http://agriprojournal.com
JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
PRENSIP
https://prensip.gen.tr

RESEARCH ARTICLE

Microstructure of *Eobania vermiculata* (Müller, 1774): SEM, F-TIR and XRD Methods

Kerim Emre Öksüz[1] • Hülya Şerefl işan[2*]

[1]Sivas Cumhuriyet University, Faculty of Engineering, Department of Metallurgical and Materials Engineering, Sivas/Türkiye
[2]İskenderun Technical University, Faculty of Marine Sciences and Technology, Department of Aquaculture, Hatay/Türkiye

ARTICLE INFO

Article History
Received: 08.06.2022
Accepted: 23.07.2022
First Published: 13.12.2022

Keywords
Characterization
Chocolate band snail
Land snail
Microstructure
SEM

ABSTRACT

In this study, Scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) analyses are used for the microstructure characterisation of *Eobania vermiculata* samples collected from Iskenderun region. The shells of land snails are discarded as waste; however, they are qualified materials with multiple use areas. To substantiate this proposition, an attempt was made to elucidate the physical and chemical properties of the shells of chocolate band snail, *E. vermiculata*. SEM observations indicated that nacre crystals are always laminated aragonite, usually presenting sharp edges. Nacre crystallites which pile up into columns vertically abreast aligned observed. The crystals are about 390-155 nm thick, and they form stacks along a fixed spacing, filled with biological matter. The XRD and FTIR observations revealed the dominance of the aragonite form of the calcium carbonate crystal in the microstructures of each snail shell with the occurrence of different shell surface functional groups. Thus, further exploration of the shell inclusive of the organic components is required to promote its possible use as a biocomposite. Nonetheless, the present study provides an overview of physical and chemical characteristics of the land snail shells and inlight their potential use in different areas in the perspective of sustainability.

Please cite this paper as follows:

Öksüz, K. E., & Şereflişan, H. (2022). Microstructure of *Eobania vermiculata* (Müller, 1774): SEM, F-TIR and XRD methods. *Journal of Agricultural Production*, *3*(2), 42-47. https://doi.org/10.56430/japro.1128026

Introduction

Eobania vermiculata (Müller, 1774) or chocolate band snail is native to the Mediterranean region but today has a worldwide distribution via anthropogenic activities (Godan, 1979). *E. vermiculata* is registered in Italy, Bulgaria (Dedov, 1998), Greece (Welter-Schultes & Williams, 1999), Turkey (Örstan et al., 2005) and Croatia (Rađa et al., 2012). It has become widespread to evaluate mollusk shells in different areas in the living world. In particular, the shell structures of Gastropoda species seem worth investigating (de Paula & Silveira, 2009).

Mollusk shells are mineralized tissues each with a unique mineral composition. In all three main mollusk classes (Cephalopoda, Gastropoda and Bivalvia) the shell consists of stratified layers (Santana & Aldana Aranda, 2021). Mollusk shells are composed of calcium carbonate and a small amount of organic matrices (Lowenstam & Weiner, 1989). Calcium carbonate has amorphs form and consist of calcite, aragonite and vaterite (Suzuki & Nagasawa, 2013). Vaterite is a mineral that is a polymorph of calcium carbonate, and it is the most unstable one; whereas, calcite is the most stable and aragonite is metastable. Accordingly, most mollusk shells consist of aragonite and/or calcite; yet, vaterite is rarely found (Spann et

* Corresponding author
E-mail address: hulya.sereflisan@iste.edu.tr

al., 2010). The periostracum is formed as a first layer in the shell formation, and the calcified layer subsequently forms on the periostracum which is not mineralized (Checa, 2000). The first calcification in the mollusk shell begins in the shell gland cells (Nielsen, 2004). The mantle, which supplies the periostracum and calcified layers with inorganic ions and organic matrices through the extrapallial fluid creates mollusk shell (Waller, 1980). The periostracum consists of three layers: inner, middle and outer. Among them inner layer is secreted by the mantle epithelium (de Paula & Silveira, 2009). Nacre which is widely distributed in mollusks is the most studied aragonitic and has stratified microstructure. Stratified microstructure which make to nacre luster occurring it one of the most studied hard tissues and has been of great interest to the pearl industry (Wang et al., 2013). In the gastropods, the aragonite nanocrystals of the nacre which exhibits specific growth pattern and mechanism are stacked in the form of towers (Romana et al., 2013). Nacre is a biomineral consisting (by weight) of 95% aragonite ($CaCO_3$) with the remaining 1-5% being organic matrix (Zhang & Li, 2012). Its microstructure is one of layered "brick" (aragonite tablets) and "mortar" (protein-polysaccharide matrix) (Machado et al., 1991; Santana & Aldana Aranda, 2021).

The microstructure features of the mollusk shell thanks to scanning electron microscopy is in use to the determine the phylogenetic evolution of the mollusk (Machado et al., 1991; Hedegaard, 1997; Lopes-Lima et al., 2010). Infrared spectroscopy which investigated composition of both inorganic and organic materials provides the identification of characteristic functional groups in molecules that correspond to specific molecular vibrations in the molluscan shells (Dauphin, 1999; Wang et al., 2013; Dauphin et al., 2018). The X-ray diffraction method (de Paula & Silveira, 2009) provided us the information about the complex microstructure of different types of deposited calcium carbonate crystals (Lowenstam & Weiner, 1989).

The objective of the present study was to analyze the micro and nanostructure of *E. vermiculata* (Müller, 1774) which is commonly found in Turkey. Analysis was conducted by scanning electron microscopy, its chemical composition was identified by X-ray diffraction Fourier Transform Infrared Spectrometry. The aim of this research will be provides theoretical data for the use of *E. vermiculata* in the biomaterials area and represented useful criteria for studies of the phylogeny of mollusca.

Materials and Methods

In this study, chocolate band snail *Eobania vermiculata*, a pulmonate stylommatophoran gastropod of the *Helicidae* was investigated. The snails were collected at the beginning of March 2018 in the Iskenderun region. Collected individuals were brought to the laboratory and the soft tissue was carefully separated from the shell. Shells were washed with tap water followed by distilled water to remove the mud, sand and other impurities. The shells were kept under the sun for 3 days to dry (Singh & Purohit, 2011).

Fourier Transform Infrared Spectroscopy (F-TIR) Analysis

The samples of raw materials from the chocolate band snail shell powders (*E. vermiculata*) were analyzed at 4000-650 cm^{-1} using a model Jasco/FT/IR-6700 equipped with ATR (Attenuated Total Reflection) Spectrometer.

X-Ray Diffraction Analysis (XRD)

X-ray diffraction analysis (XRD) was conducted to detect the crystallinity of the raw materials from shell powder. The XRD measurements on powder samples were done at 5°-40° with XRD (Malvern Panalytical EMPYREAN 3rd generation, United Kingdom) equipped with Ni-filtered Cu Kα radiation (λ = 1.5406 Å). It was operated with 1°/min deviating and receiving slit at 40 kV and 30 mA and continuous scan was carried out with step size of 0.015° and step time of 0.2 s.

Scanning Electron Microscopy (SEM) Analysis

The samples were mounted on stubs with conductive double-sided carbon tape and coated with gold/palladium in a sputter coater (Polaron SC7620, UK) for 90 s at 9 mA. The samples were examined and photographed using a JEOL JSM 5500 scanning electron microscope (SEM) at an accelerating voltage of 5 kV.

Results and Discussion

Fourier Transform Infrared Spectroscopy (F-TIR)

Infrared characterization was carried out for the sample to study the spectral characteristics indicative of the chemical bonding in the snail shell powder. The infrared spectra of *Eobania vermiculata* shells powder is shown in Figure 1.

The peak around 2969 cm^{-1} appeared due to the CH_2 stretching bonds of aliphatic chains. The F-TIR spectrum revealed lower intensity organic bands; the band at 1786.52 cm^{-1} was attributed to the carboxylate (carbonyl) groups of the acidic proteins in the organic matrix. Four bands characteristic of aragonite, corresponding to the CO_3^{-2} ions, were identified: ν1 at 1082.83 cm^{-1}; ν2 at 855.19 cm^{-1}; ν3 at 1448.49 cm^{-1}; and ν4 at 699.91-712.63 cm^{-1} (Figure 1). The ν1 band to the symmetric stretch mode and the ν4 band corresponds to the planar flexion mode of carbonate vibration.

Also, the characteristic carbonate ν4 bands of aragonite were at 712.63 and 699.91 cm^{-1} and the characteristic carbonate ν2 band of aragonite at 855.19 cm^{-1} revealed the availability of aragonite form of calcium carbonate in the shell powders from snails (Anjaneyulu et al., 2015; Hossain et al., 2015). This was also supported by the study of Weir and Lippincott (1961), Focher et al. (1992), Marxen et al. (2003), Cárdenas et al. (2004), Agbaje et al. (2017), and Parveen et al. (2020).

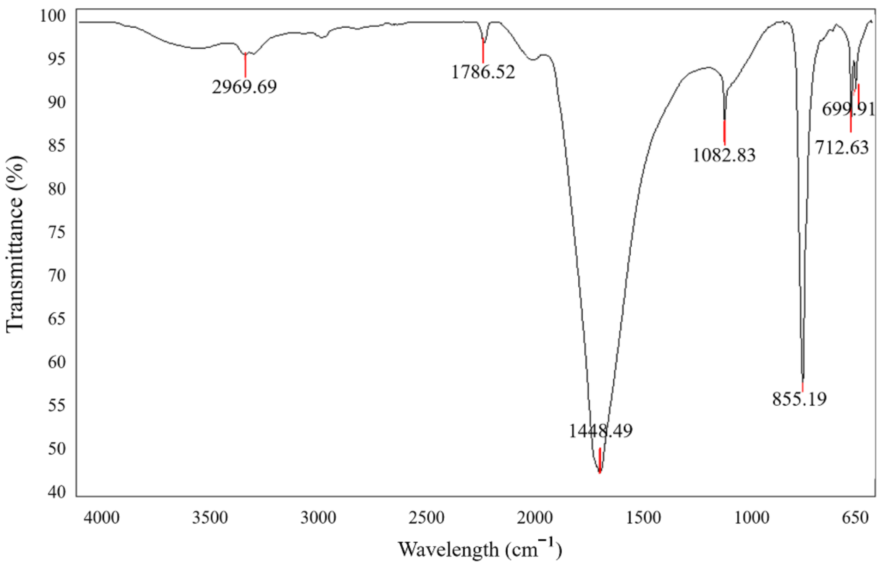

Figure 1. F-TIR spectra of *Eobania vermiculata* shells powder.

X-Ray Diffraction Analysis (XRD)

In order to understand the phase components of chocolate band snail, XRD patterns were conducted. The XRD patterns of shells of the chocolate band snail revealed similarities in crystalline peaks, confirming the existence of the aragonite form of calcium carbonate (Figure 2). The X-ray diffraction data were acquired in steps of 0.015° at scattering angels (2θ) ranging from 5° to 80°. The XRD phase analysis of aragonite, shown in Figure 2, carry high intensity peaks at 2θ = 26.43°, 27.09°, 31.12°, 33.81°, 36.23°, 38.17°, 46.32°, 52.29°, 53.07°, 66.31° with monochromatic Cu-Kα radiation (λ = 1.5406 Å). The XRD pattern (Figure 2) reflected the intense peaks at (26.43°) and (31.12°) planes, providing evidence of the existence of orthorhombic aragonite phase. The XRD parameters of biological aragonite due to the intracrystalline biopolymers, has been confirmed as a widespread phenomenon in chocolate band snail shell (Ren et al., 2009; Li & Zeng, 2012; Parveen et al., 2020).

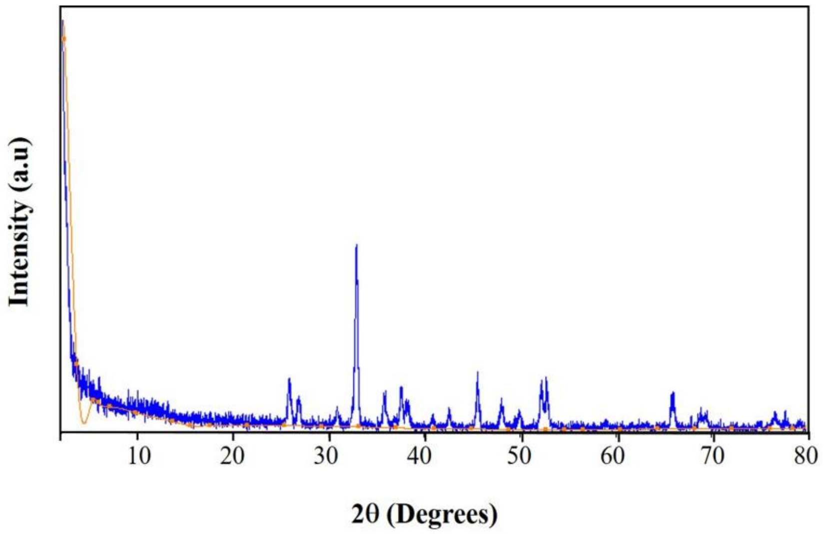

Figure 2. XRD patterns of *Eobania vermiculata* shells powder.

This crystal conformance of aragonite in nacre is frequently build in biological mineralization. This is due to the presence of fibrils and the polypeptide chains that control crystal improvement (Feng et al., 2000).

Scanning Electron Microscope (SEM)

The arrangement of the crystal layers in mollusk shells has two types. The sheet nacre model of Bivalvia and Cephalopoda mimick so called "brick-and-mortar wall"; on the other hand, Gastropoda mimick "columnar stacking" model (Watabe, 1988). Nacre crystallites pile up into columns vertically abreast aligned in Gastropoda. Accordingly, the vertical piling-up of crystals in these shells promotes the formation of columns or pyramids. These shell calcification sequences will represent useful criteria for studies of the phylogeny of Mollusca. In this study, the crystallized structure of *E. vermiculata* is in the form of vertical columns as stated in the literature. Nacre crystals are always aragonitic, laminated, usually presenting sharp edges (Figure 3).

According to the SEM results, the crystal columns are between about 390-155 nm and their edges are sharp looking. Nacre is one of the leading mechanical properties of shell components of mollusks (Currey, 1988). Addadi et al. (2006) reported that the crystals form a stacks along about 500 nm thick, and they form stacks along a fixed spacing of some 30 nm, filled with biological matter. Placing the crystals at regular intervals with the organic matrix creates a strong material with high tensile and bending strength.

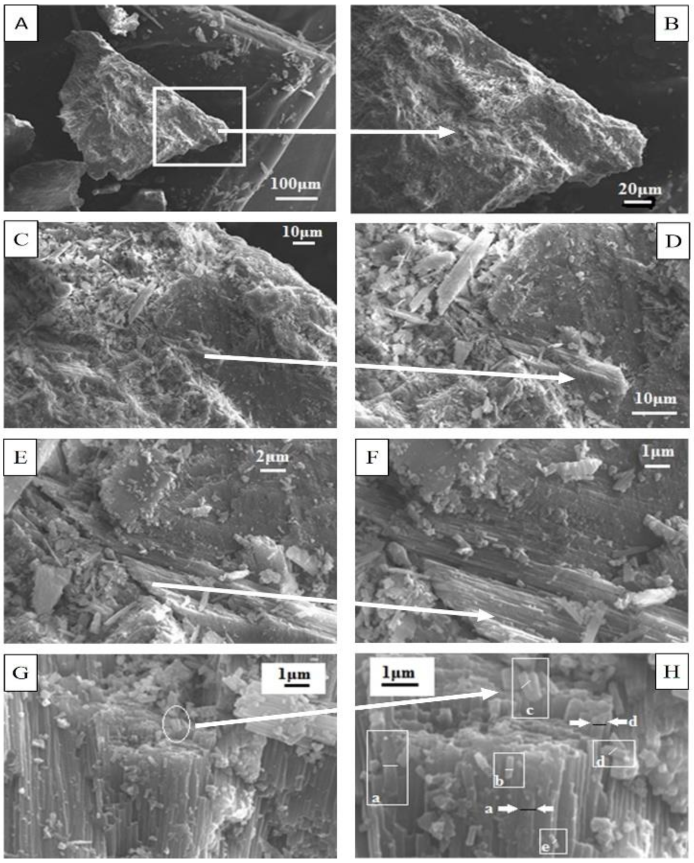

Figure 3. SEM images of crossed lamellar layers occurring in shell of *Eobania vermiculata.* A, B, C, D, E and F the point determined on the *E. vermiculata* shell fragment at 100, 20, 10, 2, 1 micron, respectively. G: Thin prisms from lamellar of *E. vermiculata* at 1 µm (Mag: 20.00 KX). H: Elongate, lath like, thin prisms from acrossed-lamellar layers of *E. vermiculata* at 1 µm (Mag: 40.00 KX): Cross-layered layers are 370 nm, 160 nm, 360 nm, 355 nm, 155 nm thick, respectively.

Conclusion

The results of SEM showed that land snail has species-specific arrangement patterns of calcium carbonate crystals in the diverse layers of shells. Characteristic of Gastropoda shell is the "columnar stacking" model. Accordingly, the vertical piling-up of crystals inside shells promotes the formation of columns. The XRD and FTIR observations revealed the dominance of the aragonite form of the calcium carbonate crystal in the microstructures of each snail shell with the occurrence of different shell surface functional groups.

Species-specific variations in the shell morphology, microstructure, and shell content were prominent for the land snails considered for the study. Nonetheless, the shells of *E. vermiculata* are qualified biological materials that could be used in many fields like bioremediation, biocatalyst, biomedical applications, and a source of lime. Since the shells of the land snails are discarded as waste, subsequent use as a biological material will support the "waste made useful" paradigm in sustainability, both from ecologic and economic perspectives.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

References

Addadi, L., Joester, D., Nudelman, F., & Weiner, S. (2006). Mollusk shell formation: A source of new concepts for understanding biomineralization processes. *Chemistry - A European Journal*, *12*(4), 980-987. https://doi.org/10.1002/chem.200500980

Agbaje, O. B. A., Wirth, R., Morales, L. F. G., Shirai, K., Kosnik, M., Watanabe, T., & Jacob, D. E. (2017). Architecture of crossed-lamellar bivalve shells: The southern giant clam (*Tridacna derasa*, Röding, 1798). *Royal Society Open Science*, *4*(9), 170622. https://doi.org/10.1098/rsos.170622

Anjaneyulu, U., Pattanayak, D. K., & Vijayalakshmi, U. (2015). Snail shell derived natural hydroxyapatite: Effects on NIH-3T3 cells for orthopedic applications. *Materials and Manufacturing Processes*, *31*(2), 206-216. https://doi.org/10.1080/10426914.2015.1070415

Cárdenas, G., Cabrera, G., Taboada, E., & Miranda, S. P. (2004). Chitin characterization by SEM, FTIR, XRD, and ^{13}C cross polarization/mass angle spinning NMR. *Journal of Applied Polymer Science*, *93*(4), 1876-1885. https://doi.org/10.1002/app.20647

Checa, A. (2000). A new model for periostracum and shell formation in Unionidae (Bivalvia, Mollusca). *Tissue and Cell*, *32*(5), 405-416. https://doi.org/10.1054/tice.2000.0129

Currey, J. D. (1988). Shell form and strength. In E. R. Trueman & M. R. Clarke (Eds.), *The Mollusca: Form and function* (pp. 183-210). Academic Press. https://doi.org/10.1016/B978-0-12-751411-6.50015-1

Dauphin, Y. (1999). Infrared spectra and elemental composition in recent biogenic calcites: Relationships between the upsilon 4 band wavelength and Sr and Mg concentrations. *Applied Spectroscopy*, *53*(2), 184-190.

Dauphin, Y., Brunelle, A., Medjoubi, K., Somogyi, A., & Cuif, J. P. (2018). The prismatic layer of Pinna: A showcase of methodological problems and preconceived hypotheses. *Minerals*, *8*(9), 365. https://doi.org/10.3390/min8090365

de Paula, S. M., & Silveira, M. (2009). Studies on molluscan shells: Contributions from microscopic and analytical methods. *Micron*, *40*(7), 669-690. https://doi.org/10.1016/j.micron.2009.05.006

Dedov, I. (1998). Annotated check-list of the Bulgarian terrestrial snails (Mollusca, Gastropoda). *Linzer Biologische Beiträge*, *30*(2), 745-765.

Feng, Q. L., Cui, F. Z., Pu, G., Wang, R .Z., & Li, H. D. (2000). Crystal orientation, toughening mechanisms and a mimic of nacre. *Materials Science and Engineering: C*, *11*(1), 19-25. https://doi.org/10.1016/S0928-4931(00)00138-7

Focher, B., Naggi, A., Torri, G., Cosani, A., & Terbojevich, M. (1992). Structural differences between chitin polymorphs and their precipitates from solutions-Evidence from CP-MAS ^{13}C-NMR, FT-IR and FT-Raman spectroscopy. *Carbohydrate Polymers*, *17*(2), 97-102. https://doi.org/10.1016/0144-8617(92)90101-U

Godan, D. (1979). *Schadschnecken und ihre Bekämpfung*. Ulmer.

Hedegaard, C. (1997). Shell structures of the recent Vetigastropoda. *Journal of Molluscan Studies*, *63*(3), 369-377. https://doi.org/10.1093/mollus/63.3.369

Hossain, A., Bhattacharyya, S. R., & Aditya, G. (2015). Biosorption of cadmium by waste shell dust of fresh water mussel *Lamellidens marginalis*: Implications for metal bioremediation. *ACS Sustainable Chemistry & Engineering*, *3*(1), 1-8. https://doi.org/10.1021/sc500635e

Li, T., & Zeng, K. (2012). Nano-hierarchical structure and electromechanical coupling properties of clamshell. *Journal of Structural Biology*, *180*(1), 73-83. https://doi.org/10.1016/j.jsb.2012.06.004

Lopes-Lima, M., Rocha, A., Gonçalves, F., Andrade, J., & Machado, J. (2010). Microstructural characterization of inner shell layers in the freshwater bivalve *Anodonta cygnea*. *Journal of Shellfish Research*, *29*(4), 969-973. https://doi.org/10.2983/035.029.0431

Lowenstam, H. A., & Weiner, S. (1989). Biomineralization processes. In H. A. Lowenstam & S. Weiner (Eds.), *On biomineralization* (pp. 26-49). Oxford University Press. https://doi.org/10.1093/oso/9780195049770.003.0005

Machado, J., Reis, M. L., Coimbra, J., & Sá, C. (1991). Studies on chitin and calcification in the inner layers of the shell of *Anodonta cygnea*. *Journal of Comparative Physiology B*, *161*(4), 413-418. https://doi.org/10.1007/BF00260802

Marxen, J. C., Becker, W., Finke, D., Hasse, B., & Epple, M. (2003). Early mineralization in *Biomphalaria glabrata*: Microscopic and structural results. *Journal of Molluscan Studies*, *69*(2), 113-121. https://doi.org/10.1093/mollus/69.2.113

Nielsen, C. (2004). Trochophora larvae: Cell-lineages, ciliary bands, and body regions. 1. Annelida and Mollusca. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *302*(1), 35-68. https://doi.org/10.1002/jez.b.20001

Örstan, A., Pearce, T. A., & Welter-Schultes, F. (2005). Land snail diversity in a threatened limestone district near

Istanbul, Turkey. *Animal Biodiversity and Conservation*, *28*(2), 181-188.

Parveen, S., Chakraborty, A., Chanda, D. K., Pramanik, S., Barik, A., & Aditya, G. (2020). Microstructure analysis and chemical and mechanical characterization of the shells of three freshwater snails. *ACS Omega*, *5*(40), 25757-25771. https://doi.org/10.1021/acsomega.0c03064

Rađa, B., Rađa, T., & Šantić, M. (2012). The shell characteristics of land snail *Eobania vermiculata* (Müller, 1774) from Croatia. *The Online Journal of Science and Technology*, *2*(3), 66-70.

Ren, F., Wan, X., Ma, Z., & Su, J. (2009). Study on microstructure and thermodynamics of nacre in mussel shell. *Materials Chemistry and Physics*, *114*(1), 367-370. https://doi.org/10.1016/j.matchemphys.2008.09.036

Romana, L., Thomas, P., Bilas, P., Mansot, J. L., Merrifiels, M., Bercion, Y., & Aranda, D. A. (2013). Use of nanoindentation technique for a better understanding of the fracture toughness of *Strombus gigas* conch shell. *Materials Characterization*, *76,* 55-68. https://doi.org/10.1016/j.matchar.2012.11.010

Santana, P., & Aldana Aranda, D. (2021). Nacre morphology and chemical composition in Atlantic winged oyster *Pteria colymbus* (Röding, 1798). *PeerJ*, *9*, e11527. https://doi.org/10.7717/peerj.11527

Singh, A., & Purohit, K. M. (2011). Chemical synthesis, characterization and bioactivity evaluation of hydroxyapatite prepared from garden snail (*Helix aspersa*). Journal of Bioprocessing & Biotechniques, *1*, 104. https://doi.org/10.4172/2155-9821.1000104

Spann, N., Harper, E. M., & Aldridge, D. C. (2010). The unusual mineral vaterite in shells of the freshwater bivalve *Corbicula fluminea* from the UK. *Naturwissenschaften*, *97*(8), 743-751. https://doi.org/10.1007/s00114-010-0692-9

Suzuki, M., & Nagasawa, H. (2013). Mollusk shell structures and their formation mechanism. *Canadian Journal of Zoology*, *91*(6), 349-366. https://doi.org/10.1139/cjz-2012-0333

Waller, T. R. (1980). *Scanning electron microscopy of shell and mantle in the order Arcoida (Mollusca: Bivalvia)*. Smithsonian Institution Press. https://doi.org/10.5479/si.00810282.313

Wang, S. N., Yan, X. H., Wang, R., Yu, D. H., & Wang, X. X. (2013). A microstructural study of individual nacre tablet of *Pinctada maxima*. *Journal of Structural Biology*, *183*(3), 404-411. https://doi.org/10.1016/j.jsb.2013.07.013

Watabe, N. (1988). Shell structure. In E. R. Trueman & M. R. Clarke (Eds.), *The Mollusca: Form and function* (pp. 69-104). Academic Press. https://doi.org/10.1016/B978-0-12-751411-6.50011-4

Weir, C. E., & Lippincott, E. R. (1961). Infrared studies of aragonite, calcite, and vaterite type structures in the borates, carbonates, and nitrates. *Journal of Research of the National Bureau of Standards-A. Physics and Chemistry*, *65*(3), 173-183. https://doi.org/10.6028%2Fjres.065A.021

Welter-Schultes, F. W., & Williams, M. R. (1999). History, island area and habitat availability determine land snail species richness of Aegean islands. *Journal of Biogeography*, *26*(2), 239-249.

Zhang, G., & Li, X. (2012). Uncovering aragonite nanoparticle self-assembly in nacre-A natural armor. *Crystal Growth & Design*, *12*(9), 4306-4310. https://doi.org/10.1021/cg3010344