

Additional data on *Acanthochitona pilosa* Schmidt-Petersen, Schwabe & Haszprunar, 2015 (Polyplacophora: Acanthochitonidae)

HERMANN L. STRACK

Institute of Systematics, Evolution, Biodiversity (ISEB), Muséum National d'Histoire Naturelle,
57 rue Cuvier, 75005 Paris, France; hermann.strack@orange.fr [corresponding author]

LUCREZIA LEOTTA

Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome,
Viale dell'Università 32, 00185 Rome, Italy; lucrezia.leotta@uniroma1.it



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The status of *Acanthochitona pilosa* Schmidt-Petersen, Schwabe & Haszprunar, 2015 as a distinct species is firmly confirmed by additional discriminating morphological characters and genetic analysis. Furthermore, new information has become available on its habitat, biology, and distribution range. The genetic analysis also shows the urgent need for a more comprehensive, large scale molecular phylogeny and species delimitation of European / North African Acanthochitonidae.

INTRODUCTION

At present five living species of the genus *Acanthochitona* are known from Europe. The best known and most common are *Acanthochitona fascicularis* (Linnaeus, 1767) and *A. crinita* (Pennant, 1777). According to Kaas (1985) another species lives in north-western Europe: *A. discrepans* (T. Brown, 1827). This species is not well known and often misinterpreted. A recent study (Vončina et al., 2023) has firmly established this species status as valid using both morphological and molecular data.

A fourth species *A. oblonga* Leloup, 1981 was described but was considered a variety of *A. crinita* by several authors (Kaas, 1985; Kaas & Van Belle, 1998; Dell'Angelo & Smriglio, 2001). Later it was recognized as a distinct species (Bonfitto et al., 2011), based on morphological and molecular data. Recently a fifth species was described: *A. pilosa* Schmidt-Pe-

tersen, Schwabe & Haszprunar, 2015. Its description was based on morphological data only.

In the early eighties the senior author (HLS) got an interesting collection of loose chiton valves that were collected in shell grit from the Bay of Algeciras (Spain). His identifications were used in a paper on the malacofauna of the region (Aartsen et al., 1984). All this loose valve material indicated a diverse and rich (12 species) chiton fauna. Among the material received at the time were about a hundred valves HLS identified as *A. crinita* but proved to be valves of *A. pilosa*. These looked somewhat peculiar inasmuch that the articulamentum was always reddish in colour.

In 1984 HLS went to the Bay of Algeciras to collect the same species alive. The living *Acanthochitona* material collected during this voyage differed from regular *A. crinita* and these specimens were kept apart for future study. Later HLS became convinced that it was an undescribed species of *Acanthochitona*, and in 2001 a new voyage was undertaken to the Bay of Algeciras to collect more specimens, gather data and to take photographs in situ. By the time HLS started the description the species was already described. However, since much additional data was gathered, it was thought useful to publish this information, even more since there was the opportunity to add molecular data.

MATERIAL AND METHODS

Material studied. — This study is mainly based on material collected by the senior author in Spain (Punta Carnero and Tarifa), and from material received from Italy collected by Giacomo di Paco. All material was preserved in alcohol (ethanol). The first collections from Spain and Italy on 70% and the 2001 collection made in Spain was preserved on 95% alcohol (these were used for DNA sequencing). A total of 54 alcohol preserved specimens, 2 dry preserved specimens and 58 loose shell valves were examined.

Abbreviations. — ZSM = Bavarian State Collection of Zoology, Munich; CSIC = Museo Nacional de Ciencias Naturales, Madrid; MNHN = Muséum national d’Histoire naturelle, Paris; RMNH = Naturalis Biodiversity Center (formerly Rijksmuseum van Natuurlijke Historie), Leiden; spms = specimens.

Laboratory analysis. — A piece of tissue was dissected from alcohol-preserved specimens and DNA was isolated with a ‘salting out’ protocol (modified from Aljanabi & Martinez, 1997, following Fassio et al., 2023). The 658 bp barcode fragment of the mitochondrial gene cytochrome oxidase subunit I (*cox1*) was amplified using the primers LCO-1490 5’-GGTCAACAAATCATAAAGATATTGG and HCO-2198 5’-TAAACTTCAGGGTGACCAAAAAATCA (Folmer et al., 1994). PCR reactions were performed with 1 µL of undiluted DNA template in 25 µL reactions (5 µL of Reaction Buffer, 0.75 µL of 50 mM MgCl₂ solution, 1 µL of 10% BSA solution, 0.4 µL of each 25 pM primer solution, 0.5 µL of 10 mM nucleotide mix solution and 0.12 µL of GOTAQ DNA Polymerase). PCR conditions were performed following Nocella et al. (2024). PCR products were purified using the ExoSAP-IT protocol (USB Corporation) and sequenced at MacroGen Europe (Milan Genome Center), in both directions.

Bioinformatic analysis. — Sequences were manually inspected and aligned with MAFFT v.7 (Katoh & Standley, 2013; Katoh et al., 2019) using the G-INS-i algorithm. All the available *cox1* sequences of Mediterranean and Atlantic *Acanthochitona* spp. were downloaded from the GenBank and aligned with the newly produced sequences. The alignment was partitioned by codon with PAUP* v.5.0 (Wilgenbusch & Swofford, 2003). The best fitting substitution model for each partition was estimated with JModelTest2 v2.1.10 (Guindon & Gascuel, 2003; Darriba et al., 2012) on the CIPRES Science Gateway (phylo.org, Miller et al., 2010), using the Bayesian Information Criterion (BIC; TrN 1st codon position; HKY, 2nd codon position; TIM3 3rd codon position). Maximum Likelihood analyses were done with IQ-TREE (Nguyen et al., 2015) obtaining branch supports with the ultrafast bootstrap (Hoang et al., 2018) on 10,000 replicates. The tree was rooted using sequences of *Lepidochitona caprearum* (Scacchi, 1836) as the outgroup, and visualized with FigTree v.1.4.4. (Rambaut et al., 2018).

A first analysis was carried out to detect potential misplacement of individual sequences: where taxonomic identification was not consistent with phylogenetic results, a correction and/or the removal of some GenBank sequences followed. Then, a second and final analysis was performed.

The genetic distance matrix was obtained using Hooty (Princic & Chiappa 2024, <https://princic-1837592.github.io/Hooty/>), which computes the minimum and maximum K2P (Kimura, 1980) distance between predetermined groups in an alignment and outputs results in a matrix format.

Species delimitation followed an iterative approach, where individuals were initially identified morphologically yielding morphospecies; then morphospecies were tested for their reciprocal monophyly in a phylogenetic analysis of the *cox1* alignment; and eventually pairwise genetic distances were checked for congruence, expecting intraspecific distances to be consistently lower than interspecific ones.

SYSTEMATIC PART

Class Polyplacophora J.E. Gray, 1821

Subclass Neoloricata Berghayn, 1955

Superfamily Cryptoplacoidea H. Adams & A. Adams, 1858

Family Acanthochitonidae Pilsbry, 1893

Genus *Acanthochitona* J.E. Gray, 1821

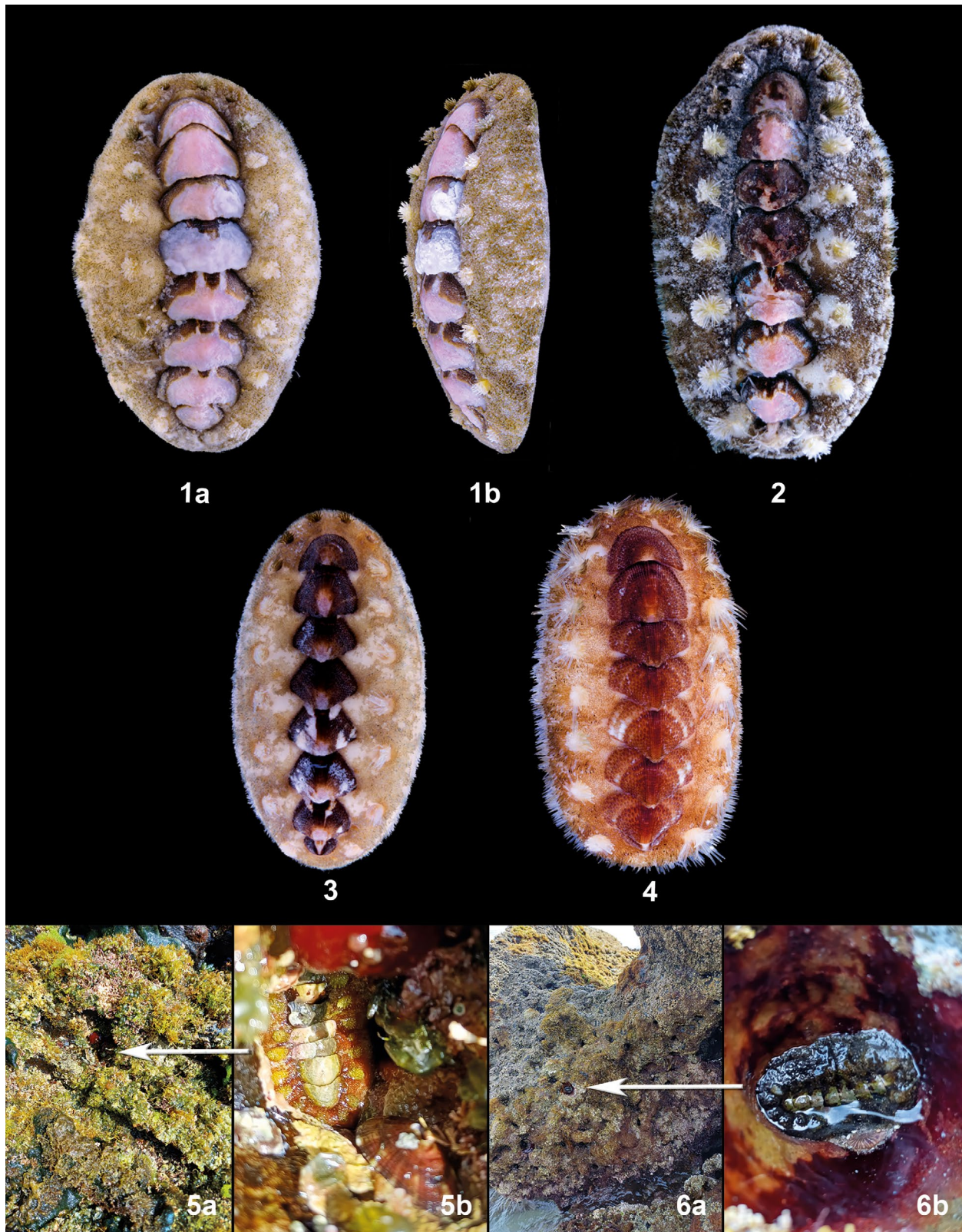
***Acanthochitona pilosa* Schmidt-Petersen, Schwabe & Haszprunar, 2015**

Figs 1-7, Tables 1-2

Material studied. — Spain, southside Bay of Getares, Punta Carnero, wedged in solid rock crevices relatively high in the intertidal zone, 22/23-viii-1984, H.L. Strack leg., 18 spms (RMNH 9 spms, ZSM 9 spms). Spain, Tarifa, wedged in crevices of a large solid rock on the beach in front of the city near the port, mid to high intertidal zone, 24/25-viii-1984, H.L. Strack leg., 14 spms (RMNH 7 spms, ZSM 7 spms). Spain, Tarifa, rock outcrops east of the harbor, mid to high intertidal zone, wedged in solid rock crevices, 30-v-2001, H.L. Strack leg., 5 spms (MNHN). Spain, Playa de Getares, in shell grit, 9-iv-1980, H.P.M.G. Menkhorst leg., 14 valves (RMNH), Spain, Getares, in shell grit, iv-1981, H.P.M.G. Menkhorst leg., 44 valves (RMNH). Spain, south of Granada, Marina del Este, rocks, photophilic algae, 1993, J.L. Rueda leg., 2 spms, dry and rolled up (CSIC), Spain, south of Granada, Marina del Este, stones, photophilic algae, 0-0.2 m, 6-iv-2021, Inmaculada Garcia leg., 7 spms on alcohol (CSIC).

Italy, Livorno, Il Romito, on solid rocks, intertidal among cirripeds and *Mytilus galloprovincialis* Lamarck, 1819, 24-ii-1985, Giacomo di Paco leg., 10 spms (RMNH 5 spms, ZSM 5 spms).

Size. — Except for one 20 mm long specimen in the original description and a 23 mm long specimen (Renda et al., 2020), all material recorded so far seems to be juveniles or young adults (< 16 mm). This species grows considerably larger than previously described. The two largest specimens examined were both 30.5 mm (Punta Carnero, Spain and Livorno, Italy). Specimens collected in Tarifa in 1984 ranged between 21 and 27 mm in length. Specimens collected in Tarifa in 2001 ranged between 15 and 30 mm, those



Figs 1-6. *Acanthochitona pilosa* Schmidt-Petersen, Schwabe & Haszprunar, 2015. **1a.** Tarifa, Spain, 1984, length 27 mm, dorsal view, eroded specimen. **1b.** Idem, lateral view. **2.** Tarifa, Spain, 2001, length 30 mm, dorsal view, eroded specimen. **3.** Livorno, Italy, 1985, length 20 mm, dorsal view, apical areas eroded. **4.** Punta Carnero, Spain, 1984, length 10 mm, dorsal view, non-eroded specimen. **5a-b.** Tarifa, Spain, 2001. **5a.** Habitat of first specimen of *A. pilosa*. The arrow marks the actual position of the specimen depicted in the next figure. **5b.** Detail of habitat showing living specimen in situ. **6a-b.** Tarifa, Spain, 2001. **6a.** Habitat of second specimen of *A. pilosa*. The arrow marks the actual position of the specimen depicted in the next figure. **6b.** Detail of habitat showing living specimen in situ.

of Punta de Carnero between 9 and 30.5 mm, and those of Livorno between 11 and 30.5 mm.

On the other end of the spectrum, we examined a lot from Marina del Este where only juvenile specimens were collected. Here 7 specimens ranging from 2–6 mm were found together with 26 specimens of *A. crinita* ranging from 1.5–7 mm. It is unknown if both species shared exactly the same habitat. Specimens under 5 mm are difficult to discriminate from *A. crinita* as some characteristics like the valves 3–7 with their triangular posterior are not yet visible. At first sight they differ only from *A. crinita* by their uniform brown tegmentum, except for the white jugal area of the tail valve. The girdle is very uniform light brown, whereas in *A. crinita* the girdle is much lighter in colour often with darker coloured bands.

The adult size is of importance as specimens over 20 mm show changes in their morphology due to their mode of life. The main changes are due to erosion, with the characteristic triangular posterior areas of valves 3–7 becoming straighter and the valves thus more rectangular, and when the dark brown tegmentum is worn the reddish articulation becomes prominently visible, giving a completely different aspect to this species.

Colour of valves. — Very consistent, up to about 5 mm in length the tegmentum is uniformly light to dark brown with just the jugal part of the tail valve being white; as the animal grows the tegmentum starts to show more white patches and small light blue spots on other valves as well. In rare cases a shell valve may be predominantly white with small brown spots or patches. Often white patches occur on both sides of the anterior jugal part just as described by Schmidt-Petersen et al. (2015: 12). However, patches can also be found on the lateropleural areas, and these patches can occur on every shell valve (see also Amati & Oliverio, 2019: fig. 1), except on the head valve which always remains uniformly brown.

In the original description it is stated that the articulation is bright brown (Schmidt-Petersen et al., 2015: 13). This might be true in smaller specimens. One disarticulated 10 mm long specimen from Livorno (Italy) showed a translucent articulation in which the dark brown colour of the tegmentum shines through. However, two valves with a tegmental area with larger patches of white showed a reddish hue that will become more intense overtime. In fact, the part of the articulation underneath the tegmentum (thus not the apophyses) in adult specimens is always reddish (mostly salmon or pink). This can also be seen dorsally when the tegmentum is eroded (Figs 1–3). The salmon/pink coloured articulation is characteristic for adults of this species. Of the 58 loose valves of different sizes studied, all except two had this reddish articulation.

Girdle. — Colour uniformly light green, beige or light brown, rarely a darker brown, often with scattered lighter

spots. Girdle thick and fleshy (Fig. 1b), much more than in *A. crinita* and *A. discrepans*, which girdle remains relatively flat and thin. We presume that all girdle elements and radula measurements in the original description were taken from the 13 mm long holotype. As could be expected girdle elements are somewhat larger in larger specimens. The largest needles in the tufts of a 30.5 mm long specimen measured $1500 \times 90 \mu\text{m}$. Girdle element measurements taken from a 22 mm long specimen are as follows. Largest tuft-needles $1600 \times 80 \mu\text{m}$, the second type of tuft-needles, which are slender and shorter, grow up to $500 \times 20 \mu\text{m}$. Perinotum clothed with two types of spicules. The smallest and pointed type of spicules measure up to $120 \times 20 \mu\text{m}$. The larger and much thicker type as in original description, and resembling much the marginal fringe spicules that differ only in being longer, up to $400 \mu\text{m}$ (and not $40 \mu\text{m}$ as stated in the original description). The study of the girdle of a 10 mm long specimen showed not only that in younger specimens all girdle elements are smaller, but also that the second larger type of spicules on the perinotum is less abundant and that they are completely lengthwise striated and sharply pointed. Apparently, part of the striation and the point wears off with time.

Radula. — As described in original description, except for the measurement of the width of the central tooth which is not $3 \mu\text{m}$, but $30 \mu\text{m}$ in a 10 mm long specimen, increasing up to $80 \mu\text{m}$ in a 22 mm long specimen. Major lateral tooth with a tricuspid head, denticles sharply pointed, the central one decidedly longer than the outer ones. Measurements of the head are $85 \times 105 \mu\text{m}$ (10 mm long specimen) and $180\text{--}185 \times 205\text{--}210 \mu\text{m}$ (22 mm long specimen). The 10 mm long specimen had 38 rows of teeth of which 29 were mature.

Gills. — The gills were not described in the original description nor in Amati & Oliverio (2019). Although the arrangement of gills is similar in all members of the genus *Acanthochitona*, being abanal and merobranchial, gill counts can differ considerably between species. As the number of gills is partially correlated with specimen length, we here give gill counts for specimens of different lengths. The gills were counted of 10 specimens (one side only): 6 mm – 8 gills; 9 mm – 15 gills; 15 mm – 16 gills; 15 mm – 20 gills; 16 mm – 16 gills; 20 mm – 20 gills; 25 mm – 19 gills; 25 mm – 21 gills; 30.5 mm – 20 gills; 30.5 mm – 23 gills. It seems that specimens of 20 mm and larger have between 19 and 23 gills.

Habitat and mode of life. — According to the original description (Schmidt-Petersen et al., 2015: 13–14) *A. pilosa* lives exclusively in the encrustations of the red algae *Lithophyllum tortuosum*, but this seems to be a local habitat preference not necessarily shared with data in other localities. In the Bay of Getares and Tarifa it was found on rocky outcrops covered with barnacles and short algae in relatively high energy zones (Figs 5–6). All specimens were found

Species	Voucher	Locality	Legit	N° local-ity	References	COI
<i>Acanthochitona pilosa</i>	MNHN-IM-2019-25067	Spain, Tarifa	H. L. Strack	1	This paper	PP748469
<i>A. pilosa</i>	MNHN-IM-2019-25068	Spain, Tarifa	H. L. Strack	1	This paper	PP748466
<i>A. pilosa</i>	MNHN-IM-2019-25069	Spain, Tarifa	H. L. Strack	1	This paper	PP748467
<i>A. pilosa</i>	MNHN-IM-2019-25070	Spain, Tarifa	H. L. Strack	1	This paper	PP748468
<i>A. pilosa</i>	MNHN-IM-2019-25071	Spain, Tarifa	H. L. Strack	1	This paper	PP748470
<i>A. pilosa</i>	ZSM20120166	France, Banylus-sur-Mer; 42°28'52"N 3°08'14"E	K. Vončina	1	This paper	PP748481
<i>A. discrepans</i>	MNHN-IM-2019-25078	France, Finistère, Molène Island	H. L. Strack	1	This paper	PP748476
<i>A. discrepans</i>	MNHN-IM-2019-25079	France, Finistère, Molène Island	H. L. Strack	1	This paper	PP748477
<i>A. discrepans</i>	MNHN-IM-2019-25080	Ireland, Donegal, Machaire Rabhartaigh	H. L. Strack	1	This paper	PP748478
<i>A. discrepans</i>	MNHN-IM-2019-25081	Portugal, Praia de Galapinhos	H. L. Strack	1	This paper	PP748479
<i>A. discrepans</i>	MNHN-IM-2019-25082	France, Finistère, Molène Island	H. L. Strack	1	This paper	PP748480
<i>A. fascicularis</i>	SMF 373028	Ireland, Galway, Bealadangan; 53°18'43"N 9°37'26"W	C. Morrow	1	Vončina et al., 2023	OR526597
<i>A. fascicularis</i>	360506-4	Portugal, Azores, Galway, Ponta Delgada, Rosto do Cão; 37°44.567'N 25°38.167'W	J. Sigwart, L. Sumner-Rooney, N. Carey	1	Vončina et al., 2023	OR145409
<i>A. fascicularis</i>	SMF 373029	Ireland, Galway, Bealadangan; 53°18'43"N 9°37'26"W	C. Morrow	1	Vončina et al., 2023	OR526598
<i>A. fascicularis</i>	SMF 373030	Ireland, Galway, Bealadangan; 53°21'21"N 9°38'45"W	C. Morrow	1	Vončina et al., 2023	OR526596
<i>A. fascicularis</i>	SMF 373027	Ireland, Galway, Bealadangan; 53°18'43"N 9°37'26"W	C. Morrow	1	Vončina et al., 2023	OR526595
<i>A. fascicularis</i> cf.	CROBB139	Southern Istria, Adriatic Sea	/	9	Buršić et al., 2021	MT920117
<i>A. crinita</i>	LMBSCt11-001	(W) Portugal	/	/	Lobo et al., 2013	KF369104
<i>A. crinita</i>	CSUF_DE_6679	(W) France, Pointe du Chay	/	/	Eernisse et al., 2018	MG680023
<i>A. crinita</i>	CSUF_DE_6701	(NW) Spain	/	/	Eernisse et al., 2018	MG680030
<i>A. crinita</i>	CSUF_DE_6685	(W) France, Les Pierrieres	/	/	Eernisse et al., 2018	MG680024
<i>A. crinita</i>	CSUF_DE_6686	(W) France, Les Pierrieres	/	/	Eernisse et al., 2018	MG680025
<i>A. crinita</i>	CSUF_DE_6700	(NW) Spain	/	/	Eernisse et al., 2018	MG680029
<i>A. crinita</i>	CSUF_DE_6692	(NW) Spain	/	/	Eernisse et al., 2018	MG680026
<i>A. crinita</i>	CSUF_DE_6678	(W) France, Loctudy	/	/	Eernisse et al., 2018	MG680022
<i>A. crinita</i>	CSUF_DE_6698	(NW) Spain	/	/	Eernisse et al., 2018	MG680027
<i>A. crinita</i>	CSUF_DE_6677	(W) France, Loctudy	/	/	Eernisse et al., 2018	MG680021
<i>A. crinita</i>	CSUF_DE_6699	(NW) Spain	/	/	Eernisse et al., 2018	MG680028
<i>A. crinita</i>	LMBRUI5-001	(W) Portugal	/	/	Lobo et al., 2013	KF369103
<i>A. crinita</i>	/	(N) Iberian Peninsula, Asturias; 43°20'N 6°00'W	/	14	Miralles et al., 2016	KU695277

Table 1. Information on the samples used for the genetic analysis.

Species	Voucher	Locality	Legit	N° local-ity	References	COI
<i>A. crinita</i>	MCZ100109	(NE) Spain, Girona	/	/	Giribet & Wheeler, 2002	AF120627
<i>A. crinita</i>	SMF 363941-2	Portugal, Azores, São Miguel, Ponta Delgada, Rosto do Cão; 37°44.567'N 25°38.167'W	J. Sigwart, L. Sumner-Rooney, N. Carey	1	Vončina et al., 2023	OR145407
<i>A. crinita</i>	SMF 373026	England, Falmouth; 50°08'38"N 5°03'46"W	R. Ang, J. Dermody	1	Vončina et al., 2023	OR526579
<i>A. crinita</i>	SMF 363942-2	Portugal, Azores, São Miguel, Capelas, São Vicente; 37°50.4'N 25°40.8'W	J. Sigwart, L. Sumner-Rooney, N. Carey	1	Vončina et al., 2023	OR145405
<i>A. crinita</i>	SMF 363942-1	Portugal, Azores, São Miguel, Capelas, São Vicente; 37°50.4'N 25°40.8'W	J. Sigwart, L. Sumner-Rooney, N. Carey	1	Vončina et al., 2023	OR145404
<i>A. crinita</i>	SMF 363943	Portugal, Azores, Ilha de São Miguel, Ponta Delgada, Rosto do Cão; 37°44.567'N 25°38.167'W	J. Sigwart, L. Sumner-Rooney, N. Carey	1	Vončina et al., 2023	OR145403
<i>A. crinita</i>	SMF 363941-1	Portugal, Azores, Ilha de São Miguel, Ponta Delgada, Rosto do Cão; 37°44.567'N 25°38.167'W	J. Sigwart, L. Sumner-Rooney, N. Carey	1	Vončina et al., 2023	OR145406
<i>A. crinita</i>	ZSM20150336	France, Brittany, Finistère, Roscoff; 48°43'40"N 3°59'21"W	G. Haszprunar	1	Vončina et al., 2023	OR145411
<i>A. crinita</i>	SMF 363941-3	Portugal, Azores, Ilha de São Miguel, Ponta Delgada, Rosto do Cão; 37°44.567'N 25°38.167'W	J. Sigwart, L. Sumner-Rooney, N. Carey	1	Vončina et al., 2023	OR145408
<i>A. discrepans</i>	SMF 373036	Northern Ireland, Down, Strangford Lough; 54°29'34"N 5°39'03"W	C. Morrow	1	Vončina et al., 2023	OR526585
<i>A. discrepans</i>	SMF 373032	Northern Ireland, Down, Strangford Lough; 54°23'38"N 5°34'40"W	C. Morrow	1	Vončina et al., 2023	OR526582
<i>A. discrepans</i>	SMF 373024	Northern Ireland, Down, Strangford Lough; 54°29'23"N 5°32'15"W	J. Sigwart	1	Vončina et al., 2023	OR145402
<i>A. discrepans</i>	SMF 373031	Northern Ireland, Down, Strangford Lough; 54°23'38"N 5°34'40"W	C. Morrow, B. Picton, J. Sigwart	1	Vončina et al., 2023	OR526580
<i>A. discrepans</i>	SMF 373033	Northern Ireland, Down, Strangford Lough; 54°23'38"N 5°34'40"W	C. Morrow	1	Vončina et al., 2023	OR526581
<i>A. discrepans</i>	ZMBN140331	Norway, Vestland, Puddefjorden; 60°22'51"N 5°19'30"E	N.T. Mikkelsen	1	Vončina et al., 2023	OR526589
<i>A. discrepans</i>	SMF 373035	Northern Ireland, Down, Strangford Lough; 54°29'34"N 5°39'03"W	C. Morrow	1	Vončina et al., 2023	OR526584
<i>A. discrepans</i>	SMF 373034	Northern Ireland, Down, Strangford Lough; 54°29'34"N 5°39'03"W	C. Morrow	1	Vončina et al., 2023	OR526583
<i>A. discrepans</i>	ZMBN140296	Norway, Trondelag, Hopavågen; 63°35'34"N 9°32'02"E	N.T. Mikkelsen	1	Vončina et al., 2023	OR526593
<i>A. discrepans</i>	ZMBN140333	Norway, Vestland, Espegrend; 60°16'11"N 5°13'19"E	N.T. Mikkelsen	1	Vončina et al., 2023	OR526588
<i>A. discrepans</i>	ZMBN140330	Norway, Trondelag, Hopavågen; 63°35'35"N 9°32'E	N.T. Mikkelsen	1	Vončina et al., 2023	OR526586
<i>A. discrepans</i>	SMF 373025	Northern Ireland, Down, Strangford Lough; 54°29'23"N 5°32'15"W	J. Sigwart	1	Vončina et al., 2023	OR145401
<i>A. discrepans</i>	ZMBN46755	Norway, Vestland, Sævrøysund; 60°48'14"N 4°48'29"E	N.T. Mikkelsen	1	Vončina et al., 2023	OR526594
<i>A. discrepans</i>	ZMBN140293	Norway, Vestland, Bakkasund; 60°07'51"N 5°05'31"E	N.T. Mikkelsen	1	Vončina et al., 2023	OR526590
<i>A. discrepans</i>	ZMBN140332	Norway, Trondelag, Hopavågen; 63°35'35"N 9°32'0"E	N.T. Mikkelsen	1	Vončina et al., 2023	OR526587

Table 1, continued.

Species	Voucher	Locality	Legit	N° locality	References	COI
<i>A. discrepans</i>	ZMBN140295	Norway, Trondelag, Hopavågen; 63°35'34"N 9°32'02"E	N.T. Mikkelsen	1	Vončina et al., 2023	OR526592
<i>A. discrepans</i>	ZMBN140294	Norway, Vestland, Bakkasund; 60°07'51"N 5°05'31"E	N.T. Mikkelsen	1	Vončina et al., 2023	OR526591
<i>Lepidochitona caprearum</i>	MNHN-IM-2019-25072	Spain, Tarifa	H. L. Strack	1	This paper	PP748471
<i>L. caprearum</i>	MNHN-IM-2019-25074	Spain, Tarifa	H. L. Strack	1	This paper	PP748472
<i>L. caprearum</i>	MNHN-IM-2019-25075	Spain, Tarifa	H. L. Strack	1	This paper	PP748473
<i>L. caprearum</i>	MNHN-IM-2019-25076	Spain, Tarifa	H. L. Strack	1	This paper	PP748474
<i>L. caprearum</i>	MNHN-IM-2019-25077	Spain, Tarifa	H. L. Strack	1	This paper	PP748475

Table 1, continued.

Genetic distances						
	<i>A. fascicularis</i>	<i>A. cf. fascicularis</i>	<i>A. crinita</i>	<i>A. pilosa</i>	<i>A. discrepans</i>	<i>L. caprearum</i>
<i>A. fascicularis</i>	0 - 1.78					
<i>A. cf. fascicularis</i>	14.76 - 15.92	/ - /				
<i>A. crinita</i>	14.72 - 16.76	13.61 - 14.39	0 - 0.48			
<i>A. pilosa</i>	14.48 - 16.05	13.47 - 13.77	11.07 - 12.43	0 - 0.48		
<i>A. discrepans</i>	14.50 - 17.35	12.60 - 14.05	10.48 - 12.39	7.26 - 8.53	0 - 1.55	
<i>L. caprearum</i>	19.43 - 21.29	20.34 - 22.61	21.26 - 24.16	19.67 - 22.73	21.46 - 24.67	0 - 1.05

Table 2. Minimum and maximum pairwise genetic distances, obtained using Hooty, between individuals belonging to the different monophyletic lineages in the *cox1* tree.

quite high in the intertidal zone. The pulmonate gastropod *Otina ovata* (T. Brown, 1827), which lives just below high water mark, was found crawling on one of the specimens collected in 2001. Turned loose stones in the area did produce many specimens of *A. fascicularis* (Linnaeus, 1767) but no specimens of *A. pilosa*. In Punta Carnero and Tarifa *A. pilosa* is not abundant and is rather difficult to find, although the common occurrence of loose valves in shell grit from the area suggest otherwise.

Probably (but more field study is needed to prove it) juvenile and subadult specimens are free dwelling until they find an ideal spot to settle. Once settled they live sedentary in rock crevices or depressions which are never left. When removed from its substrate *A. pilosa* moves very little and has difficulty to lower its girdle to flatten tightly when placed on a new substrate. This inert behaviour seems to be the result of its life style. Because of this behaviour and its habitat preference adult specimens have often very eroded valves. Homing behaviour can not totally be excluded, but wandering large specimens were never observed in the Bay of getares and Tarifa.

A very similar sedentary behaviour has been observed (HLS) in a possibly undescribed species of *Acanthochitona* from New Caledonia, which lives in the same kind of habi-

tat (except that it lives in old coral boulders and not on solid rock). It might also be found, although not yet confirmed, in other *Acanthochitona* species like *A. shirleyi* Ashby, 1922 from east Australia and a further undescribed *Acanthochitona* species from Japan (pers. comm. Hiroshi Saito, 31-10-2018).

Distribution. — At present known from **France**: Banyuls sur Mer (= type locality); **Italy**: Riomaggiore (Dell'Angelo & Smriglio, 2001 – as *A. crinita*), Catania and Calambrone (Renda et al., 2020), Baia del Saraceno, Varigotti (Granpoder et al., 2023) and Livorno (herein); **Spain**: Cadaques, Girona (Amati & Oliverio, 2019), Marina del Este, Punta Carnero (Bay of Algeciras) and Tarifa (all herein); **Algeria**: Port of Oran (Amati & Oliveiro, 2019). *Acanthochitona pilosa* probably occurs in the whole western Mediterranean. As it lives in the Strait of Gibraltar (Tarifa and Punta Carnero) it would be quite possible that this species can be found in Atlantic localities too.

Sequence data. — Our samples were morphologically identified by the senior author. For the sequences retrieved from the GenBank their identification was corrected in a single case (AF120627), evidently a case of a specimen of *A. fascicularis* misidentified as *A. crinita*.

The final molecular dataset was composed of 61 sequences. Among these, 15 sequences were newly pro-

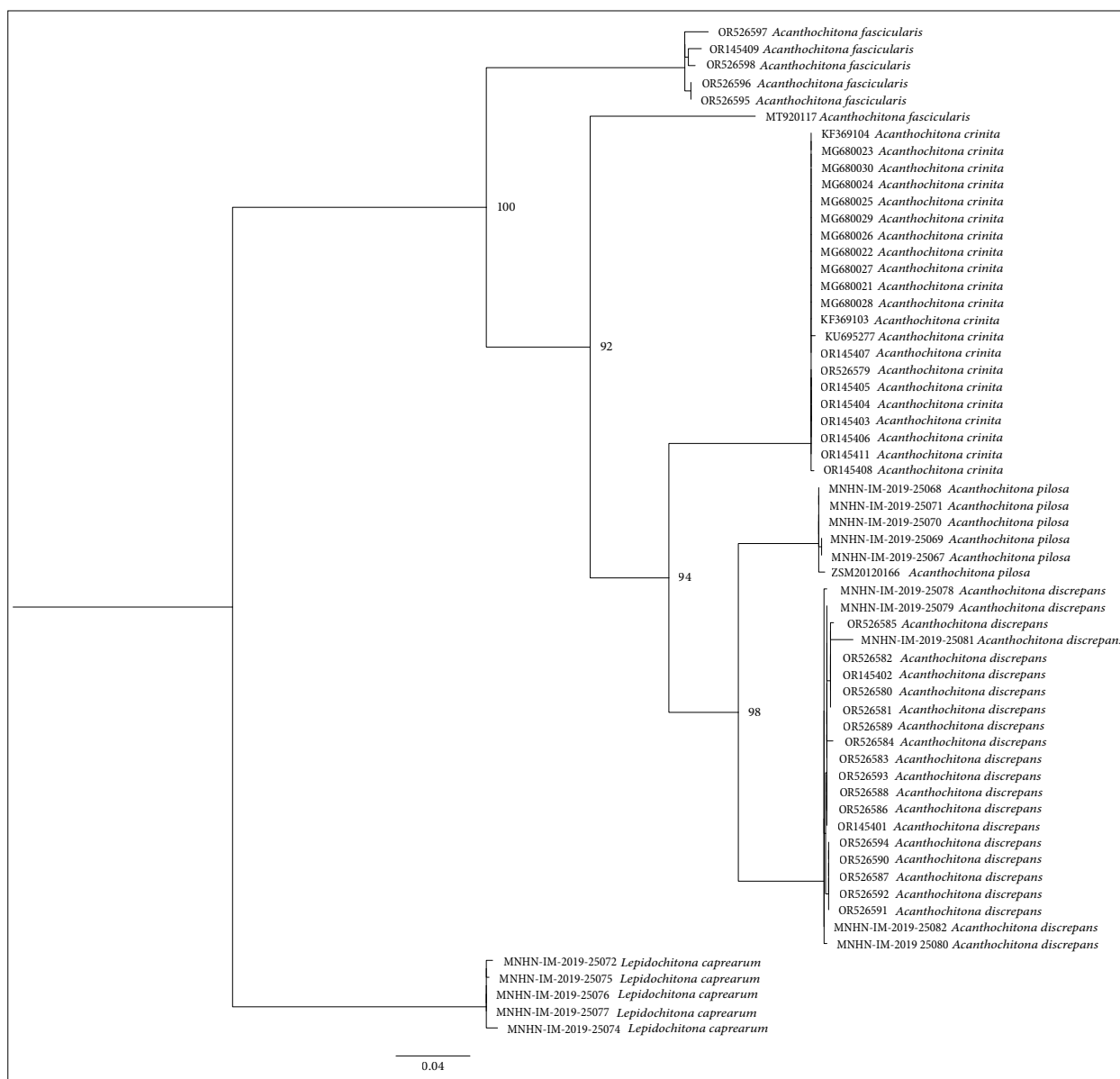


Fig. 7. Phylogenetic relationships of the Mediterranean and Atlantic *Acanthochitona* spp. Maximum Likelihood tree based on *cox1* data set. Numbers at nodes indicate branch support values [ultrafast bootstrap (Ufb) values].

duced: 5 sequences of *Acanthochitona pilosa*, 5 sequences of *A. discrepans*, 5 of *Lepidochitona caprearum* (GenBank accession numbers: PP748466-PP748481). These sequences were aligned with the 46 publicly available belonging to Mediterranean and west Atlantic *Acanthochitona* species (Table 1) and with a sequence of *A. pilosa* provided by Katarzyna Vončina (ZSM20120166).

The Maximum Likelihood tree showed six well-supported clades (bootstrap > 92) (Fig. 7) corresponding to: *A. fascicularis*, *A. cf. fascicularis*, *A. crinita*, *A. pilosa*, *A. discrepans* and the outgroup *Lepidochitona caprearum*.

Maximum and minimum pairwise genetic distances between individuals belonging to the different monophy-

letic lineages are reported in Table 2. All interclade distances were consistently higher than 7%, whereas all intraclade comparisons were consistently lower than 1.8%, with a remarkable gap between interspecific and intraspecific distances (Fig. 7).

In particular, *A. pilosa* showed a genetic distance with its closest taxon, *A. discrepans*, ranging 7.26% - 8.53%, and even higher with the more distantly related *A. fascicularis* (14.48% - 16.05%), *A. cf. fascicularis* (13.47% - 13.77%), and *A. crinita* (11.07% - 12.43%).

Sequences ascribed to *A. fascicularis* have been split into two distinct lineages, with distances ranging from 14.76% to 15.92%.

DISCUSSION

A more comprehensive description of morphological characters (e.g. size and colour of articulamentum) discriminating *A. pilosa* from other European *Acanthochitona* species is provided. Also, more information has become available on its habitat, biology, and its distribution range. The new data confirms the status of *A. pilosa* as a distinct species.

The genetic analysis presented here offers valuable insights into the relationships within the genus *Acanthochitona*, focusing on the taxonomic status of *Acanthochitona pilosa*. Our results highlight the significant genetic differentiation between *A. pilosa* and other closely related species.

Among the species assayed genetically herein, *A. pilosa* positioned as the sister to *A. discrepans*. We could not assay any specimen of *A. oblonga* (and the sequences used in Bonfitto et al., 2011 were not available in the GenBank nor from the authors).

A single specimen morphologically identified as *A. fascicularis* proved genetically distinct from the others under this name (not monophyletic and with a genetic distance of c. 15%), suggesting that *A. fascicularis* might need a taxonomic revision; the sequences of *A. fascicularis* (Vončina et al., 2023) were from specimens of the northeast Atlantic, while the single specimen of *A. cf. fascicularis* (Buršić et al., 2021) has been sampled in the Adriatic Sea.

At first, we wanted to compare *A. crinita* to *A. pilosa*, but the five specimens selected (MNHN-IM-2019-25078/82), which were morphologically identified as *A. crinita* (from Northern Ireland, northwest France, and Portugal), proved to form a monophyletic clade with *A. discrepans*, with genetic distances between individuals in this clade lower than 1.55%. The assumption (Vončina et al. 2023), that *A. discrepans* is a northern European species, can no longer hold. It is evident that more research is needed to elucidate what might be called the *A. crinita* complex.

Overall, our integrative investigation provided compelling evidence for the recognition of *Acanthochitona pilosa* as a distinct species and underscores the need for integrative taxonomy to accurately delineate species boundaries and elucidate evolutionary relationships within chiton taxa.

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