1 **How did the carrier shell *Xenophora crispa* (König, 1825) build its shell? Evidence from the**

2 **Recent and fossil record**

3 Short title: *X. crispa* shell microstructure and agglutination

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7 The genus *Xenophora* comprises species of marine gastropods (Cretaceous-Recent) able to add

8 fragments of various origins to their shells surface. Agglutination potentials vary, from species

9 lacking attachments to species completely covered by agglutinated materials, as in the

10 Mediterranean species *Xenophora crispa*. Here, we analyse Recent and fossil specimens of

11 *Xenophora crispa* from the Mediterranean area using SEM and XRD, to better understand their

12 biomineralization patterns and the mechanisms leading to the agglutination of shells, bioclasts and

13 lithoclasts, and their evolution in time. We also provide new data on poorly studied gastropod shell

14 microstructures. We conclude that: a) most of the *Xenophora crispa* shell consists of an aragonitic

15 crossed lamellar fabric, but fibrous to spherulitic prismatic fabrics, seemingly of calcite, have been

16 found in the columella and peripheral edge (the thickest parts of the shell); b) the objects attachment

17 is mediated by a prismatic microstructure, indicating that this may be the most functional fabric in

18 attachment areas in molluscs; c) the functional purpose of the agglutination in *Xenophora crispa*

19 may be related to a snowshoe strategy to successfully colonize muddy substrates, coupled with

20 tactile and olfactory camouflage. Indeed, this species secretes in the columella and peripheral edge a

21 less dense and a more organic rich calcitic fabric, possibly to lighten the shell thickest parts in order

22 not to sink in soft sediments and to facilitate the shell raising from the substrate to create a protected

23 feeding area. This behaviour seems to have been maintained by *X. crispa* over 2 My time span.

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25 *Key-words: Gastropod, Scanning Electron Microscope, X-Ray Powder Diffraction,*

26 *Biomineralization*

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The animal kingdom offers several examples of organisms forming their exoskeletons by selecting and agglutinating objects from the surrounding environment (Linsley & Yochelson 1973). Among molluscs, the microgastropod genus *Scaliola* Adams, 1860 attaches sand grains to its shell (Bandel & El-Nakhal 1993; Al Shuaibi & Mahmoud 2018) and the bivalves *Granicorium indutum* Hedley, 1906 and *Samarangia quadrangularis* (Adams & Reeve, 1850) have a shell coated by siliciclastic and bioclastic particles taken from adjacent sediments (Taylor *et al.* 1999; Braithwaite *et al.* 2000). On a micrometrical scale, agglutinated foraminifera have their tests formed by foreign particles glued together with a variety of cements (e.g. Hemleben & Kaminski in Hemleben *et al.* 1990). Also, agglutination occurs in unrelated groups such as insects: several aquatic larvae of taxa belonging to the order Trichoptera possess cases made of silk and hardened with gravel, sand, twigs or other debris found in the surrounding environment (e.g. Wiggins 2004).

Among the most spectacular of these agglutinating organisms is the carrier shell *Xenophora* Fischer von Waldheim, 1807, a genus of marine gastropods. This taxon comprises species, known from the Cretaceous to the Recent, which have the ability to form their shells cementing various kinds of objects with different origins: coral skeletons and bivalve, gastropod, brachiopod and foraminifera shells, bioclasts and, in some species, also siliciclastic sand grains and rock fragments (e.g. Ponder 1983; Lebrun *et al.* 2016). As observed by Braithwaite *et al.* (2000) for *G. indutum* and *S. quadrangularis*, this is not a secondary incrustation, but it is a primary constructional feature of the shell.

The process of agglutination by *Xenophora* is not well understood, and has only rarely been directly observed and described (Morton 1958; Shank 1969; Zhu 1984). Morton (1958) and Shank (1969) illustrated the process in *Xenophora neozelanica* Suter, 1908 and *Xenophora conchyliophora* (Born, 1780) respectively, observing that both species are very active when looking for objects to attach, but during agglutination they experience a long period of inactivity that can last up to 10 hours, exposing the organism to predators. Although it is still an unresolved issue, two main groups of theories have emerged regarding the functional purpose of agglutination (Feinstein & Cairns 1998

78   and reference therein): A) a defensive strategy provided either by visual, tactile and olfactory

79   camouflage coupled with discontinuous or slow movements (this is especially valid for species

80   living above the photic zone) or by an increase in size and thickening of the shell cementing objects

81   to form an armour against predators; B) a functional support strategy provided by increasing the

82   weight and the stability against wave and current action, or by enlarging the area of the shell base,

83   raising the aperture from the substrate and avoiding sinking and suffocation in fine-grained

84   sediments (snowshoe strategy; see Copper 1992).

85   Species of *Xenophora* show different agglutination potentials, from species lacking object

86   agglutination to species completely covered by agglutinated materials, as is the case for *Xenophora*

87   *crispa* (König, 1825), the only species of the genus *Xenophora* currently living in the Mediterranean

88   Sea. This taxon is widely distributed over the entire central and western Mediterranean Sea and in

89   the western Atlantic Ocean, from France to Angola (Poppe & Goto 1991). As a fossil, the species

90   has been extensively recorded from the Pliocene and Pleistocene of the Mediterranean area,

91   especially from Italian outcrops (Caprotti 1967; Ponder 1983; Manganelli *et al.* 2004). Despite this,

92   the (palaeo)ecology and life habits of *Xenophora crispa* are still poorly known (Manganelli *et al.*

93   2004). According to some authors (Ponder 1983; Kreipl & Alf 1999; Nappo & Nappo 2014), the

94   species lives on muddy or sandy substrates in waters, from 20-30 m up to 1400 m, whereas others

95   (e.g. Adam & Knudsen 1955; Poppe & Goto 1991) reported a maximum depth of only 300 m. Also,

96   the shell microstructure is poorly described and only Bøggild (1930) briefly referred to the shell

97   fabric of the family Xenophoridae. This lack of information can be extended also to gastropod shell

98   microstructures, where knowledge is generally limited to a relatively small number of specific

99   taxonomic groups (e.g. Bøggild 1930; MacClintock 1967; Taylor & Reid 1990; Fuchigami &

100   Sasaki 2005; Füllenbach *et al.* 2014). In addition, there have been few observations of the cement

101   used by the *Xenophora* organism to agglutinate bioclasts and lithoclasts to its shell. According to

102   Zhu (1984) the object is initially glued with mucus secreted by the mantle; the growth of new

103   mineralised shell and the definitive cementation of the object then proceed simultaneously. But

104 there is no information in the literature about the microstructure of the biomineral cementing the

105 object.

106 Here, we analysed shell sections of Recent (Mediterranean Sea, Spain) and fossil specimens (~1.8 to

107 1.2 Ma, lower Pleistocene, Arda and Stirone River sections, Italy) of *X. crispa* using the Scanning

108 Electron Microscope (SEM) and powder samples from different parts of the shell by means of X-

109 Ray Powder Diffraction (XRD). These allow us to better understand the biomineralization and the

110 mechanisms leading to the agglutination of shells, bioclasts and lithoclasts in *X. crispa*, comparing

111 the function and behaviour of the agglutination in the same species through time, in this case over

112 about 2 millions of years; besides this, we provide new data on gastropod shell microstructures in

113 general terms.

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115 **Geological setting**

116 The Arda and Stirone River marine successions, located in Northern Italy, belong to the

117 Castell'Arquato wedge-top basin, that developed from the late Messinian to the Pleistocene after the

118 north-eastward migration and the fragmentation of the Po Plain-Adriatic foredeep (Roveri &

119 Taviani 2003; Ghielmi *et al.* 2013).

120 These sections belong to the upper part of the Castell'Arquato Formation (Pliocene-lower

121 Pleistocene), cropping out along the homonym rivers, respectively close to the towns of

122 Castell'Arquato and Salsomaggiore Terme at the margin of the northern Apennines facing the Po

123 plain (Fig. 1 A-D). The marine sediments (Fig. 1 C, D) correspond to a subaqueous extension of a

124 fluvial system affected by hyperpycnal flows triggered by river floods, whose terrigenous input is

125 mainly supplied by an increase in the Apennine uplift and erosion, especially starting from 1.80 Ma

126 (e.g. Amorosi *et al.* 1996; Bartolini *et al.* 1996; Argnani *et al.* 1997, 2003; Dominici 2001, 2004;

127 Crippa *et al.* 2016a, 2018, 2019).

128 Marine deposits are composed by an alternation of siltstones, sandstones and mudstones, recording

129 lower order transgressive and regressive cycles with shifts from lower foreshore-shoreface to

offshore transition settings and water depths ranging between 5 and 50 m (Crippa *et al.* 2018). The

marine succession ends with alluvial conglomerates, that represent a sea-level drop and the

establishment of a continental environment with freshwater molluscs and vertebrate faunas (Cigala

Fulgosi 1976; Pelosio & Raffi 1977; Ciangherotti *et al.* 1997; Esu 2008; Crippa & Raineri 2015;

Esu & Girotti 2015; Crippa *et al.* 2018, 2019). Based on calcareous nannofossil and foraminifera

biostratigraphy these successions have been given a Calabrian age (early Pleistocene), ranging from

~1.8 to 1.2 Ma (Crippa *et al.* 2016a, 2019).

Materials and methods

14 Recent and fossil specimens of the species *Xenophora crispa* were analysed in this study. The

material is housed in the Dipartimento di Scienze della Terra "A. Desio" and registered with

reference numbers consisting of a prefix MPUM followed by a five digit number.

Recent specimens [(id numbers: #35450 (MPUM 11857), #35453 (MPUM 11858), #35459

(MPUM 11859)] were trawled by fishing boats at 100-120 m water depth in muddy substrates of

the Mediterranean Sea, offshore from Sant Carles de la Ràpita (Spain) (Fig. 1A).

Fossil specimens were collected from the lower Pleistocene (Calabrian) part of the Castell'Arquato

Formation, cropping out along the Arda and Stirone Rivers in northern Italy [id numbers, Arda

section: ACG11 (MPUM 11846), ACG24 (MPUM 11847), ACG133 (MPUM 11848), ACG199

(MPUM 11849), ACG236 (MPUM 11850); Stirone section: STR1 (MPUM 11851), STR2 (MPUM

11852), STR3 (MPUM 11853), STR4 (MPUM 11854), STR5 (MPUM 11855), STR6 (MPUM

11856)]. Specimens of *Xenophora crispa* were sampled in situ from several stratigraphic beds along

the sections mainly in fine-grained massive siltstones and mudstones, rarely in massive sandstones

(Fig. 1 C, D); these sediments were deposited around 20-40 metres of water depth, according to the

sedimentary structures and the associated fauna (Crippa *et al.* 2016a, 2018, 2019).

Fossil specimens were first cleaned from the sediment using a scalpel and a brush, then washed and

air dried, being careful to preserve the attached objects to the shell. Recent and fossil shells were

then described, focusing on the types and numbers of agglutinated material. To characterise the

microstructure shell sections were investigated using the Scanning Electron Microscope [(SEM

Cambridge S-360 with lanthanum hexaboride (LaB_6) cathodes], and to define the mineralogical

composition shell powders were analysed at the X-Ray Powder Diffraction (XRD, Panalytical

X'pert Powder Diffractometer). For SEM sample preparation we follow the procedure proposed by

Crippa *et al.* (2016b) for brachiopod shells, with a few modifications (i.e. higher exposure time to

hydrochloric acid). Five specimens (ACG11, ACG199, STR1, STR6 and #35450) were cut in half

longitudinally along the major axis and one section of each shell was embedded in a transparent

bicomponent epoxy resin forming small blocks; further transversal sections were then obtained

from each block, to analyse the microstructure along the growth direction. Every block was ground

smooth using Silicon Carbide (SiC) powder of two different granulometries and etched with 5%

hydrochloric acid for 15-20 seconds in order to reveal the detail of the microstructure. After

washing with demineralised water and drying, each block was coated with gold and observed using

SEM at Dipartimento di Scienze della Terra 'A. Desio', Università di Milano.

Small amounts of powders (~0.1 gr) were collected from six specimens (ACG11, ACG199, STR1,

STR6, #35450 and #35459) and from different parts of the shell (columella, inner surfaces of

different whorls, ornamentation on the adapical and abapical surface, surface of the object casts and

peripheral edge) using a microdrill (Dremel 3000) equipped with a 300-μm tungsten carbide drill

bit. Powders collected from the columella and the peripheral edge were sampled in the sectioned

surface (beneath the shell surface). The powders were then deposited on low-background sample

holders (made with specifically cut silicon single crystals), fixed with acetone and then analysed

using XRD at Dipartimento di Scienze della Terra 'A. Desio', Università di Milano. The X-ray tube

(Cu Kα wavelength) was set at 40 kV and 40 mA, and data were collected between 5 and 90° 2θ,

with a step size of about 0.02° 2θ and a counting time per step of 30 sec. The incident slit was fixed

at 1/2°, with an antiscatter of 1/2°; the detector is a multistrip X'Celerator.

182   **Results**

183   *Specimen descriptions*

184   Recent and fossil specimens of *Xenophora crispa* have a dextral, trochoid shell of small size [Arda

185   specimens, height: (9)21-26 mm, diameter: (12)35-38 mm; Stirone specimens, height: 22-34 mm,

186   diameter: 34-52 mm; Recent specimens, height: 21-22 mm, diameter: 32-38 mm] with a pointed

187   apex, narrow peripheral flange and generally 6-8 whorls progressively increasing in size (Fig. 2).

188   The spiral angle is 90°-95°. The suture varies from shallow to deep and the whorl profile –

189   observable only where attached objects are missing – ranges from flat to slightly convex. The shell

190   surface is ornamented by irregular and curved collabral and spiral costellae, sometimes forming a

191   net. The base is flat to slightly convex, ornamented by thin and dense collabral growth lines and

192   spiral grooves, crossing each other and giving a granular appearance to the surface. A deep

193   umbilicus is visible in Recent specimens, but in nearly all the fossil ones is covered by a variably

194   thick callus. The basal ornamentation is sometimes visible where the callus layer is thin. 70-80% of

195   the spire surface is covered by objects or object scars (from 20 to 30 in number for each specimen).

196   Fossil specimens generally preserve the agglutinated objects, but when these are absent the shapes

197   of their casts usually allow the type of object to be determined. The casts of agglutinated objects are

198   usually deep, although a few specimens show poor preservation and weak corrasion (ACG236,

199   STR1, STR3). Agglutinated objects may be small or proportionally very large and consist

200   predominantly of valves or shell fragments of bivalves [*Aequipecten opercularis* (Linnaeus, 1758)*,*

201   *Chamelea gallina* (Linnaeus, 1758)*, Timoclea ovata* (Pennant, 1777)*, Corbula gibba* (Olivi, 1792)*,*

202   *Astarte sulcata* (da Costa, 1778)*, Anadara* sp., *Nucula* sp., *Acanthocardia* sp.], together with a few

203   lithoclasts, and numerous fragments of determinable (*Ditrupa* sp.) and undeterminable taxa (e.g.

204   gastropods, echinoids). Bivalve shells are agglutinated with the concavity-upward and increase in

205   size along the growth vector, but there is no preferential orientation with respect to the umbo.

206   Encrusting bryozoans, serpulids and corals are present on both the original gastropod shell surface

207   and on attached objects in fossil specimens.

208

Shell microstructure

210 SEM analyses of longitudinal and transversal sections of specimens of *Xenophora crispa* show that

211 Recent and fossil shells have the same microstructure. Almost all of the shell of *Xenophora* consists

212 of the most common fabric among molluscs, that is an aragonite crossed lamellar microstructure

213 (Fig. 3 A-E), occurring as alternating layers of simple crossed lamellae (SCL) and irregular

214 complex crossed lamellae (ICCL).

215 The hierarchical organization of simple crossed lamellae, which defines a sort of "zebra pattern", is

216 easily discernible. First order lamellae (~20-30 μm thick) appear as a series of linear oriented

217 lenses, rarely branching, that frequently change their orientation (Fig. 3 A). These are easily

218 recognised on SEM images thanks to the alternating brightness - linked to differences in electron

219 scattering (Tschudin 2001) - of the adjacent first order lamellae, inclined in two opposite directions

220 (Crippa 2013). The boundary between simple crossed lamellae and irregular complex crossed

221 lamellae is usually well defined (Fig. 3 B, C). It is difficult to find a particular pattern in the

222 distribution of the alternating layers of SCL and ICCL; these alternate irregularly without any

223 specific organization. In some cases, a three-layered pattern occurs from the inner to the outer part

224 of the shell, passing from ICCL to SCL and then again to ICCL; in others there is a two-layered

225 distribution of ICCL and SCL (Fig. 3 B,C).

226 In this otherwise monotonous crossed lamellar shell, we have noted the presence of additional

227 fibrous to spherulitic prismatic layers in the thickest parts of the shell, in area such as the columella

228 and the peripheral edge, in both Recent and fossil specimens (Fig. 3 D-H; Fig. 4 A-C). These are

229 sometimes interleaved with crossed lamellar layers (Fig. 3 E; Fig. 4 C). The prisms composing

230 these layers are very narrow, 0.2-0.5 μm. In the inner part of the peripheral edge, these layers follow

231 the curvature of the shell (Fig. 3 H). The fibrous prismatic layers are not always distinct in both the

232 columella and the peripheral edge of all the specimens; in some of them a fine crossed lamellar

233 fabric with a banded appearance occurs, like if the fibrous prismatic fabric is superimposed on,

234   slightly masking, the crossed lamellar fabric (Fig. 3 G; Fig. 4 B). This is particularly evident in the

235   transitional zones between fibrous prismatic and CL fabrics, indicating a gradual passage between

236   the two microstructures. In the contact region between two different shell whorls, which has usually

237   an undulose appearance, the fibrous prismatic fabric may grade into a spherulitic prismatic

238   microstructure (Fig. 3 D).

239   The objects attached to *Xenophora* shell are firmly included in the shell itself (Fig. 4 D). The

240   attachment area of the object to the shell comprises a thin irregular prismatic layer of variable

241   thickness (~2-6 μm) between the agglutinated object and the underlying crossed lamellar fabric; this

242   layer is present along the entire contact surface. Similar layers are present in both Recent and fossil

243   specimens, although not always so clearly defined; they are formed by a stockade of parallel

244   elongated irregular simple prisms in which the prism cross sections are highly variable along their

245   lengths (Carter *et al.* 2012) and are arranged perpendicularly to the contact surface (Fig. 4 E-H).

246

247   *Mineralogical composition*

248   XRD analysis allows us to differentiate aragonite and calcite. Aragonite is a metastable form of

249   calcium carbonate that is commonly replaced by calcite during diagenesis (Casella *et al.* 2017).

250   XRD analyses of both Recent and fossil specimens of *X. crispa*, indicate that aragonite is the major

251   mineral component of the shell; however, calcite occurs sporadically in both Recent and fossil

252   specimens, although mainly restricted to the thickest parts of the shell (columella and peripheral

253   edge; Figs. 5, 6, Table 1). Indeed, a low-intensity peak corresponding to the (210) peak of calcite

254   (d=3.037 Å, 2Θ=29.45° with Cu Kα) is present in the XRD patterns of almost all specimens,

255   including the Recent ones. Table 1 shows the approximate weights % of calcite in the samples. The

256   area of calcite main peak (with d=3.037 Å) was divided by the sum of the peak areas of calcite and

257   aragonite (with d=3.401 Å) and then multiplied by 100. As these are the only two components of

258   the mixture and because calcite and aragonite have the same chemical composition (and therefore

259   the same X-ray absorption coefficient), this can be considered a valid approximation.

260   Calcite is found in every specimen in both the columella, except in STR6, and the peripheral edge.

261   A large amount of calcite is present in the peripheral edge of fossil specimens (ACG11, ACG199,

262   STR1), and also in Recent ones (#35450 and #35459). A small amount of calcite, but still well

263   above the detection limit, is present in the peripheral edge of STR6. The columella is particularly

264   rich in calcite in samples ACG11, ACG199, STR1 and #35450. Calcite is less abundant or not

265   detected in the other analysed shell parts (cast surface in STR1; columella and ornamentation on

266   adapical surface in STR6). There are, however, exceptions in the cast surface in ACG11 and

267   ACG199, and the ornamentation on adapical surface in ACG11, ACG199 and #35450 where calcite

268   is more abundant.

269

270   **Discussion**

271   *Shell microstructure and the agglutination process*

272   The microstructural organization of the shell of *Xenophora crispa* is complex and irregular; it

273   consists of several alternating layers of crossed lamellar fabric (simple and irregular complex

274   crossed lamellae) without a definite distribution pattern. According to Bøggild (1930) this

275   complexity mainly reflects the irregular form of the shells that causes a great variation in the

276   distribution of the shell layers in the different regions of the same specimen. Two important features

277   where missing from the Bøggild (1930) description of the microstructure of the Xenophoridae and

278   are here recorded for the first time in both Recent and fossil specimens: i) the presence of fibrous to

279   spherulitic prismatic layers in the columella and peripheral edge and ii) the presence of a thin

280   prismatic layer (~2-6 μm in thickness) beneath agglutinated objects.

281   Carter (1990) suggested that the shells of most gastropods consists of an aragonitic crossed lamellar

282   microstructure, in agreement with our observations in *X. crispa* shells. He also observed that many

283   gastropod taxa (e.g. *Strombus*) show intercalations of fibrous prismatic layers with crossed lamellar

284   ones, structurally continuous with overlying and underlying crossed lamellar fabrics. This fibrous

285   prismatic microstructure may grade into a spherulitic prismatic fabric, especially in areas where the

shell is thickened (e.g. in apertural lips and within the shell interior). This feature is also recorded in *Xenophora crispa*, where the columella and peripheral edge, which represent the thickest parts of the shell, consist of fibrous to spherulitic prismatic layers.

The second important character reported here has important implications for the agglutination process of *Xenophora crispa*. Below the cemented object there is an irregular prismatic layer (~2-6 μm thick), which then gives origin towards the shell interior to the crossed lamellar microstructure (Bandel 1979; Carter 1990; Wilmot *et al.* 1992). As pointed out by several authors (e.g. Taylor *et al.* 1969; Waller 1980; Crippa 2013), in bivalve shells the irregular simple prismatic fabric is commonly associated with muscle-attachment areas (e.g. adductor and pedal retractor muscles, pallial line); here, it is associated with object attachment to the shell. This led us to hypothesize that this fabric may represent the most functional microstructure for such areas. The prismatic fabric has a higher organic content than the crossed lamellar microstructure (Taylor & Layman, 1972; Checa *et al.* 2005; Esteban Delgado 2008; see also next paragraph); considering the same shell volume, it has a higher organic/inorganic ratio and thus more space (volume) occupied by the organic content than by the mineral. Also, the prisms are arranged perpendicularly with respect to the contact surface; as each prism is surrounded by an organic envelope a greater organic volume is exposed than if the prisms were parallel oriented. This may create a larger surface area/volume available for the mucus secreted by the mantle (Zhu 1984) to promote and strengthen the attachment of the object to the shell.

In the case of *X. crispa*, after having glued the bio/lithoclasts with organic material, the organism continues the biomineralization of new carbonate shell to solidly cement and embed the object (see Fig. 4 D). Our data suggest that *Xenophora crispa* attaches foreign particles to its shells in a way that is different from that observed by Shank (1969) in *Xenophora conchyliophora*. The latter species fills the gaps between the mantle and the agglutinated object with grains of sands, whereas no sand grain has been detected between the attached object and the shell in *Xenophora crispa*. Linsey & Yochelson (1973) observed that the methods of manipulation and implantation of foreign

312   material in *X. conchyliophora* and *X. neozelanica* is completely different, suggesting that the

313   agglutination process may differ from species to species and is a species-specific behaviour.

314

315   *Occurrence of calcite in* Xenophora crispa *shell*

316   The major mineral component of the shell of *Xenophora crispa* is aragonite, as indicated by XRD

317   analysis; however, calcite, which is rarely found in gastropod shells and is generally limited to the

318   outer shell layers (Blandel in Carter 1990; Taylor & Reid 1990), has been found to occur in both

319   Recent and fossil analysed specimens. Calcite has also been detected in species of the order

320   Littorinimorpha (Lowenstam 1954; Taylor & Reid 1990), the same order to which *Xenophora*

321   belongs.

322   However, the presence of calcite in fossil shells may not be of primary origin. Aragonite is a

323   metastable form of calcium carbonate and when the shell undergoes diagenetic alteration it is

324   commonly replaced by calcite (Casella *et al.* 2017). Nevertheless, the occurrence of calcite in the

325   same parts of the shell of Recent specimens, implies that it may be of primary origin also in fossils.

326   Furthermore, samples from the columella and the peripheral edge in both Recent and fossil

327   specimens were collected on the sectioned innermost surfaces (beneath the shell surface) which are

328   supposed to have lacked a direct contact with diagenetic fluids. So, the presence of calcite in the

329   columella and peripheral edge in fossil specimens should have a primary origin. However, we take

330   into account the possibility that traces of calcite occurring in other parts of the shell, as in the

331   surface of the inner whorl, of the abapical and adapical ornamentation and below casts of attached

332   objects in fossil specimens, may be due to diagenetic alteration. As testified also by studies on other

333   fossil organisms (e.g. brachiopods; Romanin *et al.* 2018) the shell outermost parts may be more

334   directly affected by diagenetic fluids and thus be more prone to be altered. However, it is not

335   possible to discern between primary or diagenetic calcite and quantify the different proportion of

336   the two.

337 Most of the shell of *X. crispa* consists of a crossed lamellar fabric, a typically aragonitic

338 microstructure (e.g., Bøggild 1930; Taylor *et al.* 1969; Carter 1990), in agreement with the results

339 of XRD. However, the columellas and the peripheral edges, of both Recent and fossil specimens,

340 include fibrous prismatic layers. Notwithstanding the difficulty of precisely relating the presence of

341 calcite to a particular microstructural type using only SEM, we suggest that these fibrous prismatic

342 layers are probably made of calcite. Taylor & Reid (1990) and Carter (1990) suggested that this

343 microstructural type is of aragonite, although Pérez-Huerta *et al.* (2011) recorded calcite forming

344 fibrous prismatic fabric in the gastropod genera *Haliotis* and *Concholepas*. To try to explain why

345 calcite occurs in specific parts of the shells, we need to consider the physical properties of the two

346 polymorphs, the organic contents of the different shell microstructures and the ecological behaviour

347 of *X. crispa*. Calcite is less dense and less hard compared to aragonite (2.71 g/cm^3 and 3 Mohs'

348 scale compared to 2.95 g/cm^3 and 3.5-4 Mohs' scale) (MacDonald 1956; Table 1 in Thenepalli *et*

349 *al.* 2015). In addition, the prismatic fabric has a higher organic content than the crossed lamellar

350 microstructure that has only 1 wt% of organic content, most of which is present as thin organic

351 sheaths enveloping the third order lamellae (e.g. Uozumi *et al.* 1972; Suzuki *et al.* 2011; Rodríguez-

352 Navarro *et al.* 2012). Organic content values reported by Taylor & Layman (1972) and Checa *et al.*

353 (2005) for prismatic fabrics are higher (4–6 wt%) (Esteban Delgado 2008), with each prism

354 surrounded by an organic envelope. Rhoads & Lutz (1980) suggest that the porosity and the

355 percentage of organic matrix significantly affect shell density, and observe, together with Taylor &

356 Layman (1972), that, despite differences in organic content in the shell microstructures, calcitic

357 shell layers are generally less dense than aragonitic ones.

358 Thus the presence of the less dense polymorph (calcite) in the thickest parts of the shell (columella

359 and peripheral edge) may have a functional and adaptative significance. *Xenophora crispa* lives on

360 loose muddy substrate, commonly below the photic zone, where the use of object attachment as

361 visual camouflage is unlikely. Instead, a snowshoes strategy would be more beneficial together with

362 tactile and olfactory camouflage. As observed by Copper (1992), epibenthic organisms adapt in

363 different ways to soft muddy bottoms mainly finding strategies to prevent sinking; one of these

364 includes the increase of the surface area. Through object attachment, *Xenophora crispa* enlarges the

365 area of the shell base (see Fig. 2 D, F and specimens figured by Nappo & Nappo, 2014) and lifts

366 itself from the sea bottom, avoiding sinking in soft sediment and, at the same time, creating a

367 protected feeding area where the animal can graze; also, although *Xenophora* has a sedentary

368 lifestyle (Feinstein & Cairns, 1998), the shell lifting allows the animal's body to remain suspended

369 from the substrate and to leave, when it moves, discontinuous scent trails to protect itself from

370 predators.

371 In addition, secretion in the columella and in the peripheral edge of a less dense, less hard and more

372 organic rich calcitic microstructure, lighten these thickest parts of the shell, possibly representing a

373 further adaptation to muddy substrates and aiding the lifting of the shell for feeding and olfactory

374 camouflage. The scattered presence of calcite in other parts of the shell may indicate further

375 attempts to locally lighten the shell. A similar strategy is followed by another deep water species of

376 Xenophoridae, *Stellaria solaris* (Linnaeus, 1764), which possesses long and hollow, thus light,

377 spines on the edge of the shell that lift the animal from the muddy substrate.

378 The same behaviour has been recorded in Pleistocene and Recent specimens of *X. crispa* living in

379 muddy environments, suggesting that object attachment was a strategy pursued and beneficial to the

380 species for over 2 million of years.

381 Understanding why *Xenophora* attaches objects to its shells still remain a complex issue. According

382 to Feinstein & Cairns (1998) camouflage was possibly the original function, but the degree to which

383 the other functions are derived is still unknown; as above mentioned, this is likely a species-specific

384 behaviour, where different species exposed to different predation pressure and environmental

385 conditions may have adapted the basic attachment to various purposes. The new findings here

386 presented (i.e., the occurrence of calcite) can help to add another piece to this intriguing puzzle.

387

388 **Conclusion**

389 The study of biominerals of marine organisms provides invaluable information in different fields of

390 palaeontology (e.g. the comprehension of evolutionary taxonomy and of biomineralization

391 processes, the detection of shell diagenetic alteration, palaeoclimatic and palaeoenvironmental

392 reconstructions), so it has to be applied more often. The present study, besides providing new and

393 taxonomically useful data on *Xenophora crispa* shell microstructure, has implications for the

394 understanding of the agglutination process and its significance through time.

395 The analyses of Recent and fossil *Xenophora crispa* specimens using the SEM and XRD indicate

396 that:

a) *Xenophora crispa* has a predominantly aragonitic crossed lamellar shell, although calcite is
397

398 present in the thickest parts of the shell (i.e. the columella and peripheral edge). In these

399 regions a fibrous to spherulitic prismatic fabric occurs, suggesting this fabric to be made by

400 calcite.

b) Object attachment requires a prismatic microstructure, which may be thus the most
401

402 functional fabric in attachment areas in molluscs, although further studies are required to

403 confirm this hypothesis.

c) The functional significance of the attachment of foreign material is species-specific and
404

405 linked to different ecological behaviours. For *Xenophora crispa* a snowshoe strategy is

406 suggested, coupled with tactile and olfactory camouflage. This species secretes in the

407 columella and in the peripheral edge a less dense, less hard and more organic rich calcitic

408 microstructure, possibly to lighten these thickest parts of the shells in order to adapt to

409 muddy substrates and to lift the aperture from the substrate.

d) *Xenophora crispa* shell microstructure and the agglutination purpose do not changed in the
410

411 last 2 millions of years.

412

413 **Acknowledgement**

420

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613

Captions

Figure 1. A) Schematic map showing collection sites of Recent (star with stripes; offshore from Sant Carles de la Ràpita, Spain) and fossil (white star; Arda and Stirone River sections, Italy) specimens of *Xenophora crispa*. B) Geological map of northern Italy, showing the position of the Arda and Stirone River sections (modified after Crippa *et al.* 2018). C) Log of the Arda river section (base at 44°51′18.52″N, 9°52′26.7″E) with position of shell layers (modified after Crippa *et al.* 2016a). D) Log of the Stirone River Section (base at 44°50′38.87″N; 9°58′38.37″E) with position of shell layers (modified after Crippa *et al.* 2019).

Figure 2. Fossil (A-C, E) and Recent (D) specimens of *Xenophora crispa*. A1-3) Fossil specimen, Arda River section; apical, abapical and apertural views respectively (ACG133). B1-3) Fossil specimen, Arda River section; apical, abapical and apertural views respectively (ACG236). C1-3) Fossil specimen, Stirone River section; apical, abapical and apertural views respectively (STR5). D1-3) Recent specimen, offshore from Sant Carles de la Ràpita, Spain, Mediterranean Sea; apical, abapical and apertural views respectively (#35453). E1-3) Fossil specimen, Stirone River section, apical, abapical and apertural views respectively (STR3). F1-3) Recent specimen, offshore from Sant Carles de la Ràpita, Spain, Mediterranean Sea; apical, abapical and apertural views respectively (#35459).

Figure 3. Scanning electron microscope images showing the microstructure of *Xenophora crispa* shells; CL: crossed lamellar fabric; Fp: fibrous prismatic fabric; ICCL: irregular complex crossed lamellar fabric; SCL: simple crossed lamellar fabric; Sph: spherulitic prismatic fabric. A) First order lamellae changing in orientations in the simple crossed lamellar fabric. Recent specimen (#35450). B) Alternation of irregular complex crossed lamellae and simple crossed lamellae. Recent specimen (#35450). C) Boundary between simple crossed lamellar and irregular complex crossed lamellar fabric. Recent specimen (#35450). D) Contact between two different

640 whorls; the contact is undulose and a spherulitic prismatic fabric is present. The lower dark contact

641 is in correspondence of a growth line. Growth lines are organic rich; in fossil specimens the decay

642 of the organic matrix leave many voids making this part more delicate and prone to fracture (e.g.

643 during specimens cutting). Fossil specimen, Stirone River section (STR6). E) Spherulitic band

644 crossing the simple crossed lamellar fabric, possibly representing a growth band. Fossil specimen,

645 Stirone River section (STR6). F) Fibrous prismatic fabric in the columella. Fossil specimen, Arda

646 River section (ACG199). G) Fibrous prismatic fabric to banded crossed lamellar fabric in the

647 columella. Fossil specimen, Arda River section (ACG199). H) Fibrous prismatic fabric in the

648 innermost part of the peripheral edge. Fossil specimen, Stirone River section (STR6).

649

650 Figure 4. Scanning electron microscope images showing the microstructure of *Xenophora crispa*

651 shells; CL: crossed lamellar fabric; Fp: fibrous prismatic fabric; Pr: prismatic fabric; Obj: object

652 agglutinated; SCL: simple crossed lamellar fabric. A) Fibrous prismatic fabric in the innermost part

653 of the peripheral edge. Fossil specimen, Stirone River section (STR6). B) Banded crossed lamellar

654 fabric in the innermost part of the peripheral edge. Recent specimen (#35450). C) Alternated layers

655 of fibrous prismatic and crossed lamellar fabrics in the innermost part of the peripheral edge. Fossil

656 specimen, Stirone River section (STR6). D) Object cemented to the shell. Fossil specimen, Arda

657 River section (ACG11). E-H) Prismatic layer below the agglutinated object. Fossil specimens, Arda

658 River section (ACG11, Fig. E; ACG199, Fig. F), Stirone River section (STR6, Fig. H), Recent

659 specimen (#35450, Fig. G).

660

661 Figure 5. Diffractograms of Recent specimens showing the main peaks of calcite and aragonite.

662 Legend: C: columella; OAS: ornamentation on the abapical surface; PE: peripheral edge.

663

664 Figure 6. Diffractograms of fossil specimens showing the main peaks of calcite and aragonite.

665 Legend: CS: cast surface; PE: peripheral edge; WIS: whorl inner surface.

666

667   Table 1. Distribution and approximate % weight of calcite in the different parts of the shell of

668   *Xenophora crispa*. X: no calcite.

669