the presence of the egg coils. The egg spawn of *K. pictoni* n. sp. is very distinctive among those of the Onchidorididae: it is a flat mucous ribbon forming an almost perfect Archimedean spiral containing egg capsules (Figs. 9, 10). Sometimes two different individuals spawn in the same place and it may happen that part of the last laid ribbon surrounds the other one (Fig. 1). In aquarium, at 18 °C, one specimen was observed spawning on 6th of April 2015 at 7.00 PM (Fig. 9). On 8th of April at 10.00 AM the coil was full of 9248 egg capsules, while 3 veligers were swimming nearby (Fig. 10). At 8.30 PM of the same day there was only a slight trace of the coil and a large number of veligers was swimming in the entire mass of water.

DISCUSSION

A recent phylogenetic reassessment of the family Onchidorididae by Hallas & Gosliner (2015) proposed some systematic changes regarding the genera *Adalaria*, *Onchidoris* and *Knoutsodonta*. According to this revision, we described a new species of this group through an integrative approach. Morphological evidences revealed the absence of the rachidian tooth in the new species, positioning it in the genus *Knoutsodonta*, while all the molecular analyses performed confirmed the assignment of the Mediterranean and Atlantic specimens to the new species *K. pictoni* n. sp. Additionally, the COI DNA barcoding allowed to identify one sequence present in GenBank (COI accession number KP340411) and corresponding to the new species here described. Interestingly, phylogenetic analyses here proposed (although based on the single COI marker), revealed *K. pictoni* n. sp. as sister to (yet not conspecific with) a specimen (COI accession number KR084801) previously ascribed to *O. bilamellata* that needs further analyses. The study by Hallas & Gosliner (2015) could not provide a complete definition of the genus *Knoutsodonta* and, furthermore, it included only two out of the five Mediterranean species of this genus. The species of Onchidorididae are mainly distributed in the northern hemisphere, and, as depicted in figure 7, for the total of 54 accepted species the highest diversity area is the North Atlantic with the presence of 25 species from the NW Atlantic to the Mediterranean Sea. Twelve species are distributed in the NE Pacific, while eight are in the NW Pacific. Four species are distributed in the SW Pacific, one in Central W Pacific, three in SW Atlantic and one in SE Atlantic-S Indian Ocean.

With the description of *K. pictoni* n. sp., the Onchidorididae of the Mediterranean Sea raise to 8 species, two of which endemic, whose generic attribution needs further investigation. For all these reasons a further integrative study is desirable to understand the systematic position of some critical Onchidorididae taxa and to investigate on the validity of the genus *Knoutsodonta*.

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