ESPE
UNIVERSIDAD DE LAS FUERZAS ARMADAS
INNOVACIÓN PARA LA EXCELENCIA
100 años

REVISTA Ciencia

ISSN 1390-1117 • ISSN E: 2697-343X

VICERRECTORADO DE INVESTIGACIÓN, INNOVACIÓN Y TRANSFERENCIA DE TECNOLOGÍA

VOLUMEN 24, No . 1
Enero- Marzo 2022

REVISTA
Ciencia
ISSN 1390-1117 • ISSN E: 2697-343X

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CIENCIA - QUITO, Volumen 24, No. 1,
Enero-Marzo 2022
ISSN: 1390-1117, ISSN ELECTRÓNICO: 2697-343X
Revista Trimestral- 50 ejemplares

Diagramación IMPREVEL Artes Gráficas
Impresión Imprenta Universitaria ESPE
UNIVERSIDAD DE LAS FUERZAS ARMADAS-ESPE
Distribución gratuita

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Normas de Publicación **16**

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ISSN: 1390-1117, ISSN E: 2697-343X.
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Caracterización morfométrica y estructural de las lapas costeras *Nacella concinna* (Nacellidae) encontradas en la estación antártica ecuatoriana

Morphometric and structural characterization of coastal limpets *Nacella concinna* (Nacellidae) found in ecuadorian antarctic station

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Recibido: Diciembre, 2021
Aceptado: Marzo 2022

Doi: https://doi.org/10.24133/ciencia.v24i1.2700

RESUMEN

Este estudio examina la especie más abundante de moluscos sublitorales en la Estación Antártica Pedro Vicente Maldonado: las lapas *Nacella polaris*. Colectadas durante la XXI expedición, mediante cuadrantes formando un transecto litoral lineal. Las conchas se analizaron mediante morfometría y microscopía electrónica de barrido (SEM). La morfometría de las lapas es variable con un rango de 22.88 ± 53.92 mm. El 85.60% (98/115) de las lapas son de gran tamaño, y se encontraron principalmente en el Cementerio de Ballenas. Las más pequeñas se localizaron sobre todo en la zona del León Dormido, y suponen el 1.36% (2/115) de la muestra. Mediante microscopía electrónica de barrido se observaron características en su borde externo inferior: en forma de estrías transversales, lineales a ligeramente onduladas con espesores definidos, una ultraestructura cristalina en líneas de crecimiento superficiales lineales a ligeramente onduladas, formando un patrón microestructural en las capas externas de las conchas, de columnas de calcita en forma de escalera que soportan las líneas de crecimiento. Los datos morfométricos y ultramicroscópicos relativos de las conchas indican que su arquitectura externa es análoga y que la población muestra examinada se comporta como una población con ligero polimorfismo de tamaño. Paralelamente se analizaron someramente otros Moluscos de diferentes familias de la diversidad litoral en la estación.

Palabras claves: altura, longitud, microscopía electrónica de barrido, morfometría, patrón ultraestructural, sublitoral.

ABSTRACT

This study examines the most abundant species of sublittoral mollusks at the Pedro Vicente Maldonado Antarctic Station: the *Nacella Polaris* limpets. It was collected during the XXI expedition through quadrants forming a coastal transect. The shells were analyzed using morphometry and scanning electron microscopy (SEM). The morphometry of limpets was variable, with a range of 22.88 ± 53.92

mm. The 85.60% (98/115) of the limpets were large, and these were found mainly in the Whale Cemetery. On the other hand, the smaller ones were found primarily in the Sleeping Lion area and accounted for 1.36% (2/115) of the sample. With the use of the scanning electron microscopy, characteristics were observed on its lower outer edge: in the form of transverse, linear to slightly wavy striations with defined thicknesses, a crystalline ultrastructure in linear to slightly wavy surface growth lines, forming a microstructural pattern in the outer layers of the shells, of calcite columns in the form of a ladder that supports the growth lines. The morphometric and relative ultramicroscopic data from the shells indicate that their exterior architecture is analogous and that the sample population examined behaves as a population with small size polymorphism. At the same time, other mollusks of different families of the coastal diversity in the station were briefly analyzed.

Keywords: height, length, morphometry, scanning electron microscopy, sublittoral, ultrastructural pattern.

1. INTRODUCTION

Antarctica is an important natural laboratory, unique from the environmental and biological point of view. As a result, Antarctica plays a crucial role in understanding the effects of global warming, climatic influences, and ecological and biogeographic interrelationships that link the continent with the rest of the world (Lurcock, Pontus, & Florindo, 2017). In addition, due to its territorial remoteness and the fact that the area is still in a pristine state, it plays a symbolic role worldwide (Murphy, et al. (2012).

It is a source of resources that have not yet been exploited. Antarctica holds a strategic value among other integrated environmental factors due to its position and proximity to Pacific, Atlantic, and Indian interoceanic crossings (Cardenas-Calle, 2009) and millions of years of biogeographic isolation.

Countries with national and international geopolitical interest in the scientific exploration of the locality, such as Argentina and Chile, conducted systematic investigations at their respective Antarctic stations on Antarctic intertidal fauna. These investigations are conducted under Ecuador's Antarctic Research Program in the Pedro Vicente Maldonado (PVM) scientific station. The countries presented summaries of these investigations at the VI Argentine Symposium and the III Latin American Symposium on Antarctic Research, 2007. In addition, these investigations have been conducted under Ecuador's Antarctic Research Program at the Pedro Vicente Maldonado (PVM) scientific station.

As for Antarctic marine mollusks, studies on intertidal fauna have been conducted. These studies also address mollusks found in the areas of the Pedro Vicente Maldonado scientific station (Calderón & Jaramillo, 1998; Cárdenas et al., 2013; Castillo & Rozbaczylo, 1985; Cruz, 1990), as well as other benthic communities (Torres & Calderón, 2013) of macroinvertebrates, macroalgae, heavy metals, and hydrocarbons encountered in the mesolitoral zone in Ensenada Guayaquil and Punta Fort Williams during the austral summer in 2008 (Cardenas-Calle, 2009). Antarctic coastal limpets of the *Nacellidae* family were also found in the localities mentioned above (Calderón & Jaramillo, 1998; Cárdenas et al., 2013; Castillo & Rozbaczylo, 1985; Cruz, 1990)

Nacellidae family is well studied as it is the representative and dominant family in the ice-free areas of the Pedro Vicente Maldonado station, related areas of the Greenwich Islands, and the South Shetland Islands. In Antarctica, they are an essential group ecologically, economically, and scientifically because of their diversity and abundance (Torres & Calderón, 2013). Another thing that makes them significant is that their natural predators are primarily waterfowls. Both spatially and evolutionarily, these limpets have biogeographic affinity and taxonomic and ecological interrelationship with the limpets found in the extreme points of southern South America (Valdovinos & Ruth, 2005).

The antarctic plays a vital role in the Antarctic food chains. Therefore, various pollutants or stressors (e.g., hydrocarbons or others) that affect limpets are crucial. These pollutants and stressors cause different alterations in

limpets, mainly coastal limpets found in rocky areas in Ecuador (Gentile et al., 2007; González & Puntarulo, 2007). These species are essential for the ecosystems, the Antarctic territory, and potential research on marine resources and current environmental interaction.

This study aims to determine the morphometric and structural characteristics of the *Nacella concinna* species. The study focuses on Antarctic malacological biodiversity, Ecuadorian station Pedro Vicente Maldonado, and the Antarctic Expedition XXII (2018). Furthermore, the samples of limpets and other mollusks taken during the expeditions of 2012 (XVI), 2013 (XVII), 2017 (XXI) are also included. The samples were taken at the same sampling points: Sleeping Lion, Whale Graveyard, Prat, and Quito Glacier. This study, along with the aspects related to the PVM station, is one of the DIGEIM-FUNDEMAR biodiversity objectives (General Directorate of Maritime Interests - Foundation for Maritime Fluvial and Lacustrine Development). Furthermore, it serves future biodiversity research, the Mollusks Framework Project of Ecuador, which has been carried out for several years to comprehensively describe the terrestrial and fluvial malacofauna found in continental, insular (Correoso, 2002, 2008),and Antarctic Ecuador

2. MATERIALS AND METHODS

The field phase was conducted in the coastal intertidal area of the Supralittoral Mesolitoral and Infralittoral zone, ice-free regions of the northern parts of Greenwich Island (an approximate surface of the island is 1300 km2 (2% of the site). Rocky substrate can be observed in summer), Punta Ambato, and the Pedro Vicente Maldonado PVM scientific station.

Transepts were made parallel to the coastline, laid in 100-m quadrants, and joined together while considering the parameters of the habitat, low-tide periods, and rocks in the intertidal zone, where habitat variations were found. The live and dead mollusks were collected manually in the intertidal zone and in the areas where habitats (rocky substrates or others) with differences and discontinuities, variations of species/habitat, and biogeographic limits of the intertidal species were found. Individuals of maximum and minimum sizes ≥ 0.5 cm were randomly chosen. According to the previous abundance assessment, the average number of selected visuals ranges from 15 to 40. The task was executed with one researcher and no more than three researchers. It included lifting rocks and avoiding causing environmental damage by returning the rocks to their original position. The samples were taken in ecological localities or environments. The localities were equidistant and distributed to ultimately form an approximate transect line (Correoso, 2004) (C. Osorio, Peña, Ramajo, & Garcelon, 2006). A base map of the collection sites at the PVM station is included in this paper (figure 1).

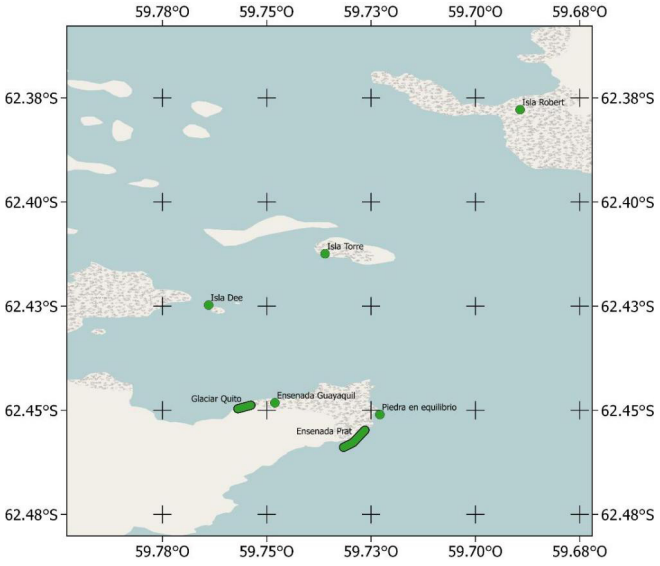

Figure 1 Map of the PVM station

The coordinates of the sampling sites on the map were changed into latitude and longitude coordinates since the initial UTM plane coordinates obtained at high latitudes had errors, as shown in table 1. Also, the GPS signal is distorted in Antarctica.

Table 1.
Coordinates of sampling sites

Site N°	Sampling sites	Latitude	Longitude	Altitude (MSL)	H_2O Temp. [°C]	Ambient Temp. [°C]
1	Prat Base	62º27'25,363''S	59º43'51,837''W	1	1	-4
2	Prat Base	62º27'23,291''S	59º43'46,509''W	2	1	-4
3	Prat Base	62º27'17,667''S	59º43'41,477''W	2	1	-4
4	The Balancing Stone	62º26'59,020''S	59º43'24,606''W	2	1	-6
5	Quito Glacier	62º26'54,758''S	59º45'17,086''W	1	1	-2
6	Quito Glacier	62º26'58,922''S	59º45'23,769''W	1	1	-2
7	Guayaquil Base	62º26'52,021''S	59º45'4,017''W	-1	2	-2
8	Prat Base	62º27'14,411''S	59º43'36,150''W	3	2	-2
9	Tower Island	62º24'44,709''S	59º44'9,643''W	3	-----	------
10	Dee Island	62º25'32,134''S	59º45'47,438''W	1	1	-3
11	Robert Island, Risopatrón Base	62º22'40,176''S	59º41'21,455''W	1	1	-3
12	Whale Grave-yard	62º26'35''S	59º43'32''W	2	-----	-2
14	The Sleeping Lion	62º26'40,372''S	59º43'54,501''W	10	-----	-2
15	The Sleeping Lion	62º26'37,116''S	59º43'42,661''W	10	-----	-2
16	The Sleeping Lion	62º26'39,485''S	59º43'25,494''W	10	-----	-2
17	Rio Culebra	62º26'53,805''S	59º43'47,454''W	7	-----	-2

2.1. STATISTICAL ANALYSIS

The following statistical software evaluated the morphometric correlation between length, apex heigh, and the maximum diameter of a population of 115 limpets from different geographical locations from the PVM Station. The statistical package Minitab 18 was used for the Anderson-Darling normality test, and the correlation analysis was conducted using critical values (p-value and a-square). Furthermore, JMP® 13.2.1 (Statistical Discovery. ™From SAS) software was also used. The aim was to determine if there are significant morphometric variations in the population of *Nacella concinna* found in the different localities of the area. In addition, the variables of length were correlated with multivalent methods and comparisons between numerical/ordinary size categories encountered at the sampled points. However, it is essential to note that the sampling was not performed on all the ice-free zones in the Station area and on Dee Island.

2.2. MORPHOMETRIC LABORATORY ANALYSIS

Morphometric/morphological measurements were taken to assess the species taxonomically and confirm the malacological biodiversity of the area. The sizes of *Nacella concinna* limpets were taken with a MESS-SCHIEBR

digital caliper. The tool's precision is 0.02mm. The limpets were observed under a stereoscopic microscope (range 20-150x). The mollusks were measured and photographed in batches. The process was conducted in the laboratories of the UDLA University by sampling locations, protocols for each taxon, and fundamental references.

2.3. MICROSCOPIC LABORATORY ANALYSIS

The ultramicroscopic analysis of the shells was conducted to define the external surface structure of limpets and other mollusks found in the sampling area. The study was conducted at the Center of Nanoscience and Nanotechnology (CENCINAT) of ESPE University using a scanning electron microscope (SEM). Initially, the sectioned samples were adhered (TED PELLA INC.) to the support of the scanning electron microscope with conductive double-sided carbon tape. Next, they were covered with a layer of gold (99.99% pure) 20 nm thick. Finally, the layers of gold were applied using an evaporator (sputtering evaporator Quorum Q150R ES). The samples were then observed under a scanning electron microscope (FEG-SEM, TESCAN model MIRA 3) using the backscattered electron detector at different magnifications, from a panoramic view to almost 2000X.

3. RESULTS

The morphometric correlation between length, apex heigh, and the maximum diameter of a population of 115 limpets were evaluated according to different geographical locations from the PVM Station. The Anderson-Darling test was adjusted for the taxonomic group (*Nacella* spp.). The variables adjusted are Total Length (mm), Maximum Diameter (mm), and Apex Height (mm). All the parameters offered by the software were taken into consideration, and the significance of the least standardized and atypical values, such as mean, standard deviation, A-squared, and p-value (Anderson-Darling), was highlighted. As for the analysis and measurements of the sample, the standard deviation of the total length is 6.94, and it is greater than the value of the maximum diameter, which is 5.16. Apex height is 4.08. (See Figures 2 to 4) The confidence interval is 95%. The mean is correlated with p and A-squared values (See Figures 2 to 4). It is essential to highlight that the total length is variable in the *N. concinna* population, and its range is 22.88 ± 53.92 mm, depending on the population's age distribution. Based on the population's morphology (Total Length, Maximum Diameter, and Apex Height), a more significant concentration of giant limpets (L (G); L (XG)) was found in the Whale Graveyard area, and they account for 85.60% of the sample.

On the other hand, smaller Limpets (L (P)) are predominantly found in the Sleeping Lion area, representing 1.36% of the sample. The dispersion of the *Nacella* population based on their morphology (Total Length (mm), Maximum Diameter (mm), Apex Height (mm)) is shown in a boxplot (See figure 5).

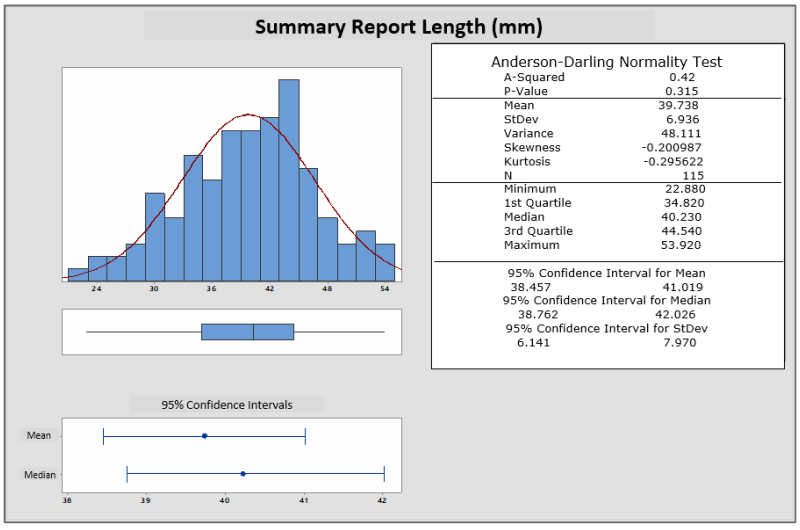

Figure 2 Length Summary Report (Source: Minitab 18)

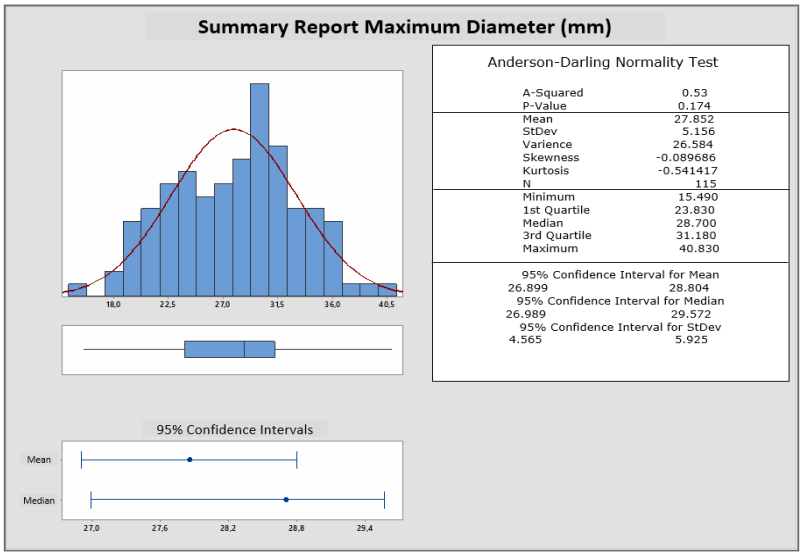

Figure 3 Maximum Diameter Summary Report (Source: Minitab 18)

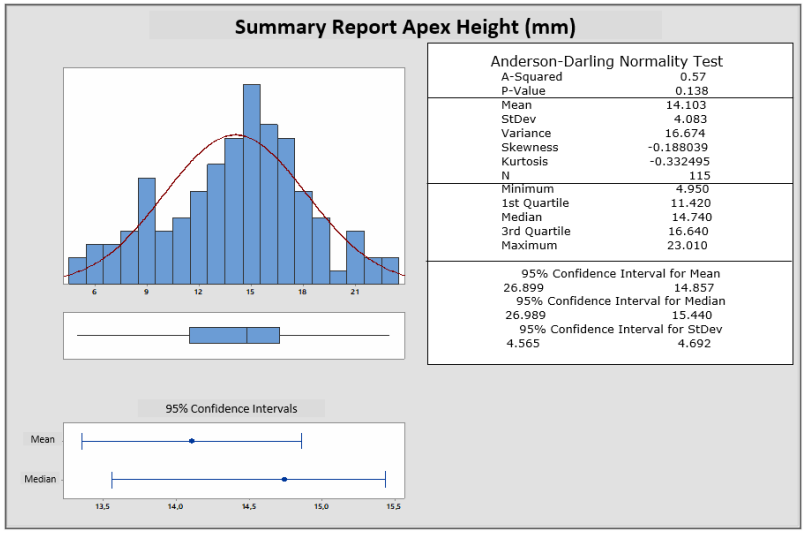

Figure 4 Apex Height Summary Report (Source: Minitab 18)

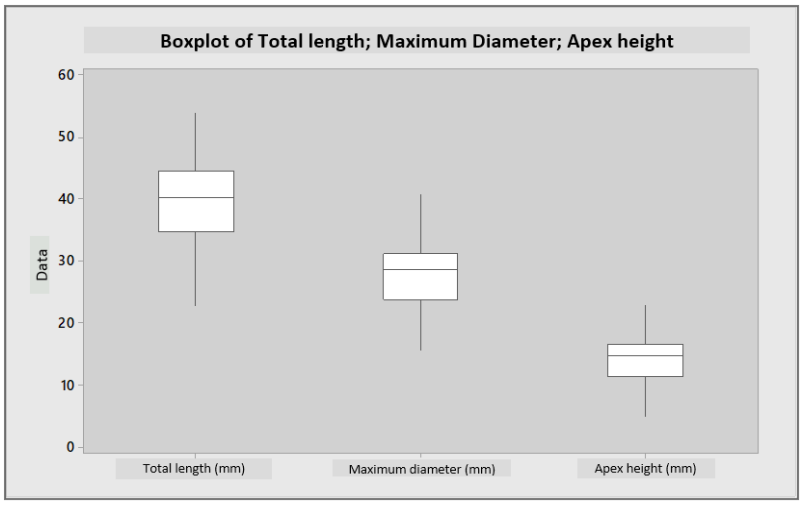

Figure 5 Boxplot – Length, Maximum Diameter, Apex Height (Source: Minitab 18)

Revista CIENCIA 2022 • ISSN: 1390-1117 • ISSN ELECTRONICO: 2697-343X

The samples of *Nacella concinna* were taken from different localities. However, when comparing their behavior, we can conclude that they all function as the same population or one single species. Significant population discrepancies between the sampled points were not observed. Nevertheless, it should be considered that the sampling did not cover all the ice-free zones in the Station area and on Dee Island. Statistics are based on the morphometric data of the shells of *N. concinna*; see figures 6 and 7.

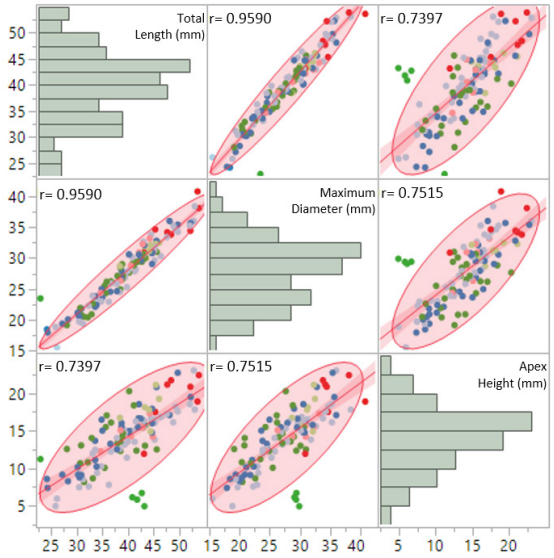

Figure 6 Source: JMP® 13.2.1 (Statistical Discovery. ™ From SAS). Multivariate analysis method applied to Numerical categories: Total Length (mm), Maximum Diameter (mm), Apex Height (mm).

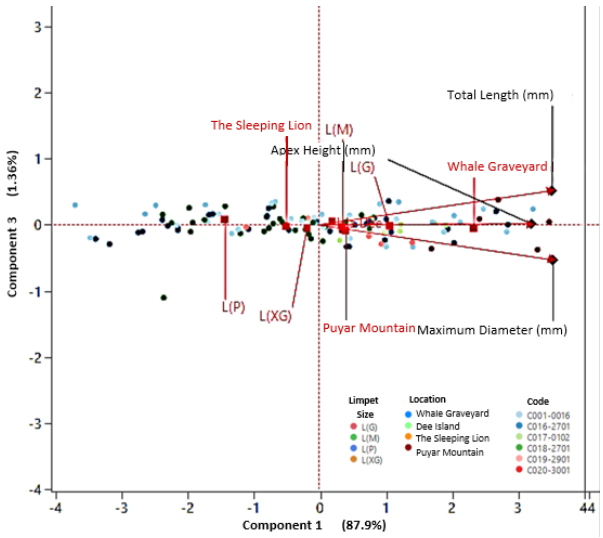

Figure 7 Source: JMP® 13.2.1 (Statistical Discovery. ™ From SAS.
Multivariate analysis method. The relationship between numerical and ordinal categories.
Numerical: Total Length (mm), Maximum Diameter (mm), Apex Height (mm); Ordinal: Location vs Limpet Size.

3.1. OBSERVATIONS OBTAINED WITH SCANNING ELECTRON MICROSCOPE (SEM)

Electron microphotographs of the limpets *Nacella concinna* from the PVM
The station was observed. The electron microphotographs focus on the lower shell right region and left edge. A panoramic magnification from 200 to 2000X was used to obtain fine details. The following characteristics were observed. Initial, the external transverse superficial striations have slightly wavy linear shapes and fairly well-defined

thicknesses. These characteristics are by shell growth. Specific morphological differences, such as more defined lines and diffuse or darker colors, were observed, and these differences depend on the season (Antarctic summer and winter). Under magnifications above 500 and up to 1000X, we distinguished the crystalline ultrastructure of the shells, which is characterized by simple growth lines in the aragonitic layer and slightly wavy linear sculpture. This can be considered an element typical for this species, recognizable under the magnification from 200x to 1000x. As the magnification increases, the presence of small vertical calcite pillars that appear architecturally to support or sustain the growth lines stands out. This pattern can be described as similar to a lying staircase (figure 8).

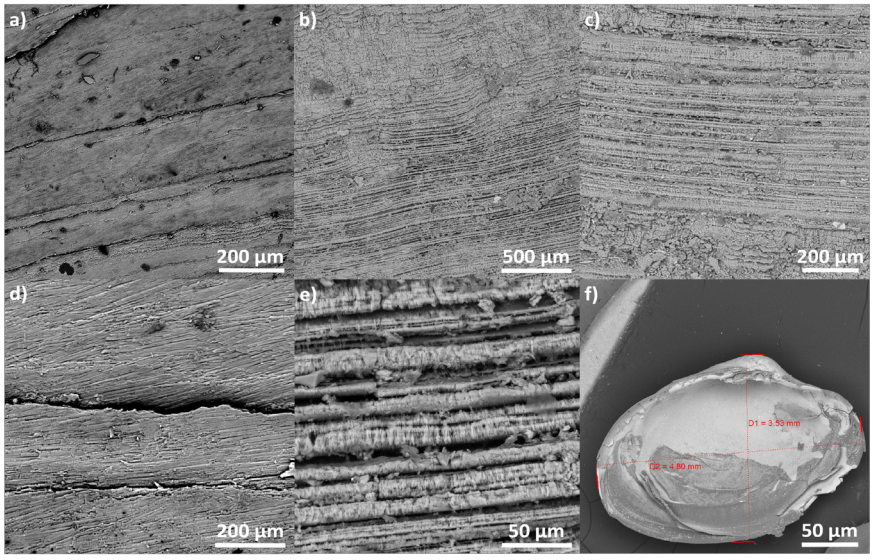

Figure 8 a) Ultrastructure of Nacella concinna. PEVIMA station on Dee Island. Delicate lines of surface growth identifiable at 200X magnification, b) Ultrastructure of *Nacella concinna*, c) Ultrastructure of *Nacella concinna*, d) Nacella convincing shell ultrastructure. Areas of slower growth separate narrow linear to slightly wavy superficial growth lines—a recognizable pattern of this species and different from the Ecuadorian species. Identifiable at 200x magnification, e) Ultrastructure of *Nacella concinna,* f) Ultrastructure and measurements (4.8 x 3.5mm) analyzed with SEM. Micro clams *Kidderia sp.*

3.2. MALACOLOGICAL BIODIVERSITY

The samples obtained at the PVM Station during the XXI expedition show the preliminary diversity of mollusks are taxonomically composed of limpets *Nacella polaris* (Hombron & Jaquinot, 1841) or *Nacella polaris concinna* (Strebel, 1908), the most abundant species and dominant intertidal mollusks (No. sampled approx. 150X). The majority of the samples are dead shells, as indicated by previous authors (Cruz, 1990) (See Photographs, second row), small clams *Kidderia sp* (Bivalvia); they are abundant in rocky areas with pools of water. (See figure 8, refer to f).They are a single specimen of clam X. Mollusks from other families found in rocky coastal regions of the station were collected for this study as well. The number of these samples is scarce. The sampled species belong to the *Buccinidae, Littorinidae, and Olividae*, found in the mesolitoral and infralittoral zones, see table 2.

There is a total of six (6) families and eight (8) species (morpho-species) pending more precise taxonomic determination. The taxonomically disaggregated samples are: six (6) families, four (4) gastropods, including the well-known limpets *Nacella*, and two (2) Bivalvia mollusks were found. These constitute eight (8) preliminary morpho-species found in the coastal area (see annexed digital images). Empirically speaking, these figures are still low compared to similar projects with a higher number of samples and more in-depth sampling, such as those carried out on Greenwich Island by INOCAR, PEVIMA, and Chilean researchers (Torres & Tania Calderón, 2003); (Valdovinos & Ruth, 2005). Therefore, the diversity of intertidal mollusks sampled from the station is still insufficient,

and a more significant number of more specific samplings is required. However, this small and significant sublittoral sample allows to contextualize the *N concigna* limpets within the diversity of mollusks of the station in general. The diversity that is denoted is similar to other adjacent Antarctic localities (Warwick & Laybourn-Perry, 2008), (Torres & Calderón, 2006; Valdovinos & Ruth, 2005).

Table 2.

Table of primary taxonomy and relative abundance in biodiversity transects at the station.

Taxonomy/ Class	Taxonomy/ Family	Species/Morphotypes	Relative abundance
Gastropods	Nacellidae	*Nacella polaris concinna*	Abundant
Gastropods	Buccinidae	*Morphospecies 1*	Rare
Gastropods	Buccinidae	*Morphospecies 2*	Rare
Gastropods	Littorinidae	*Littorina*	Rare
Gastropods	Olividae	*Morphospecies*	Rare
Bivalvia	Heterodonta Veneroida Cyamidae	*Kidderia sp*	Common
Bivalvia	Heterodonta Veneroida	*Morphospecies 2*	Rare (incomplete adult specimen)

4. DISCUSSION

It is considered that the external morphology of the shells is the fundamental taxonomic aspect for the species of mollusks and limpets, e.g., *Nacella polaris* and *Nacella concinna.* However, the morphometry of this and other species is somewhat variable, as indicated by (Valdovinos & Ruth, 2005). Therefore, morphometric correlation and ultramicroscopic characterization were used in this study rather than genetic and molecular validation analysis methods to avoid taxonomic confusion (González-Wevar, et al ; 2018). Unfortunately, they are currently not available within our reach but will be irreplaceable in the future.

The samples were taken from different localities, but we can conclude that they all function as the same population or one single species when comparing their behavior. Significant population discrepancies between the sampled points were not observed. Nevertheless, the sampling did not cover all the ice-free zones in the Station area and on Dee Island.

Most of the *Nacella concinna* limpets found at the PMV station present a normal correlation between length/ diameter/height and the total length. Their total length is variable, and its range is 22,88 + - 53.92 mm. The mean sizes are larger than in previous studies, such as 28 to 37 mm reported by Torres & Calderón (2006), 37 mm reported by (Cruz, 1990), and other researchers who in previous studies reported finding 28 to 32.30 mm limpets on Robert Island (Castillo & Rozbaczylo, 1985). These small population discrepancies in our study are because we did not segregate dead shells from alive ones at the different sampling sites.

The data show few anomalies in the three variables, length, diameter, height. The only animals found were already excepted due to the average growth with age. These results agree with previous studies (Cruz, 1990; Torres & Calderón, 2006). However, these studies used mostly shell length, numerical correlations with other dimensions were not established. It is confirmed that the largest limpets, in terms of variables Total Length, Maximum Diameter, and Apex Height, are primarily found in the Whale Cemetery area. They account for 85.60% of the total number

of samples found in the room with abundant dead shells. The height of the site (+ 2 MSL) above the tidal line and the number of remains indicate that this is a feeding area and a place where different animals that ingest Limpet's dump waste, such as kelp gulls (*Larus dominicanus*). The site with smaller limpets L (P) is in the Sleeping Lion area (+2 MSL), accounting for 36% of the samples. Feeders and "feeding dumpsters" were also found in this area. These extreme size points also confirm the ecological and trophic role that *N. concinna* has for seabirds, mainly gulls in the station area (Castillo et al., 2017). This also corroborates a suggestion made by some authors, such as (Guaiquil, Leppe, Rojas, & Canales, 2017). There are several types of valve morphology in the littoral or sub-littoral zone where they live.

The observations of the shells' structure and architecture with the SEM Scanning Electron Microscope, specifically the lower right/left edges of the *Nacella concinna* limpets, show that the observed specimens correspond to each other of their crystalline ultrastructure and the pattern of their transverse striations. Transverse striations were linear to slightly wavy and with defined thickness. Under the magnification of 200, 500 to almost 1,000X, lines of superficial growth in the aragonitic layer and linear to slightly wavy forms were observed. These augmentations are highlighted by the presence of small calcite pillars that support the growth lines, and their shape is remarkably similar to a ladder. The electron microphotographs from 200-1000x allowed us to document the calcite columns that support the ladder-shaped growth lines, sculpture, or recognizable patterns typical for *Nacella concinna* limpets found in the station area (See figure 8, refer to c, d, e). This data can also be used for comparison with related species from Antarctica (C. Osorio et al., 2006) (C. Osorio et al., 2006) (Valdovinos & Ruth, 2005) in future and more in-depth studies.

The limpets sampled from the feeding sites were not samples of limpets in their typical habitat. Hence, to compare and avoid statistical and morphological bias, samples were taken from another area far from the station: Robert Island. The sampling on this island, a remote location from Greenwich Island, showed that the size of *Nacella concinna* found in that area is almost 25% larger than those observed in the study area. Nevertheless, significant differences in shape or color were not observed.

As for the shell samples of *Nacella concinna* from the station at **Rissopatron**, far north of PVMA (Robert Island). This morphological aspect is related to the more significant increase in the size of these limpets and the geographical location, which is further north of the Ecuadorian Antarctic station. Several observations showed relatations between Patagonian species located biogeographically further north (C. Osorio et al., 2006), while other related species are found in a small size polymorphism and external microstructural pattern. This pattern observed in a few specimens is slightly different from the one found in species from Ecuadorian Antarctic waters, and the design is differentiable at + 200x. However, the observed samples are not enough to confirm that this is another different species. A more detailed study of the anatomy and genetics of the shells in the future would allow us to establish whether they are other species or whether these are just phenotypic changes generated by different environmental and latitudinal conditions on these islands related to their biogeographic distribution (Cárdenas et al., 2013; C. Osorio et al., 2006; Valdovinos & Ruth, 2005).

5. CONCLUSIONS

It is essential to highlight that the total length is variable in the *N. concinna* population, and its range is 22.88 ± 53.92 mm, depending on the population's age distribution. Based on the population's morphology (Total Length, Maximum Diameter, and Apex Height), the more significant concentration of larger limpets (L (G); L (XG)) was found in the Whale Graveyard area, and they account for 85.60% of the sample. On the other hand, smaller Limpets (L (P)) are predominantly found in the Sleeping Lion area, representing 1.36% of the model.

Considering all the sampling lots and localities, we can conclude that all samples behave as the same population. Significant population discrepancies between the sampled points were not observed. Nevertheless, it should be

noted that the sampling does not cover all the ice-free zones in the Station area and on Dee Island. Comparatively speaking, the shells from Dee Island and other sampled localities and transepts around the station are similar in their external microscopic architecture pattern and match limpets' general morphology and morphometry. This shows that, at least in the sampled sites, there is only one species of limpets – *N. concinna*.

A different contrast or pattern is found in the shell samples from the Rissopatron comparison station. This morphological characteristic is related to the more significant increase in the size of these limpets and the geographical location, which is further north of the Ecuadorian Antarctic station. Although the observed samples are not sufficient to confirm that this is another species, this does denote a small size polymorphism and external microstructural pattern of the shells that should be considered.

The SEM ultramicroscopic observations determined the external morphology structural of the shells, characterized by fine lines of growth with a slightly wavy linear sculpture. The ultrastructure has the presence of vertical pillars that architecturally seem to support the growth lines in the form of a pattern similar to a staircase, which allows to characterize the species *N concinna*, parallel to the morphological and morphometric features analyzed.

The studies of the malacological biodiversity act as bioindicator species from Antarctic stations, such as *N. concinna*. This specie allows us to compare the malacological biodiversity encountered at the sampling points in the station area, their limnological relationship with different mollusks.

6. ACKNOWLEDGMENTS

To the logistical personnel of the National Navy, who supported the fieldwork and provided security to the Antarctic scientific personnel. A FUNDEMAR represented by its director Mr. Verónica Masson; to the University laboratory staff (UDLA) to the experts in electron microscopy Center of Nanoscience and Nanotechnology (CENCINAT) of the ESPE university; especially to its director Dr. Alexis Debut, for his professionalism and friendship. To the IGM for the maps of the scientific station. To the Engineers Álvarez, Mugliza, Jhon Panimboza for helping with geographic/statistical issues or other initial research. Special thanks to our colleague Dr. Maritza Cárdenas, antarctic researcher (ESPOL, RIEAE), to Dr. Marcela Coello (UCE) for her support for her correct suggestions. To the volunteer and anonymous reviewers of this study. Thank you for the proper/logistical support from DIGEIN, INAE, and related. The principal author wishes to indicate that he conducted this research without financial support, with only the essential use of laboratories and the goodwill of the teaching / technical staff of the universities involved.

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RESUMEN

En esta sección, se debe describir claramente y en forma sintetizada la naturaleza y objetivo del trabajo, el método utilizado, los resultados obtenidos, y las principales conclusiones e implicaciones del estudio. El resumen puede ser escrito en un máximo de 200 palabras.

En esta sección, se debe describir claramente y en forma sintetizada la naturaleza y objetivo del trabajo, el método utilizado.

Palabras claves:

Deben ser incluidas en orden alfabético y separadas por comas. Se debe incluir un máximo de ocho palabras claves.

ABSTRACT

This section describes the nature and goals of the study, the methodologic approach, the main results, conclusions, and the main implications. The abstract must be clearly and concisely detailed. The abstract has a maximum of 200 words.

This section describes the nature and goals of the study, the methodologic approach, the main results, conclusions, and the main implications.

Keywords:

The keywords must be sorted alphabetically and separated by commas. A maximum of eight keywords is allowed.

1. INTRODUCCIÓN

Escriba su texto en Arial de 10 Pts, espacio simple. No utilice el doble espaciamiento. Todos los párrafos deberán iniciar con una sangría de 1.25 cm en el primer renglón y justificados. Por favor deje un espacio en blanco entre párrafos. Se describen los fundamentos del estudio, y principalmente, el estado del arte del tema tratado. Se mencionan los estudios previos pertinentes que se hayan realizado utilizando citas en el texto.

Es importante que se indiquen las razones que han motivado la investigación y los objetivos que se pretende alcanzar con el tema.

1.1. SUBAPARTADOS

Todas las referencias bibliográficas deben ser citadas dentro del artículo. Se utiliza el formato (Apellido, año de publicación); por ejemplo: “Para realizar la caracterización reológica de la mezcla asfalto-polvo de llanta se utilizó los parámetros descritos en (Villacís, 2013)”.

2. METODOLOGÍA

Este apartado puede cambiar su título, lo cual dependerá de la investigación que se haya desarrollado.

En caso de que el estudio posea una metodología de experimentación, o pasos que se hayan seguido para llegar a los resultados, serán detallados en esta sección. Se debe incluir los instrumentos de investigación empleados, los datos que fueron tratados y cómo fueron analizados.

El lenguaje de este y los consiguientes apartados debe ser técnico - científico, de acuerdo a la particularidad del trabajo.

En el texto del artículo se debe hacer referencia a las figuras, tablas y ecuaciones, como se indica en las secciones 2.1, 2.2, y 2.3.

Si las figuras o tablas no son inéditas, es obligatorio indicar su fuente.

2.1. REFERENCIA A FIGURAS

Cada figura debe estar mencionada en el texto; por ejemplo: “En la Figura 1 se presenta la microscopía de las fibras desarrolladas con la técnica de electrohilado”.

Las figuras deben ser claras y legibles, independientemente de que la impresión se realice en blanco y negro. Además, deben tener su respectiva leyenda y numeración arábiga secuencial en la parte inferior, y estar centradas en el texto. Deberán estar incorporados en el texto de forma ordenada. En el caso de figuras (imágenes, fotografías y gráficos) deberán ser integrados en el texto en formato JPEG o TIFF, mínimo 1024x758 pixeles o 4 Megabytes (MB), numerados según el orden de aparición en el texto.

2.2. REFERENCIA A TABLAS

La numeración de las tablas sigue las mismas directrices que la numeración de las figuras, con la diferencia que la leyenda se coloca en la parte superior de la tabla. No se debe dividir ni cortar la tabla en diferentes páginas. Se debe hacer referencia a la tabla en el texto; por ejemplo: La Tabla 1 muestra las características del equipo utilizado para los ensayos reológicos.

Los títulos de la figura y de las tablas deben ser en Arial de 10 Pts. Use mayúsculas sólo en la primer palabra de cada título de las Figuras y de las Tablas.

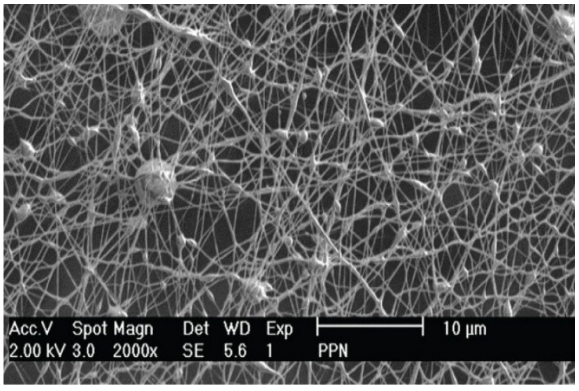

Figura 1. Microscopía de las fibras de polipirrol/óxido de polietileno/nylon 6 (Olvera, Aguilar, & Kryshtab, 2013).

Tabla 1.
Características del Reómetro Discovery HR-2

Elemento	*Características*
Tipo de rodamiento	Magnético
Diseño del motor	Copa de arrastre
Torque mínimo	2 nN.m
Torque máximo	200 mN.m
Frecuencia máxima	100 Hz

2.3. REFERENCIA A ECUACIONES

Todas las variables de una tabla o ecuación deben ser descritas en el texto. Las ecuaciones se escriben en la mitad del texto, con el editor de ecuaciones y van numeradas a la derecha entre paréntesis. No debe ir espacio entre la abertura del paréntesis y el número de identificación de la ecuación. Ejemplo: La ecuación (1) describe el fenómeno de continuidad (Mott, 1996).

$$\nabla.(u)=0 \qquad (1)$$

Donde $\mathbf{u}$ es el vector velocidad.

2.4. RECOMENDACIONES GENERALES

El texto debe redactarse en tercera persona, no utilizar lenguaje informal, procurando escribir con palabras sencillas y claras. Para palabras en otro idioma, se debe utilizar letra itálica; por ejemplo: El fluido tiene un comportamiento pseudoplástico o *shear-thickenning*.

Cuando se definan siglas, es importante, la primera vez que aparecen en el texto, colocar la definición y luego del paréntesis las siglas. Ejemplo: *American Society of Mechanical Engineers* (ASME). Después de ello, se recomienda utilizar únicamente las siglas.

Es mandatorio utilizar magnitudes del Sistema Internacional de Unidades de Medida.

3. RESULTADOS

En la sección resultados, se establecen las relaciones entre los datos obtenidos, el problema de investigación, el método y el soporte teórico de la revisión de la literatura. Los resultados deben presentarse siguiendo la secuencia lógica de presentación de la información. Se presentan únicamente los resultados relevantes de forma sintetizada en tablas, figuras, etc.

4. DISCUSIÓN

Se presenta la interpretación de los resultados en un contexto más amplio y en relación de la literatura existente del estudio específico. Por ejemplo, hacer referencia a los objetivos inicialmente planteados, así como a su eventual aplicación y los trabajos futuros que se podría realizar.

5. CONCLUSIONES

Este último párrafo presenta las conclusiones más significativas. Las conclusiones son generalizaciones derivadas de los resultados y discusión. Responden a los objetivos del estudio y están justificadas por los datos presentados.

6. AGRADECIMIENTOS

Se hace mención a las contribuciones del estudio, tales como soporte técnico, contribución crítica al manuscrito. Se coloca las fuentes de financiamiento del esxqtudio.

7. REFERENCIAS

Las referencias deben colocarse en orden alfabético, siguiendo las normas APA. Se tienen dos casos, el primero que hacer referencia a libros y el segundo a artículos.

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REVISTA Ciencia

ISSN 1390-1117 • ISSN E: 2697-343X

CIENCIA - QUITO, Volumen 24, No. 1, Enero-Marzo 2022
ISSN: 1390-1117, ISSN E: 2697-343X.
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