Structure and mineralization of the spearing mantis shrimp (Stomatopoda; Lysiosquillina maculata) body and spike cuticles

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Abstract

Stomatopoda is a crustacean order including sophisticated predators called spearing and smashing mantis shrimps that are separated from the well-studied Eumalacostraca since the Devonian. The spearing mantis shrimp has developed a spiky dactyl capable of impaling fishes or crustaceans in a fraction of second. In this high velocity hunting technique, the spikes undergo an intense mechanical constraint to which their exoskeleton (or cuticle) has to be adapted. To better understand the spike cuticle internal architecture and composition, electron microscopy, X-ray microanalysis and Raman spectroscopy were used on the spikes of 7 individuals (collected in French Polynesia and Indonesia), but also on parts of the body cuticle that have less mechanical stress to bear. In the body cuticle, several specificities linked to the group were found, allowing to determine the basic structure from which the spike cuticle has evolved. Results also highlighted that the body cuticle of mantis shrimps could be a model close to the ancestral arthropod cuticle by the aspect of its biological layers (epi- and procuticle including exo- and endocuticle) as well as by the Ca-carbonate/phosphate mineral content of these layers. In contrast, the spike cuticle exhibits a deeply modified organization in four functional regions overprinted on the biological layers. Each of them has specific fibre arrangement or mineral content (fluorapatite, ACP or phosphate-rich Ca-carbonate) and is thought to assume specific mechanical roles, conferring appropriate properties on the entire spike. These results agree with an evolution of smashing mantis shrimps from primitive stabbing/spearing shrimps, and thus also allowed a better understanding of the structural modifications described in previous studies on the dactyl club of smashing mantis shrimps.

Keywords:
- Mantis shrimps
- Cuticle
- Crustacean
- Fluorapatite
- Chitin-protein fibre
- Functional adaptation

1. Introduction

As in arthropods, the cuticle of crustaceans is recognized as one of the most complex biocomposite due to its 3D-twisted plywood organization of chitin-protein fibres. This architecture results in a characteristic lamellate appearance that was explained for the first time by Yves Bouligand (Bouligand, 1965) in the shore crab shell and by analogy with cholesteric mesophases of liquid crystals (Bouligand, 1972). This kind of fibre arrangement was later found in a large variety of natural fibrous composites and reputed to occur by self-assembly under biophysical constrains (Giraud-Guille et al., 2004; Neville, 1984; Taylor and Kennedy, 1994). More recently, the decapod carapace was described as a 3D-architecture involving up to eight hierarchical levels decreasing the anisotropy of the global mechanical behaviour (Huber et al., 2015; Nikolov et al., 2011). Each level of the hierarchical structure is finely tuned for its specific function, starting from the N-acetylglucosamine monomers, polymerising in chains assembled into α-chitin crystallites or nanofibrils (3 nm); then wrapped with protein units to form the chitin-protein fibres (7.25 nm) up to the subdivision of the proucuticle into several (sub)layers that differ in fibre-orientation and fibre association into bundles (compact, reticulate system and bundles or macrofibres (Giraud-Guille, 1984)). The cuticle is also characterised by the nature

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and importance of the mineral component that constitutes, with inter-
fibrillar proteins, the matrix part of the composite. The chitin-protein fibres are then grouped as plates which are stacked into a helicoidal plywood structure where each half rotation of the plywood is named a lamella. The mineral generally consists of high magnesium-calcite but various mineral phases and crystallinitities including amorphous calcium carbonate (ACC) and phosphate-enriched carbonate were recorded depending on the layers, the body areas and species (Al-Sawalmih et al., 2009; Kunkel and Jercinovic, 2013; Ziegler, 1994).

Form a zoological and developmental point of view (Horst and Freeman, 1993; Stevenson, 1985), the crustacean cuticle organization is almost exclusively known from ultrastructural studies on decapods (Boelsmann et al., 2007; Compere and Goffinet, 1987a; Taylor and Dirks, 2012), isopods (Strus and Compere, 1996; Vittori et al., 2016; Ziegler, 1994) and some amphipods (Putz and Buchholz, 1991; Trevisan et al., 2014). These authors divided the cuticle into three main biological layers deposited at key steps or stages of the molting cycle (Dillaman et al., 2005; Drach and Tchernigovtzev, 1967). The first distinction is between the epicuticle and the chitin-procuticle. The epicuticle is the first to be secreted in contact of the epidermal cells as a membrane-like leaflet, the cuticulin layer (Locke, 1961) or envelope (Roer et al., 2015), between the epicuticle and the chitinous procuticle. The epicuticle is thought to be universal in arthropod cuticles and it is regularly reinforced by an inner lipoproteinaceous epicuticle (Thorez et al., 1992). The procuticle is also subdivided in an exocuticle and an endocuticle. The switch between them occurring at euvization time: the first being deposited before and the second after the shedding off the old cuticle. In heavily mineralized decapod cuticle, the endocuticle includes an additional unmineralized membranous layer which transforms into a hydroscopic lubricant, the molting gell, facilitating the euvization (Putz and Buchholz, 1991; Stevenson, 1968).

Due to its low weight, complexity and high plasticity, the arthropod cuticle was seen as a tunable biomaterial that cover a wide range of mechanical properties and resistances (Fabritius et al., 2016; Ribbons et al., 2016; White and Vernerey, 2018). On the other hand, it is a source of inspiration in material conception in many fields of material conception including nanocomposite fabrication (Peng and Cheng, 2017) and architecture (Figliola et al., 2021).

One of the most outstanding examples of high impact resistant crustacean cuticle is that of the dactyl club of smashing mantis shrimps. This crustacean belongs to the Stomatopoda, a distinct order separated from other eumalacostracans since the Devonian (Hof and Briggs, 1997). The smashing mantis shrimp, Odontodactylus scyllarus (Linnaeus, 1758) was well studied especially because of its smash at high speed around 23 ms⁻¹ when hitting preys that break due to cavitation at impact point (Patek et al., 2004). The rapid movement is possible thanks to a specialized elastic cuticle, the saddle, localized in the merus (Anderson et al., 2014). The dactyl itself was first pointed out by its Ca-phosphate-based mineralization, exceptional in crustaceans (Currey et al., 1982). Recently, it was described as a formidable damage tolerant biocomposite material involving specific fibre orientations and an outer fluorapatate shell. The authors evidenced three main functional layers and regions (impact, periodic and striated) on the basis on their mechanical role and fibre architecture (Weaver et al., 2012; Yaraghi et al., 2016). However, this subdivision has no direct correspondence with the biological layers usually found in crustacean cuticle. Stomatopods also includes sparring mantis shrimps, a sister group of aggressive marine predators differing by their hunting technique and by deep modification of their anterior limbs (2nd maxillipeds). Instead of heavy dactyl clubs, sparring mantis shrimps have spiky comb-like raptorial limbs, able to grasp preys in a high velocity movement (devVries et al., 2012).

The dactyl of sparring mantis shrimps has received less attention in comparison to the crusher. Amini et al. (2014) described the same organisation of the cuticle as in dactyl clubs with functional regions but with a less developed impact region. These previous researches highlighted that mantis shrimps have adapted their dactyl cuticle to endure shocks thanks to compositional and structural modifications. Nevertheless, little is known about the spikes found on the raptorial appendage of sparring mantis shrimps which impale fishes and should resist forces during the penetration of a living prey. Moreover, important biological information is missing about the stomatopod cuticle. The organisation of the regular body cuticle from which the dactyl cuticle is thought to have been modified remains unknown. Because of this lack of stomatopod reference, previous papers compared the dactyl cuticle of mantis shrimps with the regular sclerite cuticle of decapods. While such comparison is pertinent from a biomechanical point of view, it should be refined in the evolutionary context.

Our study aims to precisely establish the regular organisation and mineral composition of the mantis shrimp body cuticle and to point out its possible differences with the classical crustacean cuticle description. A new description of the regular cuticle will then allow a better understanding of the spike cuticle found on the sparring limbs and to discriminate the biological layering hidden behind the functional regions previously highlighted in the dactyl club (Amini et al., 2014; Weaver et al., 2012). Overall, this study will highlight the deep structural and compositional modifications in the spike.

2. Material and methods

2.1. Biological specimens

The sparring mantis shrimps used in this study belong to the species, Lysiosquillina maculata (Fabricius, 1793) also called the striped mantis shrimp or ‘varo’ in French Polynesian name. Specimens (4 ind. less 10 cm in length) were collected at Maupiti (French Polynesia), dissected and fixed thanks to David Lecchini and Cécile Berthe (CRIOBE-Moorae). The other much larger specimens (3 ind. from more than 20 cm in length) come from Kendary (Indonesia) and were delivered alive by Marine Life (Paris, France). They were kept in the aquaria at the Functional Morphology Lab (ULiege) until their sacrifice. Animals were fed twice a week and fake burrow made of PVC pipes were placed into the setup, in these conditions they exhibit a normal molting cycle. The specimen size ranged from 20 to 45 cm in length measured from head to telson. To have the complete cuticle structure, three specimens were taken in aneodyis (or intermoult stage C4) as determined according the moult staging method of Reaka (Reaka, 1975) adapted for stomatopods from Drach and Tchergovtzev (1967). Animals sacrificed 24 h after the moult, i.e. at stage A were also used for comparison.

For body cuticle, pieces from the back of the cephalothorax shield and from the 4th abdominal cuticle were dissected (Fig. 1). The dactyl and its 3 first spikes of 3 spearing limbs (2nd maxillipeds) were cut and segmented using a scalpel. Samples were prepared in two ways: 1) polished block-faces of mineralized resin-embedded samples to be viewed in scanning electron microscopy using backscattered electrons.
(BSE-SEM) and in Raman microscopy, 2) demineralized resin-embedded samples to be viewed in light microscopy (semi-thin sections) and in transmission electron microscopy (TEM, ultra-thin sections).

2.2. Sample preparation

2.2.1. Polished block-faces

Samples were directly fixed in ethanol 70% and preserved until use to avoid mineral solubilization. The samples were then dehydrated in 3 baths of 30% in ethanol 100% followed by 2 baths in 1.2 propylene oxide. Resin impregnation started in a mixture propylene-epoxy resin (50/50) bath for 2 h30 followed by 1 h pure resin (EpoFix resin Kit, Struers Inc., Germany) in a vacuum oven at 20 mbar (VTR5036, Heraeus Inc., Germany) and then overnight in resin under atmospheric pressure. The resin was left to polymerize for 3 days in an oven at 60 °C. The resin blocks where grinded and polished using a polishing machine (Rotopol-2, Struers Germany) with a series of silicon carbide disks of decreasing roughness (P800, P1200, P2400, P4000 – Matador, Germany). The last polishing steps were performed on a velvet disk with diamond paste (DP-Spray P 1 µm, Struers, Germany) and colloidal silica (Eposil F, 0.1 µm – ATM, Germany) suspensions. To enhance the contrast of soft tissues, samples from cephalothorax shield and abdomen were contrasted with uranyl acetate (2.5% for 10 min).

2.2.2. Semi-thin and ultrathin sections

Samples for light microscopy (LM) and transmission electron microscopy (TEM) were fixed by immersion in 2.5% glutaraldehyde in seawater 7/10 additionned with 20 mM NaNO₂, then demineralized for 10 days in EDTA (0.2 mol/L, pH 8.0). One half of samples was rinsed in bidistilled water and post-fixed for 1 h in 1% osmium tetroxide (OsO₄) then rinsed again in water. The second half of samples was used as a negative control of OsO₄-staining. All the samples were dehydrated through graded ethanol series (30%, 50%, 70%, 90% and 100%), then epoxy resin-impregnated through propylene oxide (2 x 30 min), resin/propylene oxide mixture (1:1, 2 h30) and pure resin (1 h in a vacuum oven and overnight at atmospheric pressure). These samples were embedded in epoxy resin using the SPI-PON 812 embedding kit (hard mixture, SPI Supplies, USA) in silicon moulds to control sectioning orientation. After 3 days-polymerisation at 60 °C, the samples were cut using diamond knife (Diatome Ultra 45°, Switzerland) on an ultramicrotome (Reichert-Jung Ultra-cut E, Germany) to obtain semithin (1 µm) and ultrathin sections (70 nm). Semithin sections were then mounted in glass blades and coloured with toluidine blue (1%, pH 9.0). The ultrathin sections were mounted on copper grids and then contrasted with uranyl acetate (1% for toluidine blue and contrast agents, as well as on the mineral distribution, the procuticle exhibited an unusual lamination pattern and sub-layering that seemed more complex than the simple distinction between its high organic content which was destructed by the light source.

2.2.3. Frozen sections

EDTA-Demineralized samples of cephhalothorax shield and spike cuticle were performed with a cryostat (CryoStar NX70, Eppendorf, USA) using disposable steel blades. They were laid on glass slides and left to dry for 24 h. The sections were then stained in 1% acridine orange (Tetramethylacridine-3,6-diamine) solutions at pH 4.0 and 7.0 for 5 min then rinsed in bidistilled water.

2.3. Sample observation

Observation of semithin sections was carried out with a light microscope (CX21, Olympus, Japan). Images were taken thanks to a moticam 10+ (Motic, China) linked to the software Motic Image Plus 3.0.

Acridine orange-stained sections were observed in light microscope (Inverso-TC Trinocular, Ceti, UK) equipped with epi-fluorescence module under blue excitation light (results presented as supplementary data, Fig. S3).

Polished sections were observed in an environmental scanning microscope ESEM-PEG XL-30 (Philips™, Nederlands) in low vacuum mode (0.4 Torr) at 20 keV as accelerating voltage. Images were taken using a back-scattered electron detector (BSE-SEM).

The ultrathin sections were observed either in the JEM-1400 (Jeol, Japan) transmission electron microscope of the laboratory of Cellular and Tissular Biology (Prof. M. Thiry, GIGA-Neurosciences ULiege) or in the TEM /STEM Tecnai G2 Twin (FEI, USA) of the CAREM-ULiege (Cell for Applied Research and Education in Microscopy), both working at 80 kV accelerating voltage.

2.4. Elemental X-ray microanalysis

Elemental analyses were carried on in the ESEM-PEG XL30 an energy dispersive spectrometry (EDS) using an analytical system Bruker QUANTAX800 with a Silicon Drift detector of X-rays (SDD 129 eV, Bruker XFlash 5010, 10 mm2, Germany). X-ray spectra and elemental maps were acquired through the Quantax Esprit 2.1 software (Bruker, Germany). Semi-quantitative data were extracted from spectra using the standardless ZAF-method. For each polished section from each body parts, three spectra were probed until 50,000 counts for each layer or functional region by delimiting rectangular areas and using an operating voltage of 30 kV with a spot size of 4.

2.5. Raman spectroscopy

To determine the composition at the molecular level and the nature of the mineral, polished sections of the spike were analysed also by Raman spectroscopy imaging with a 532 nm laser source (type LabRAM 300, HORIBA Jobin Yvon, Japan). Mappings were made all along the width of the cuticle and the spectra were decomposed to separate the different constituents. Each spectrum of the Raman image was recorded for 5 s. With this technique, the body cuticle was not analysable due to its high organic content which was destructed by the light source.

2.6. Statistical analysis

To compare variation in composition of the different layers and functional regions of spikes and body parts, the elemental concentration was extracted from the elemental spectra. After the mathematical subtraction of C, O and Cl coming from the resin, the semi-quantitative data (in at.%) from EDX microanalyses were used to calculate total organic/mineral contents, the proportion of calcium carbonate and calcium (based on their molecular formula) and a Ca/P ratio for each layer-functional region. Finally, two principal component analysis (PCA) were conducted to compare the elemental composition of different layers-functional region within the abdomen, cephalothorax shield and spike cuticle. The PCAs were conducted on normalized values (i.e. reduced centred normal distribution) to minimize the variance influence of the major elements (C, O, P and Ca) and to reveal relationships between the presence and concentration of the minor elements (Na, S, N and Mg) in the different regions and layers. All statistical analyses were performed under the R Studio environment (RStudio Team, 2015).

3. Results

3.1. Structural organization

3.1.1. Body cuticle

Vertical sections in the cephalothorax shield and in abdominal tergites observed in light microscopy, SEM-BSE and TEM revealed a 2-layer organization corresponding to that of arthropod cuticles with a thin superficial epicuticle and thick fibrous procuticle. Based on their affinity for toluidine blue and contrast agents, as well as on the mineral distribution, the procuticle exhibited an unusual lamination pattern and sub-layering that seemed more complex than the simple distinction between an exocuticle and an endocuticle.
LM and SEM observation showed 4 subdivisions in the body cuticle (Figs. 2 and 3A). Fibre organization, staining properties and relative thickness differ when considering the cephalothorax or the abdominal cuticle. The outermost subdivision consists in a thick layer that intensely stains with toluidine blue, but did not show any lamellate pattern such as the rest of the procuticle (Fig. 2A and B). BSE-SEM imaging showed that this layer also has a high affinity for uranyl acetate while, without this staining, it appears as dark as the embedding resin (Fig. 2C, D and 3B). This suggests it does not contain mineral (see below) and is rather organic-rich. TEM images evidenced that it corresponds to the inner epicuticle forming a very thick, compact and fibreless layer. It only consists in a homogeneous electron-dense matrix (Fig. 4A and B). Furthermore, TEM views highlighted that this layer is overlaid by a very thin outer epicuticle structurally corresponding to the cuticulin layer, recently renamed envelope in all arthropod cuticles (Fig. 4A). The cuticulin layer is observed intact in the early post-ecdysial stage (Fig. 8A) with a thickness of about 35.76 ± 5.70 nm. It consists of a very electron-dense outer leaflet a thin electron-lucent medium leaflet and a dark inner leaflet poorly distinguished from the inner epicuticle matrix. At this early postecdysial stage it was covered by an irregular electron dense material. TEM analysis also revealed that this dense fibrous layer is crossed by pore canals with a diameter of 39.6 ± 5.2 nm (Fig. 4A and B). The thickness of the inner epicuticle was more important in the cephalothorax shield (11% of the total cuticle thickness) than in the abdomen dorsal sclerites (7%). The upper sublayer of the procuticle covers several lamellae of gradually increasing thickness. These appear as alternating stained and unstained bands depending on the fibre orientation in the helicoidal twisted plywood system. This is obvious on semi-thin sections (Fig. 2A and B) as on polished block-face (Fig. 2C and D). The staining intensity is always at least a bit more intense than that of the subjacent layers. This layer, we referred as exocuticle, is much thicker and includes more lamellae in the cephalothorax shield than in the abdominal tergites (30.5% against 25% of the total cuticle thickness) (Fig. 3C). In TEM, the exocuticle appears as a compact twisted plywood arrangement with only little spaces left between the fibres except in the mineralized zone near the lower limit (Fig. 4B and C). The lower limit of the layer is marked by a thin electron-dense band on polished block-faces respectively (Fig. 3D). This band corresponds to a densely mineralized level and is considered as the lower limit of the exocuticle (see below). At high magnification in TEM and SEM-BSE, the lower limit of the exocuticle is observed as a thin two-laminated transition region where pore canals from the endocuticle become less defined when penetrating the exocuticle (Fig. 4C). This lower region of the exocuticle was observed in early post-ecdysis when the endocuticle is absent (Fig. 8B).

From this level, the procuticle belongs to the endocuticle which represents 58% and 68% in the cephalothorax shield and abdominal tergite cuticle respectively. It can be divided into two sublayers named outer endocuticle and inner endocuticle. The outer layer has a rather low affinity for toluidine blue. It is distinguished by few thick or very thick

Fig. 2. (A-B) LM views of toluidine blue-stained semi thin sections in the abdominal tergites (A) and in the cephalic shield (B) (transversal sections) of L. maculata. BSE-SEM images of polished block-faces contrasted with uranyl acetate (right part) or not (left part) of the abdominal tergites (C) and the cephalic shield (D) (transversal sections) of L. maculata. endo, endocuticle; exo, exocuticle; epi, epicuticle; epd, epidermis.
lamellae and is mineralized in the cephalothorax shield (Fig. 2B, D). In contrast, the inner endocuticle consists of numerous lamellae slowly decreasing in thickness toward the epidermis. It stains intensely with toluidine blue but moderately with uranyl acetate (Fig. 2D and 3E). In the cephalothorax shield endocuticle, the mineral is only concentrated in the few uppermost lamellae. The lamellae thickness strongly decreases inward, measuring up to 15 µm for the thickest to 1 µm for the thinnest. In contrast, in the abdominal tergite such a distinction between outer and inner endocuticle cannot be clearly made, even if the outer region showed a reduced affinity for toluidine blue (Fig. 2A and C).

The procuticle layers are regularly crossed by numerous pore canals that are lined with vertical fibres. On TEM images, pore canals are well visible while some of them locally contain a dense osmiophilic material (Fig. 4D).

3.1.2. Spike cuticle

LM (Fig. 5), BSE-SEM (Fig. 6) and TEM (Fig. 7) observations of transversal sections in the spikes of the spearing limbs revealed a 2-layer organization with a thin superficial epicuticle and thick fibrous procuticle, but this organization is not directly obvious. What appeared first is a subdivision in six regions that cannot be linked to the subdivision into the classical layers. Comparison with body cuticle and observation of newly moulted individuals was necessary to identify the structural layers.

In spikes, the epicuticle is poorly visible, often missed and only consists in the cuticulin layer. Due to its very low thickness (25.6 ± 4.5 nm) and organic nature, this cuticulin layer is not visible in BSE-SEM at the surface of the strongly mineralized cuticle (see below). However, it can be seen as a thin toluidine blue-stained surface layer in light microscopy (Fig. 5C) and as a thin electron-dense surface layer in TEM (Fig. 7A and B). Moreover, observation of newly moulted individuals revealed the intact state of the epicuticle which is often lost or fragmentary in anec dysis (Fig. 8C). Conversely, additional observations of the dactyl club highlighted that an inner epicuticle is present on the lateral sides of the dactyl cuticle but progressively disappear in the antero-posterior sides and then in the spike cuticle (Fig. 9).

As that of the body cuticle, the exocuticle consists of two sublayers: the outer one is only lightly stained by toluidine blue (Fig. 5A and C) but appears bright on BSE-SEM images, suggesting a high mineral content (Fig. 6A and B). It was called the highly mineralized region (HMR). This outer layer forms the repetitive serrations of the spike surface that are clearly seen in longitudinal sections. Very compact mineral fills the layer but discrete holes and unmineralized vertical canals are visible (Fig. 6B). Examining their location and distribution, they were interpreted as longitudinal and transversal sections of the unmineralized vertical and horizontal pore canal lumens respectively (Fig. 6B). TEM views (Fig. 7) also support the presence of vertical and horizontal pore canals, the vertical ones having an unusual straight pathway. They also reveal thin discrete fibres (7.8 ± 2.74 nm in diameter) that are spread from each other and sectioned with various angles. Most of these fibres run parallel to the cuticle surface with various orientations but without any recognizable twisted plywood arrangement. Around vertical pore canals, some fibres run perpendicular to the cuticle surface. The pore canal lumens locally contain electron-dense osmiophilic material (Fig. 7D).
The inner exocuticle is a thin region that stains intensely in light microscopy (Fig. 5C) it is referred as the transition region. In BSE-SEM, this region appears slightly darker than the highly mineralized region but brighter than the rest of the cuticle (Fig. 6B and C). In TEM, the fibres show the characteristic twisted plywood arrangement that covers no more than one lamella in thickness. Their association is much more compact than in the outer exocuticle but the presence of spaces between irregular thick fibre bundles is rather characteristic of a reticulate system. Moreover, the spaces increase in number and size toward the outer exocuticle and the fibres are progressively spreading from each other in a helicoidal organisation that disappears in the outer region. Contrasting with their straight pathway in the highly mineralized region, pore canals adopt a helical pathway in the plywood arrangement. Broad lacunae within the fibrous reticulate system are interpreted as horizontal pore canals. Additionally, some pore canals showed a curved pattern (Fig. 7C and D). This organisation presents lacunar spaces (reaching 200 nm wide) between the compacted fibres (Fig. 7D). The start of the twisted plywood arrangement (Fig. 7E) and the mineral content (Fig. 6C) make this level congruent with the limit between the exo- and the endocuticle as previously described in the body cuticle. This was also confirmed by the observation of early post-ecdysial stages (Fig. 8D).

The OHR is the uppermost and thinnest region consisting in few lamellae (3–4) of helicoidal twisted plywood arrangement of fibres (~3 µm for each lamella) (Fig. 5C and 7F). The brightness in BSE-SEM images (Fig. 6C) suggests that their mineral content is relatively low. The second region presents a slightly higher brightness due to higher mineral content in SEM-BSE and is travelled by pore canals running perpendicularly to the surface (Fig. 6D and 7F, G). In the striated region, the fibres run parallel to each other and to the spike long axis, they appear cross-sectional on transversal sections of spikes separated by longitudinal sections of pore canals (Fig. 7G). The later follow a straight pathway in the absence of helicoidal fibre arrangement but remain surrounded by their longitudinal fibres sheath (Fig. 7F and G). This disposition produced the striated aspect of this region as seen in LM (Fig. 5). It is also visible on BSE-SEM images showing that the striated region is moderately mineralized while the pore canal lumen remains open, free of mineral (Fig. 6D). In longitudinal sections, pore canals regularly exhibit dense osmiophilic content as observed in the body procuticle, but it seemed more prevalent here (Fig. 7G). The IHR includes the lower third of the endocuticle displaying the classical twisted plywood organization with numerous lamellae of gradually decreasing thickness ranging from 10 µm to 1.5 µm at the inner side as observed in the body procuticle (Fig. 5D and 6F). This region has a weak brightness on BSE-SEM images, suggesting it is not strongly mineralized and the pore canals retrieve a twisted shape.

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Fig. 4. TEM images of the body cuticle of L. maculata. (A) Detail of the epicuticle covered by the cuticulin layer (arrow). (B) Transition between the upper and lower regions of the exocuticle. (C) Transition region of the exocuticle (TR). (D) Detail of the endocuticle showing the changes in fibre orientation according to the twisted plywood system, the very small bundles of chitin-protein interspersed by electron lucent spaces (occupied by mineral before decalcification treatment) as well as the vertical fibres associated with pore canals. Black arrow-heads: pore canals. White star: dense pore canal content. endo, endocuticle; epi, epicuticle; exo, exocuticle; TR, transition region.
3.2. Mineral content

3.2.1. Body cuticle

Qualitatively, the cephalothorax and the abdomen cuticles present similar trends in the mineral composition of their layers, but quantitatively the mineral content of the cephalothorax is significantly higher. C, O, P and Ca are the main elements found in both cuticles. Carbon is linked to both organic content and calcium carbonate mineral. Phosphorus reveals the presence of inorganic phosphate in the form of Ca-phosphate, as confirmed by the absence of P peak on EDX spectra of the demineralized samples (Fig. S1). The high Ca/P ratio however indicates that calcium carbonate is also present (Table 1). The minor constituents are N, Na, Mg, K and S. Additional U Mα and Si Kα peaks are sometimes visible due to sample staining and environmental contamination. (Fig. S2). Mg is evenly present in the mineralized layers, in agreement with the classical Ca-substitution in crustacean cuticles. Information extracted from the EDX spectra were summarized by PCA analysis (Fig. 10) which allowed to discriminate the layers in the body cuticle. The first principal component is negatively correlated to [Ca], [P], [Mg] and positively to [C], it gave an approximation of the mineral content. The second principal component is positively correlated to [S]. The epicuticle is found in the top right of the PCA graph, highlighting a low mineral content but high [S] and [C]. The exocuticle is found fairly spread along the PC1, a trend linked to the gradually decreasing mineral content as already observed on BSE-SEM images. This shows the extent of the gradient from the high mineral content the upper part to the low mineral content inward. The lower limit with the endocuticle (transition region) is close to the bottom left of the graph, because of its higher mineral content than the rest of exocuticle but with low [S]. Finally, the endocuticle is at the bottom right of the graph, sign of a low mineral content with low [S].

3.2.2. Spike cuticle

The major peaks on EDX spectra of the spike cuticle are those of C, O, P and Ca that enter the composition of calcium carbonate and phosphate minerals. The minor peaks correspond to F in addition to N, Na, Mg and S also found in the body cuticle (Fig. S2). Elemental mappings and position beam spectra of the spike cuticle evidenced compositional...
changes according to layers and functional zones. Principal Component Analysis (PCA) shows a clear discrimination of the five structural layers (the cuticulin being too thin to be analysed) (Fig. 11). The first principal component is negatively correlated with [Na], [F] and the elements linked to the phosphate mineral content (namely: P, Ca) and positively correlated with carbonated mineral [C]. The second principal component is negatively correlated with the [Mg]. In the exocuticle, the highly mineralized region presents the highest concentrations of Na, P and Ca (Fig. 11 and Table 2). The inner exocuticle or transition region, reduced to only one lamella, is barely distinguishable but presents lower [P] and [Ca]. In the endocuticle, the outer helicoidal region is characterised by the highest [C] but the lowest [Ca] and [P]. The striated region occupies the central group in the graph, presenting a medium mineralisation level and a low [Mg]. The inner helicoidal region is characterised by high [Mg] and [C] and low [P] and [Ca] linked to a weaker and carbonated mineral content as also highlighted in Table 2. In the PC analysis, [S] appears inversely correlated to [C] and seems to be less present in the inner regions (Fig. 11).

Raman spectroscopy (Fig. 12) gives evidence of three different mineral phases in the spike cuticle. In the whole exocuticle, the Raman spectra corresponds to crystalline fluorapatite with a high Raman signal at 965 cm$^{-1}$ accompanied by a small signal at 1007 cm$^{-1}$ linked with calcium sulphate traces (Fig. 12B and E). In the endocuticle, the outer helicoidal region mainly shows resin spectra (Fig. 12B and D) due to its low mineralization level and thus a high organic content (that was substituted by the resin during sample preparation). The two innermost regions (striated and inner helicoidal region) (Fig. 12C) seem to be mineralized by amorphous (Ca, Mg)-carbonate (ACC) and amorphous calcium phosphate (ACP). An obvious gradient is found between these two functional regions. In the striated region, the spectrum (Fig. 12G) shows a small signal attributed to calcium/magnesium carbonate (at 1090 cm$^{-1}$) and a high shift of calcium phosphate (960 cm$^{-1}$) hence in the inner helicoidal region, the signal change and the intensity of the phosphate shift increase while the intensity of the ACC decreases (Fig. 12F).

4. Discussion

The ultrastructural and microanalytical observation of the cephalothorax, abdomen and dactyl spikes integument in the spearing mantis shrimp, Lysiosquillina maculata, gives for the first time a precise description of the stomatopod cuticle layers, based on their ultrastructural characters, connections and deposition at key moulting stages as well as on their mineral composition and content. Comparison with the sclerite cuticle of decapod crustaceans as literature reference (Neville, 1984; Roer et al., 2015) allowed to point out homologies and adaptive characters as well as to describe the different layers of the cuticle based on the terminology accepted for structural cuticle layers in arthropods. The results established the correlation between the structural layers in the stomatopod cuticle and the functional regions previously described in the dactyl club (Amini et al., 2014; Grunenfelder et al., 2018; Li et al., 2021; Weaver et al., 2012). This part of the work allows to discuss the architectural characters of the stomatopod cuticle in relation with the phylogenetic position of the group among crustaceans and arthropods. Comparison with the inferred base cuticle architecture helps to identify the adaptive modifications undergone by the spike cuticle for harpooning preys as well as by the abdominal cuticle of Lysiosquillina mantis shrimps to live in sandy burrows. Both cases were illustrated by graphic models of the cuticle fibrous architecture (Fig. 13). It is to notice that functional specialization of cuticular regions involves all the layers, influences their structural and compositional modifications and concerns the different cuticle layers developing complementary roles.

4.1. Structure and composition of the stomatopod cuticle

The cuticle organization of both body and spike cuticle corresponds to the typical base architecture of arthropod cuticle, featuring a thin epicuticle and a thick procuticle with a helicoidal twisted plywood
arrangement of chitin-protein fibres and a branched system of twisted ribbon-shaped pore canals (Horst and Freeman, 1993). However, many characters appear unusual in the epicuticle, in the procuticle sub-divisions and plywood as well as in the mineralization of the procuticle sublayers.

4.1.1. Epicuticle

In the body cuticle of Lysiosquillina maculata, the epicuticle consists of the cuticulin layer and a supporting thick homogeneous inner epicuticle. This roughly corresponds to the classical description of the three sublayer-epicuticle in decapod crustaceans (Compère, 1995) and in some other groups such as Isopods (Compère, 1990; Hild et al., 2009) or Amphipods (Halcrow, 1978; Trevisan et al., 2014) if one except the thin covering surface coat. The crustacean inner epicuticle was described as a lipid-protein, usually fibreless, non-mineralized matrix perforated by fine epicuticular canals (47.31 ± 7.79 nm) that stop right at the lower side of the cuticulin layer (Compère, 1995; Compère et al., 2004; Neville, 1984; Stevenson, 1985). This definition perfectly corresponds to the stomatopod epicuticle as seen in the body cuticle of L. maculata. However, it differs from inner epicuticle described in the hard sclerites of many decapod crustaceans by the absence of roots anchoring it in the exocuticle. This is also the case for the inner epicuticle identified in euphausiid shrimps (Putz and Buchholz, 1991).

Several authors pointed out the tendency of the epicuticle to delineate at this level (Compère, 1995; Halcrow, 1988; Putz and Buchholz, 1991). The inner epicuticle of L. maculata is also devoid of any kind of fibres, contrasting with that of decapods were vertical 6 nm-thick fibres were seen to reinforce the epicuticular roots (Compère, 1995) and were supposed to be chitinous (Compère, 1998). The stomatopod epicuticle seems to differ from that of malacostracans by the lack of surface-coat covering the cuticulin layer (decapods (Compère, 1995; Green and Neff, 1972) amphipods (Halcrow, 1976; Trevisan et al., 2014) Isopods (Ansenne et al., 1987; Halcrow, 1988; Hild et al., 2009; Strus and Compère, 1996)). In L. maculata, the outer leaflet of the cuticulin layer appears more electron-dense and an irregular material is sometimes present at the outer surface, but its origin and its relation with a surface coat are still unclear.

In the spike cuticle, the inner epicuticle is completely absent and the cuticulin layer does not subsist for a long time because of wear on the highly mineralized serrations. An intermediate situation was observed in the dactyl cuticle where the inner epicuticle is present but with a very variable thickness. This sublayer is strongly reduced on the highly mineralized anterior and posterior sides, while it thickens on the less mineralized lateral sides. This suggests that the thickness of the inner

Fig. 7. TEM images of the spike cuticle of L. maculata. (A) General view of the outermost layers, the epicuticle with the cuticulin layer (arrow), the exocuticle (Exo) and the top of the endocuticle (Endo). (B) Detail of the cuticulin layer surrounding the highly mineralized region. (C) Detail of the upper exocuticle with vertical straight pore canals sheathed by vertical fibres (vf). Horizontal chitin-protein fibres are visible and dispersed. (D) Transition region of the exocuticle crossed by pore canals presenting local dense contents (white star) and characterized by densely compacted fibres spreading in the upper region. (E) Transition region with large lacunae interpreted as horizontal branching pore canals. (F) Outer helicoidal region of the endocuticle with twisted ribbon-shaped pore canals becoming horizontal and less defined in the upper region. (G) Striated region of the endocuticle with straight pore canals. (H) Detail of the inner helicoidal region of the endocuticle in oblique section with evident pore canal content. Black arrows: pore canals; white star: dense pore canal content. c, cuticulin layer; dhf, dispersed horizontal chitin-protein fibres; hf, horizontal chitin-protein fibres; vf, vertical chitin protein fibres; Phf, densely compacted horizontal chitin-protein fibres; HMR, highly mineralized region; TR, transition region; OHR, outer helicoidal region.
epicuticle varies inversely to the mineral content of the underlying procuticle, so the thickest inner epicuticle was seen in the relatively soft cuticle of abdominal tergites.

4.1.2. Exocuticle

As in other arthropods, the procuticle is subdivided into an exocuticle and an endocuticle defined as pre- and post-ecdysial procuticle respectively. The limit between those layers is not obvious. There was a previous effort to distinguish between exo- and endo-cuticle on the mantis shrimp uropod spike cuticle (Li et al., 2021). However, the lack of additional complementary observation of moulting stages or transition patterns, does not allow for a comprehensive identification of those layers. Here, it was definitely identified by observation of both body and spike cuticles twenty-four hours after moulting. At this time, the exocuticle is fully deposited and the endocuticle is only represented by 1–2 lamellae. Structurally, the limit between exo- and endocuticle occurs in a helicoidal procuticle region (OHR in the spike cuticle) and is materialized by a change in the mineral content but also by two thinner lamellae where pore canal became less discernible similarly in both body and spike cuticle of L. maculata. This subdivision is also be confirmed by Acridine orange staining (Fig. S3) that was known to differentiate pre- and postecdysial layers in the cuticle of decapods (Marlowe and Dillaman, 1995; Roer et al., 2015; Vatcher et al., 2015).

In the body cuticle, the exocuticle resembles to that of other eumalacostracans by its characteristic reticulate fibres pattern (Giraud-Guille, 1984; Horst and Freeman, 1993; Neville, 1984; Stevenson, 1985). It differs only by a reduced mineral content forming a gradient down to the endocuticle limit. In the spikes, it is subdivided in two differentiated zones: a strongly mineralized fibre-poor outer exocuticle and a thin lamellate inner exocuticle with a slightly lower mineral content. This interpretation can also be confirmed looking at the mineral rich areas; as the exocuticle of the body, the spike exocuticle is comprised between two mineralized lines, one below the epicuticle and a second at the lower limit of the inner exocuticle. This conformation was already observed in other decapod crustaceans and is a residue of the mineralisation front moving inward from the top to the bottom of the exocuticle (Dillaman et al., 2005). Applying this view of the exocuticle on the spike cuticle, the two mineralisation fronts are the HMR (where the line has spread downward) and the transition region. The other common traits between both body and spike exocuticle is the two lower lamellae corresponding to the “transition zone” of some authors which marks the limit between the exocuticle; i.e., the pre-ecdysial procuticle, and the endocuticle, i.e. the postecdysial procuticle (Green and Neff, 1972; Putz and Buchholz, 1991).

Fig. 8. TEM images of the body (A-B) and spike (C-D) cuticle of L. maculata in post-ecdysial stage. (A-C) Detail of the epicuticle. (B-D) Detail of the basal part of the cuticle with the first lamellae of endocuticle. White star, dense material covering the epicuticle; c, cuticuline; exo, exocuticle; endo, endocuticle; epd, epidermis; TR, transition region.
4.1.3. Endocuticle

The endocuticle of *L. maculata* is structurally similar to that of other malacostraca, with a subdivision in two sublayers and a lower mineral content than the exocuticle. Indeed, there is a clear distinction between an upper (mineralized) endocuticle and a lower (non-mineralized) endocuticle in contact with the epidermis. Structurally, this is shown as a sharp break in regularity of the lamination pattern. This is especially true for the body cuticle while the spike cuticle displays functional content entirely plugs the pore canal lumen, only leaving sometimes a content entirely plugs the pore canal lumen, only leaving sometimes a pore canals of both body and spike cuticle where it is obvious just below the cuticulin layer. In the body procuticle and spike endocuticle, this ramify in the lower part of the exocuticle and connect epicuticular ca

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**Table 1**

Composition table of the body cuticle (i.e.: cephalic shield, abdominal tergite) layers based on the EDX spectra comparing their calculated content in calcium phosphate, calcium carbonate, Ca/P ratio and total mineral content in at%.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Area</th>
<th>ACP (%at)</th>
<th>ACC (%at)</th>
<th>Ca/P</th>
<th>Total mineral content (%at)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exocuticle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalic</td>
<td>1.0</td>
<td>0.7</td>
<td>4.5</td>
<td>1.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Abdomen</td>
<td>0.5</td>
<td>0.6</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper exocuticle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalic</td>
<td>14.5</td>
<td>2.7</td>
<td>1.8</td>
<td>17.2</td>
<td>4.9</td>
</tr>
<tr>
<td>Abdomen</td>
<td>1.2</td>
<td>0.3</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower exocuticle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalic</td>
<td>3.9</td>
<td>0.2</td>
<td>1.7</td>
<td>4.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Abdomen</td>
<td>3.1</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocuticle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalic</td>
<td>16.9</td>
<td>1.2</td>
<td>1.7</td>
<td>18.1</td>
<td>5.7</td>
</tr>
<tr>
<td>Abdomen</td>
<td>0.4</td>
<td>0.1</td>
<td>4.1</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Endocuticle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalic</td>
<td>0.5</td>
<td>0.6</td>
<td>2.9</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Abdomen</td>
<td>0.3</td>
<td>0.4</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdomen</td>
<td>0.2</td>
<td>0.1</td>
<td>4.0</td>
<td>0.3</td>
<td>0.2</td>
</tr>
</tbody>
</table>

4.1.4. Pore canal system

The pore canal system is an important feature in the arthropod cuticle as it is responsible for transport to the cuticle mainly during sclerotization, wax secretion (Locke, 1961; Seidl and Ziegler, 2012; Wigglesworth, 1985) or mineralization (Compere et al., 1993; Horst and Freeman, 1993; Stevenson, 1985). As in other crustaceans, pore canals are running across the whole cuticle up to the cuticulin layer and exhibit the common twisted-ribbon shape with a vertical fibre sheath. They also ramify in the lower part of the exocuticle and connect epicuticular canals in the inner epicuticle when the latter present. However, they seem more numerous in the spike cuticle and loose they twisted shape twice, within the fibre-poor upper exocuticle and within the striated region of the endocuticle, i.e. in absence of the twisted plywood fibre arrangement. On the other hand, an electron-dense content is often found in pore canals of both body and spike cuticle where it is obvious just below the cuticulin layer. In the body procuticle and spike endocuticle, this content entirely plugs the pore canal lumen, only leaving sometimes a.
small open space (~80 nm in diameter). It could be either interpreted as cytoplasmic remains of cell processes (Neville, 1984) or as secreted material analogous to wax-canal filaments as seen in insects and myriapods (Hunger and Steinbrecht, 1998; Locke, 1961; Thorez et al., 1992). Nevertheless, such pore canal dense osmiophilic content was never observed in marine crustaceans except in some amphipods were pore canals opened to the outside (Halcrow, 1978). Thus, as in amphipods, this osmiophilic content can be interpreted as a hydrophobic plug, especially in the spike cuticle of stomatopds were it could provide additional waterproofing, when the inner epicuticle is reduced or lacking. Moreover, when the cuticulin layer and a part of the upper epicuticle are mechanically eroded by wear, this material may efficiently plug the pore canals that otherwise would be left open to the outside.

4.1.5. Mineral content

In the body cuticle, the mineral is a mixture of calcium phosphate with additional calcium/magnesium carbonate mainly localized in two floors at the top and bottom of the exocuticle. Calcium phosphate as major component could be surprising because calcium carbonate is much more widespread among crustaceans and phosphate is only locally dominant in the procuticle as in some reinforced structures as crayfish mandible (Bentov et al., 2016). Otherwise, low phosphate content is thought to be present due to co-precipitation with calcium carbonate (Becker et al., 2005; Raz et al., 2002) but is increased at mineralization initiation sites as interprismatic septae in the exocuticle of decapods (Kunkel and Jercinovic, 2013). The presence of phosphate in the cuticle implies a decrease of the particle size and the solubility of the amorphous mineral (explaining why crustacean cuticle favour ACC) (Bachra et al., 1963; Fabritius et al., 2016). The ecology of the spearing mantis shrimp does not explain the presence of ACP as a dominant component in its body cuticle, which is most of the time hidden in a burrow and should not face high mechanical loading. Its large distribution across tropical oceans also prevents us to postulate an adaptation to the accessible element (as proven by the preliminary results of this study on Polynesian specimen). The last hypothesis is phylogenic and suggests that, since stomatopods have differentiated from other Malacostracean, calcium phosphate was favoured hence it remains secondary in other groups, as suggested by Bentov (Bentov et al., 2016). It is therefore

### Table 2

Composition table of the spike layers based on the EDX spectra comparing their calculated content in calcium phosphate, calcium carbonate, Ca/P ration and total mineral content in at%.

<table>
<thead>
<tr>
<th>Region</th>
<th>ACP (%)</th>
<th>ACC (%)</th>
<th>Ca/P</th>
<th>Total mineral content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMR</td>
<td>52.6 ± 7.7</td>
<td>26.8 ± 14.5</td>
<td>2.2 ± 0.5</td>
<td>79.4 ± 8.1</td>
</tr>
<tr>
<td>TR</td>
<td>47.9 ± 3.4</td>
<td>27.6 ± 10.6</td>
<td>2.4 ± 0.5</td>
<td>75.4 ± 8.0</td>
</tr>
<tr>
<td>OHR</td>
<td>29.2 ± 6.3</td>
<td>21.9 ± 8.4</td>
<td>2.5 ± 0.4</td>
<td>51.0 ± 7.7</td>
</tr>
<tr>
<td>STR</td>
<td>36.9 ± 6.1</td>
<td>28.5 ± 9.3</td>
<td>2.7 ± 0.5</td>
<td>65.4 ± 4.4</td>
</tr>
<tr>
<td>IHR</td>
<td>23.5 ± 11.2</td>
<td>43.4 ± 15.0</td>
<td>5.2 ± 2.3</td>
<td>66.9 ± 5.7</td>
</tr>
</tbody>
</table>
reasonable to think that the presence of P in the endocuticle of the spike is not linked to any mechanical adaptation, but is rather a basic character of the stomatopod cuticle.

4.2. Ancestral characters

Comparison of the body cuticle with the spike cuticle of the spearing mantis shrimp, *Lysiosquillina maculata*, provides arguments to distinguish between base characters of the stomatopod cuticle that can be ancestral or derivative characters for the taxon from the functional character notably resulting from adaptative modifications of spikes for prey penetration. Moreover, the stomatopod cuticle showed obvious differences compare to the well-studied cuticle of decapods. This contrasts with the current opinion in the last century (Dennel, 1978) and calls for re-evaluation of the decapod sclerite cuticle which often presented as model in the Crustacea (Compere et al., 2004; Neville, 1984; Stevenson, 1985).

The phylogenetic position of the Stomatopoda as the base group of the Eumalacostraca and placed as a sister group of the Euphausiacea (Jenner et al., 2009) suggest that their cuticle could still retain ancestral characters of crustacean or arthropod cuticles. In addition, their marine and usually benthic life should be ancestral states in arthropods found in other basal groups of arthropods as horse shoe crabs (Chelicerrata, Xiphosura) while many basal groups of crustaceans are pelagic. The present ultrastructural observations of the *L. maculata* body cuticle in their lamella arrangement (Putz and Buchholz, 1991). The second argument is that the inner epicuticle is fibreless and rootless in the sclerites of both stomatopods and euphausiaceans (Buchholz et al., 1989). This character is also found in hexapods but not in the other malacostracans where the inner epicuticle is modified for anchoring into the exocuticle by short roots in isopods and long roots reinforced by vertical chitin rots in decapods (Ansenne et al., 1987; Compere, 1995). The third argument deals with mineral composition and the absence of macrofibres in the endocuticle. Phosphate-based mineralization is reputed to be primitive in marine organisms (Bentov et al., 2016; Berkovitz and Curator, 2013; Currey, 2013). Bentov et al. (2016) also suggested that a mix Ca-carbonate/phosphate primitively occurs in the Crustacea. Thus, the possibility to use Ca-phosphate in addition to carbonate should be regarded as an ancestral character for the groups as for the Arthropods. Contrasting with other groups, stomatopods should have favoured phosphate-based mineralization. In the Stomatopoda body, the exocuticle is reinforcement with small particles of ACP that dominates. In the endocuticle, the global mineral content is much lower and the proportion of ACP/ACC equilibrates. Phosphate-based mineralization should be regarded as a derivative character of the Stomatopoda. In contrast, other crustaceans favoured Ca-carbonate mineralization, with ACC and calcite, that dominate but a small subsisting proportion of Ca-phosphate does not exceed 10 % (Al-Sawalmih et al., 2009; Bentov et al., 2012). In the latter, ACP only dominates in some specialized regions (Bentov et al., 2012; Kunkel and Jercinovic, 2013). The absence of macrofibres in the stomatopod endocuticle as in that of many malacostracans suggests that this is the initial condition in

![Fig. 12. Raman analysis of the spike cuticle. (A) BSE-SEM image of the studied section. (B) Raman mapping on the transition between the highly mineralized, the outer helicoidal and the striated region. (C) Raman mapping on the transition between the striated and the inner helicoidal region. (D–E–F–G) isolated Raman spectra from the different constituent; (D) resin spectra, (E) crystalline fluorapatite, (F) amorphous ACP with minor ACC contribution, (G) amorphous ACC with minor ACP contribution.](image)
the Crustacea. Indeed, macrofibres or large chitin-protein fibre bundles in the decapod endocuticle are only associated with heavily mineralized endocuticle in hard-shelled decapods (Compère and Goffinet, 1987b; Neville, 1984).

4.3. Functional specializations

In the light of the determined ancestral characters of the cuticle it can be assumed that both abdominal and spike cuticle of *L. maculate* have undergone adaptive modifications.

4.3.1. Abdominal tergite cuticle

In comparison with the cephalothoracic shield cuticle, the abdominal tergite cuticle has a lower mineral content, a thinner epicuticle and a much thicker endocuticle. These variations may be interpreted as a weakening of the cuticle rigidity, probably linked to the burrowing mode of life of spearing mantis shrimps. The abdomen is protected in the burrow and does not need any mineral reinforcement. Moreover, the abdomen needs to be flexible to be able to turn around inside the burrow. A low mineral content also reduce the weight which could be important for efficient swimming in case of danger.

4.3.2. Spike cuticle

Natural selection linked to the hunting success has constrained the cuticle of spearing limbs mantis shrimps to adapt to the mechanical stresses endured when harpooning a prey. In previous studies, authors described the dactyl club as presenting a crystalline impact region, a periodic region and a striated region (Amini et al., 2014; Grunenfelder et al., 2018; Weaver et al., 2012). Similar regions can be found here, but their interpretation differs due to the different role of the spike and the comparison with the basic cuticle state found in the body cuticle.

In contrast to the body and dactyl cuticle, the spike cuticle lacks of inner epicuticle and the cuticulin layer remains the only present. Indeed, as a waterproof layer limiting the cuticular compartment, its presence is mandatory to allow mineral nucleation and growth. On the other hand, the disappearance of the inner epicuticle is clearly linked to the heavy mineralization of the cuticle and to the puncturing role of the spike. A soft surface layer would decrease the surface rigidity and then the efficiency of penetration. The cuticulin layer of the spike cuticle is strongly subject to wear and is then often eroded. This explains why it was never described until now even in the dactyl where a small inner epicuticle is only present at the lateral sides which were not observed in previous studies (Amini et al., 2014; Weaver et al., 2012).

The spike exocuticle thus consists of a highly mineralized fibre-poor outer part followed by a less mineralized fibrous part or a transition region. Both are Ca-phosphate mineralized in a highly crystalline state of fluorapatite. This crystalline mineral gives a hard outer shell that increases the rigidity of the structure. Crystals are resistant to compression forces and this could be important to counter bending forces during penetration. Fluorapatite is a regular component of vertebrate tooth enamel where it is reputed to afford the formation of harder crystals (Bentov et al., 2012; LeGeros and Suga, 1980). In crustaceans, fluorapatite crystallisation is only found in the mandibles of crayfish *Cherax quadricarinatus* (Bentov et al., 2016, 2012). The presence of fluorine in calcium phosphate was reported as an enhancer of the crystallisation process and was reported in teeth of some fishes (as sharks) to favour the
formation of larger crystals (Aoba, 1997; LeGeros and Suga, 1980; Miake et al., 1991). At the same time, a fundamental role of FAP is to decrease the solubility of apatite, thus enhancing the chemical stability in seawater (Moreno et al., 1974; Okazaki et al., 1998) and might ensure a slightly higher hardness compared to hydroxyapatite (Gross and Rodriguez-Lorenzo, 2004). In the mantis shrimp spikes, fluorine could also increase the crystallinity, and consequently increasing the compression resistance of the mineral layer (outer exocuticle) and thus the rigidity of the structure. In the dactyl club of smashing and spearing mantis shrimps, it has been suggested (Amini et al., 2014) that the hard fluorapatite layer was developed as an impact resistant adaptive feature. Knowing that spearing is an ancestral trait compared to smashing, the presence of strong FAP mineralization in the spike suggest that fluorapatite was earlier favoured in the spearing limbs to increase the rigidity of spikes. The adaption of smashing limbs to impact resistance could be seen as a subsequent step, tuning the thickness of the fluorapatite-mineralized exocuticle in the impact area as well as a higher fibrous content exhibiting a unique sinusoidal organisation (Yaraghi et al., 2016). As observed in the dactyl club (Amini et al., 2014), calcium sulphate (CaSO₄) traces is found within the FAP increasing the mineral stability. In our work focusing on the spike cuticle, the proportion of CaSO₄ seems to be less than that detected in the specific impact zones of the dactyl club. Owning to the different biomechanical functions of the spike (penetration and prey retention) in comparison to the impact region of the dactyl, some dissimilarity in chemical composition and mineral phases are conceivable.

In the spike cuticle, the inner exocuticle or transition region constitutes an additional unusual feature. Its role is not known but it could be proposed that it act as damper layer between the highly mineralized outer exocuticle and the moderately mineralized endocuticle. At the interface between the transition region and the highly mineralized exocuticle, chitin-protein fibres condense to form larger bundles in a reticulate lacunar structure. This arrangement could be seen as an anchoring interface, allowing a better stability between these two layers of very different mineral content. Layer anchoring is important in crustacean cuticle, particularly when the cuticle has to deal with shearing forces. While this anchoring strategy between endo and exocuticle was never observed before, other anchoring have already been described as epicuticular roots in arthrohaline membrane cuticle of decapods. These connect the inner epicuticle to the exocuticle at areas subject to shearing stresses as arthrohaline membranes (Compere and Goffinet, 1987a).

The spike endocuticle differs from the body endocuticle by the presence of longitudinal parallel chitin-protein fibre forming the striated region (STR) as described by authors in the dactyl club. This region reflects an interruption of the regular helicoidal twisted plywood arrangement of chitin-protein fibres. This arrangement is very unusual in arthropod cuticles and probably occurs for mechanical reasons. Analogous organisation were first described in the sides of the dactyl club of smashing mantis shrimps in an area called for the first time “striated region” that was thought to dissipate the impact energy in the lateral sides and to give torsional stiffness (Amini et al., 2015, 2014; Weaver et al., 2012). However, similar arrangement was found in spider fangs (Al-Sawalmih et al., 2008; Raabe et al., 2006), isopod claws (Vittori et al., 2016) and the uropod spike of the mantis shrimp (Li et al., 2021) and was rather related to penetrative and elongated cuticular structure where it affords a resistance to axial forces in the penetration direction as well as to bending forces usually occurring in such elongated structure. This last requirement corresponds better to the case of stomatopod spikes and highlights convergent evolution between elongated penetrative cuticular structures. The striated region in the dactyl club of smashing mantis shrimp should thus be considered as a remnant of the fibre arrangement that predominate in the elongated dactyl of ancestral “stabbing” mantis shrimps (Haug et al., 2016, 2010). In the smashing dactyl club, adaptation to impact resistance would include the loss of the parallel fibres arrangement below the impact region where a helicoidal plywood is restored and described as “periodic region” (Weaver et al., 2012). To prove this assumption, observation of other maxillipeds dactyls (supposed structurally close to the ancestral state or stabbing state of the raptorial appendage) highlights the generalized presence of this striated layer even if it appears reduced in thickness (Fig. S3).

5. Conclusion
Unlike what was though in last century science (Dennel, 1978), the spearing mantis shrimp cuticle demonstrate evident specific variations compared to the well-known decapod cuticle. Those variations include the absence of epicuticular roots and exocuticle macro-fibres and the mineral content dominated by calcium phosphate. From this basic structure, the mantis shrimp cuticle has highlighted structural variation linked to mechanical adaptation in its abdomen and its spike. While abdominal adaptations mainly consist in weight reduction, spikes modifications are deeper. Due to its role in the prey capture, the spike was highly selected during the evolution of spearing mantis shrimps to ensure its survival. In the present work, we highlighted that more than variation in the shape and mineral content of this spike, the internal organization and composition also present strong modifications. The main specificities of the spike cuticle are the presence of a highly mineralized superficial region reinforced with fluorapatite and a local interruption of the helicoidal plywood structure in the middle of the endocuticle. These two modifications should lead to a rigid and deformation-resistant spike required for prey penetration. Its comparison with the basic body cuticle allow to determine the classical layering subdivision of an arthropod cuticle (eпи-, exo- and endocuticle) and showed that some interpretations made in previous studies about the dactyl cuticle adaptation differs here; such as the role of the striated region or the presence of phosphorus as an adaptation to mechanical constrains. Future work shall elucidate the functional role of the sophisticated composition and ultrastructure of the spike cuticle.

CRediT authorship contribution statement

Yann Delaunois: Writing – original draft, Writing – review & editing, Visualization, Funding acquisition, Conceptualization, Formal analysis, Investigation, Resources. Sarah Smeets: Methodology, Investigation, Resources. Cédric Malherbe: Investigation, Resources, Formal analysis, Writing – review & editing. Gaëtan Goffinet: Resources, Writing – review & editing. David Lecchini: Resources, Writing – review & editing. Davide Ruffoni: Conceptualization, Writing – review & editing. Philippe Compère: Conceptualization, Investigation, Methodology, Resources, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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