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Identification of tardigrades from the Half Moon Island, Antarctic

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Abstract*– Half Moon Island is one of the Antarctic Islands of South Shetland. With an area of about four-square kilometers, it has some elevated areas such as the Cerro Capa Negra or also called Morenita Hill, which presents rocks of volcanic origin, tonalites type. There are also several species of mosses and lichens, in which the presence of tardigrades was checked. The remarkable ability of tardigrades to withstand a wide range of stressors has sparked a renewed interest in studying their presence in Antarctica. Identification of tardigrades was carried out using optical equipment such as microscope, stereoscope and camera. The following species were found: Diphascon victoriae, Diphascon rudnicki, Hypsibius conwentzii, Hypsibius dujardini, Macrobiotus aradasi, Echiniscus spp. and Hexapodibius spp. These species allowed an analysis of the diversity in Cerro Capa Negra. Further evaluations are needed to better understand the presence of tardigrades in Antarctica.

***Keywords*-- Antarctica, biodiversity, occurrence, extreme environment, lichen.**

I. Introduction

For more than 30 million years, Antarctica has been gradually glaciated and separated from other parts of the world [1]. It is one of the planet's most harsh environments, with a variety of coexisting elements including substrates, oligotrophic habitats, large temperature swings, high radiation levels, heightened salinity with variable pressure, and pH levels [2]. These elements are essential in forming the many ecological niches found in this area [3].

These kinds of environments may harbor species known as extremophiles, or those that can grow in abiotic environments that are not typically physiological for life [4]. Tardigrades are widely known for their wide distribution in natural environments with adverse conditions, making their extreme tolerance a decisive factor in conditions of extreme temperatures (high and low), high pressures, low water availability, desiccation, intense ultraviolet light, and ionizing radiation [5]. These microinvertebrates inhabit both terrestrial and freshwater ecosystems, as well as in common habitats such as different types of soil or less common ones such as mold cushions or lichen bark [6], [7].

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Tardigrades, often referred to as water bears, typically have a small size, ranging from 100 to 1200 µm, and exhibit slow movement [8]. Discrete respiratory and circulatory systems are absent in tardigrades. Instead, a chamber filled with fluid surrounds the internal organs of hydrated, active tardigrades, which may aid in the flow of different solutes [9]. Tardigrades are capable of entering a reversible metabolic suppression state known as cryptobiosis in response to extreme environments [10]. However, to remain active, they require a surrounding layer of water [11].

The remarkable ability of tardigrades to withstand a wide range of stressors has sparked a renewed interest in comprehending the molecular processes underlying the protective properties of macromolecules such as proteins and DNA [12]. Understanding the aging processes that these animals experience during anhydrobiosis is part of this. These mechanisms are highly intriguing since they provide understanding of the cellular and molecular mechanisms behind human aging and can be used as an experimental model to investigate age-related disorders [13].

The growing interest in these organisms has led to the isolation of these species and the understanding of their physiology [14], [15]. Previous studies of tardigrades in Antarctica include discoveries of new species (e.g. *Echiniscus corrugicaudatus* McInnes, 2010) [16], morphological variation of tardigrade eggs [17], ecosystem relationships with tardigrade dominance [18], evolutionary scenarios [19] to mention a few. According to McInnes & Pugh [20] in Signy Island lakes a great variety of species was reported with respect to lakes with higher temperatures.

The presence of tardigrades in lakes at different depths, as in other lentic environments, shows the wide occupation of these living beings; however, in Half Moon Island they are present in very different conditions, specifically, associated with the rocks present in the Capa Negra hill. In this perspective, the search for and characterization of tardigrades in the Antarctic Peninsula, a region of the planet characterized by extensive ice cover, a variety of igneous rocks and average temperatures of -2.1°C [21], is of interest given the extreme conditions present on the islands and the variety of ecosystems in which tardigrades are present. Thus, the present study aims to identify the different species of tardigrades present in the mosses and lichens of Cerro Capa Negra of Half Moon Island.

II. Materials and methods

A. Sampling

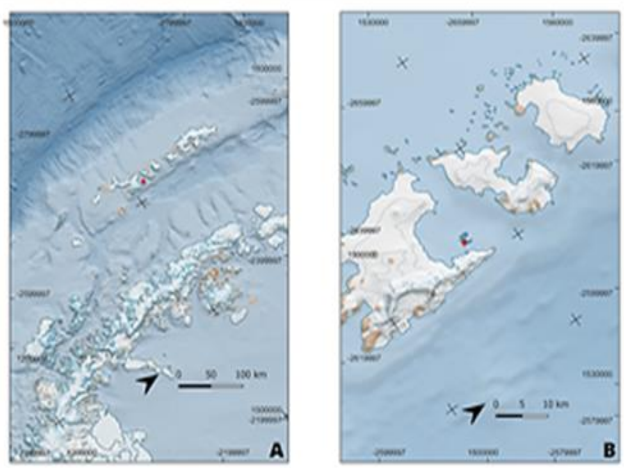

Figure 1. Location of Half Moon Island in Antarctica (a), location relative to the Antarctic Peninsula (b), scale relative to Greenwich and Livingston Islands. Data source: Quantarctica v3 [22]

Ten samples of mosses and lichens were collected during the second expedition of the Colombian Antarctic Program in the summer of 2015-2016, in the framework of international cooperation with Argentina, on Half Moon Island. This island is located in the Antarctic Peninsula area, between Greenwich and Livingston Islands, and is one of the smallest islands in the South Shetland Archipelago (Figure 1). Half Moon Island is 2 km in extent; Structurally, this island consists mostly of rock formations and is mostly ice-free. The sampling points, in Black Capa Hill or also called Morenita Hill, were selected taking into account the presence of mosses or lichens.

B. Identification of collected material.

For the identification we relied on the information obtained by the project developed by the Universidad Nacional de Colombia in the same expedition, who made the identification of specimens using optical equipment (microscope, stereoscope and camera) and specialized taxonomic keys [23], [24], [25], [26], [27], [28], [29], [30], [31], as well as, published literature was consulted for identification of tardigrades.

C. Identification of collected material.

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D. Isolation and fixation of tardigrades.

The tardigrade analysis was carried out in two ways: a) direct analysis and b) Baermann funnel filtration method [18]. In the direct analysis, the dry bryophyte sample of approximately 2 cm long and 2 cm wide was deposited in a Petri dish and moistened with distilled water for a period of 45 minutes, in order to activate the tardigrades, present in the sample [32]. On the other hand, for the Baermann funnel filtration method, the dry bryophyte sample was placed on top of the funnel and hydrated with distilled water for a period of 3 to 6 hours, so that the tardigrades precipitated [33]. After the activation time, the samples were analyzed in the DMW-143-N2GG MOTIC Digital Stereo Microscope DMW-143-N2GG MOTIC. Tardigrades were extracted using a Transferpette® S - Brand micropipette and deposited on slides for subsequent fixation.

E. Morphological identification of tardigrades.

Once the tardigrades were isolated, a brief description of the individual was made before fixation. Then, they were mounted and fixed on slides. Two mounting media were used to fix the tardigrades. One was polyvinyl alcohol mounting medium (Bioquip 6371A) [34] and the other PVA Glycerol acetic acid [33].

In order to carry out the morphological characterization of the tardigrades, first the fixations were taken to be analyzed under the Nikon Eclipse E100 microscope. Then photographs were taken to serve as a guide in the identification of any relevant aspect, in addition to the measurements of the body, mouthparts and claws [35], [36]. In the case of eggs, the diameter with and without ornamentation was measured.

Once the photographs were analyzed, a final description of the individuals was prepared with their respective photographs, measurements, morphological characteristics and the location of the collection area. In order to group those individuals with similar characteristics. In addition to this, a comparative table was made with morphologically similar tardigrade species, in order to identify the individuals studied. For the identification we followed:

(a) the keys for the taxonomic identification of tardigrade species from Mongolia proposed by Kaczmarek and Michalczyk [37], (b) the guide to common species by Kinchin [38] and, (c) the key to families, subfamilies, genera and subgenera of Eutardigrada by Pilato and Binda [39].

F. Ecological indexes

Taking into account that the observed parameter corresponds to the different species of tardigrades and the number of individuals of each of these, species richness and specific abundance were evaluated. For this, the Margalef diversity index (equation 1) and the Shannon index (equation 2) were taken into account, as follows:

Margalef diversity index (equation 1):

$$DMg = S - 1/lnN$$

where:

S = number of species

N = total number of individuals

Shannon index (equation 2):

$$H' = -\sum_{i=1}^{S} pi\ Ln\ pi$$

where:

S = number of species (species richness)

pi = proportion of individuals of species i in relation to the total number of individuals (i.e. relative abundance of species i): ni/N

ni = number of individuals of species i

N = number of all individuals of all species

III. RESULTS

A. Sampling sites and bryophyte and lichen species

Ten points were sampled (Figure 2) and determined to correspond to the lichen or moss species shown in Table 1.
In total, 168 specimens and nine free eggs were found.

Table 1. Samples collected for tardigrade isolation.

Sample code	Coordinates	Height (masl)	Moss or lichen species
M01	62°35’42.40’’S; 59°55’5.10’’W	22	*Polytrichastrum alpinum*
M02	62°35’46.5’’S; 59°55’14.11’’W	94	*Sanionia uncinata*
M03	62°35’43.50’’S; 59°55’10.30’’W	24	*Hymenoloma grimmiaceum*
M04	62°35’47.6’’S; 59°55’14.4’’W	100	*Cladonia metacorallifera*
M05	62°35’45.50’’S; 59°55’14.80’’W	49	*Psoroma hypnorum*
M06	62°35’46.5’’S; 59°55’13.1’’W	94	*Stereocaulon alpinum*
M07	62°35’43.6’’S; 59°55’15.2’’W	31	*Ramalina terebata*
M08	62°35’42.90’’S; 59°55’14.70’’W	18	*Bryum pseudotriquetrum*
M09	62°35’46.40’’S 59°55’13.60’’W	93	*Andreaea regularis*
M14	62°35’46.90’’S 59°55’10.10’’W	70	*Bartramia patens*

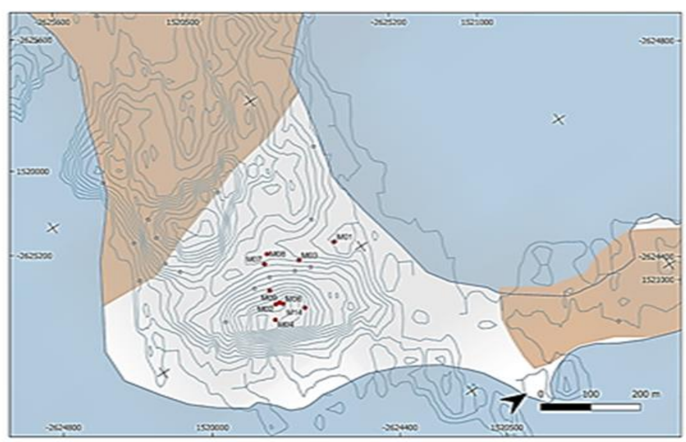

Figure 2. Sampling points at Cerro Capa Negra on Half Moon Island. Data source: Quantarctica v3 [22]

The taxonomic composition of the various tardigrade genera was as follows: 41.1% *Diphascon*, 28% *Hypsibius*, 22.6% *Echiniscus*, 7.1%, *Macrobiotus*, and 1.2% *Hexapodibius*. Five species were identified: *Diphascon victoriae* (Pilato and Binda 1999) [40], *Diphascon rudnicki* (Kaczmarek, Parnikoza, Gawlak, Esefeld, Peter, Kozeretska & Roszkowska, 2018) [41] , *Hypsibius conwentzii* **(**Kaczmarek, Parnikoza, Gawlak, Esefeld, Peter, Kozeretska & Roszkowska, 2018**)** [41], *Hypsibius dujardini* (Doyère, 1840) [42] and *Mesobiotus aradasi* (Binda, Pilato & Lisi 2005) [43]. Of which 42 individuals correspond to *Diphascon victoriae*, 27 individuals to *Diphascon rudnicki*, 26 individuals to *Hypsibius conwentzii*, 21 individuals to *Hypsibius dujardini*, 12 individuals to *Macrobiotus aradasi*, 38 individuals to *Echiniscus* spp. and 2 individuals to *Hexapodibius* spp.

B. Diphascon victoriae (Pilato and Binda 1999) [40] and Diphascon rudnicki (Kaczmarek, Parnikoza, Gawlak, Esefeld, Peter, Kozeretska and Roszkowska, [41].

The genus *Diphascon* is characterized by a drop-shaped thickening between the oral and pharyngeal tubes [39]. Two species linked to this genus were identified, namely *D. rudnicki* (Figure 2) and *D. victoriae* (Figure 3). Features such as drop-shaped thickening between the buccal and pharyngeal tubes, macroplacoid sequence of 1 = 2 <3, where the first and second are located very close to each other, a septum and apophysis present in the pharyngeal bulb are aspects discovered in the investigated individual, and characteristic of the species *D. rudnicki*.

The description and creation of the keys to the species *D. victoriae* was done by Pilate and Binda [40]. When the row of macroplacoids is longer than half the length of the buccal tube, the third macroplacoid is longer. Furthermore, the species *D. victoriae* is known to be endemic to Antarctica and exclusive to Victoria Land in West Antarctica [44].

However, previous studies by [45] and the present work conducted on Half Moon Island show that this species is not exclusive to the aforementioned area, as in Antarctica *D. victoriae* could be identified on the peninsula.

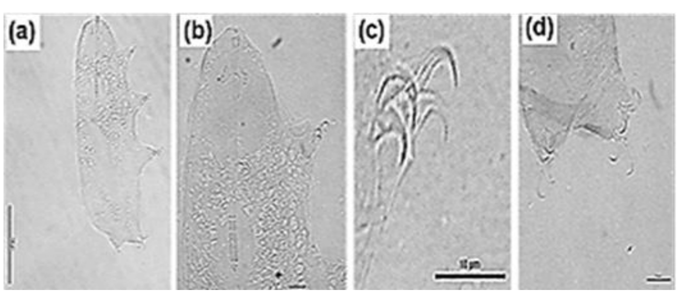

Figure 3. *Diphascon victoriae*. to. Body, 20X; b. Buccal-pharyngeal apparatus, 100X; c. Leg I, 100X; d. Leg IV, 100X.

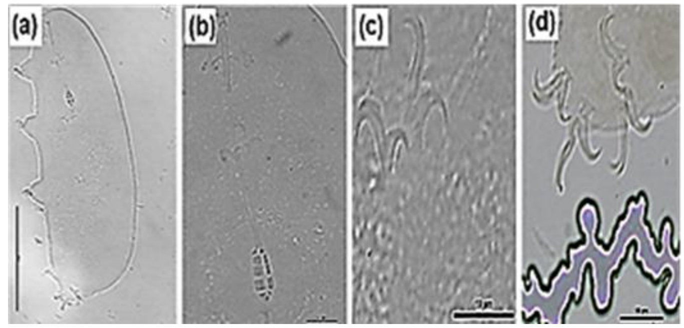

Figure 4. *Diphascon rudnicki*. to. Body, 20X; b. Buccal-pharyngeal apparatus, 100X; c. Leg II, 100X; d. Leg IV, 100X

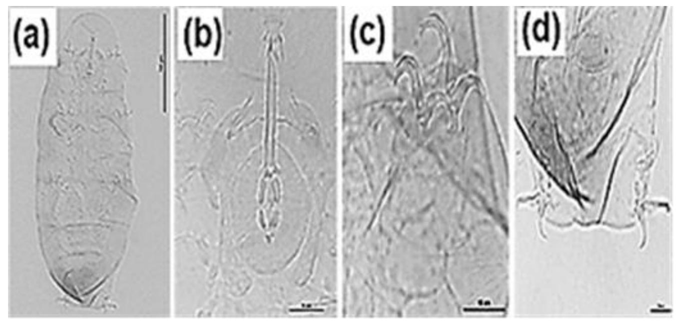

Figure 5. *Hypsibius conwentzii*. to. Body, 20X; b. Buccal-pharyngeal apparatus, 100X; c. Leg III, 100X; d. Leg IV, 100X. (cb) cuticular bar in leg IVs

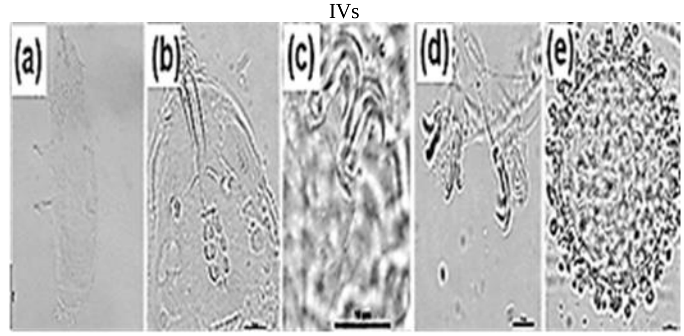

Figure 6. *Macrobiotus aradasi* Binda, Pilato & Lisi, 2005. a. Body, 20X; b. Buccal-pharyngeal apparatus, 100X; c. Leg II, 100X; d. Leg IV, 100X; and. Egg, 100X

C. *Hypsibius conwentzii (Kaczmarek, Parnikoza, Gawlak, Esefeld, Peter, Kozeretska & Roszkowska, 2018) [41] and Hypsibius dujardini (Doyère, 1840) [42].*

Within the genus *Hypsibius*, the species *H. conwentzii* and *H. dujardini* were identified. The identification were determined by observation of eyes, a pair of claws on each leg and a mouthpart without a flexible tube or ventral lamina [39]. The characterization of *H. conwentzii* was based on the description of [41]. The sequence of its macroplacoid length is 1> 2, a trait it shares with the individual analyzed. Also, the holotype studied was observed to have a soft cuticle, presence of eyes, septum, apophysis, and accessory points on all primary claws.

The characterization of *H. dujardini* was performed by comparing the measurements of this species with those of the holotype studied for this species. Features such as the presence of eyes, septum and cuticle bars on the hind legs are aspects that the studied individuals have in common [46]. However, it should be noted that although the two species found in the genus *Hypsibius* have similar morphological characteristics, they present different morphometric characteristics. Specifically, *H. dujardini* has a smaller buccal tube and septum than *H. conwentzii* (Figure 5).

D. Mesobiotus aradasi Binda, Pilato & Lisi, 2005 [43]

Using the illustrated identification key of Bingemer and Hohberg [47] and an article by Pilato and Binda [39], species identification of the genus *Mesobiotus* was achieved by identifying a rigid mouth tube that is not subdivided into a rigid posterior portion and a flexible posterior portion, lamellae, ventral lamina, and lunule on all claws presents. The identification of the species *M. aradasi* was made by comparing the measurements of the individuals with those described by Binda et al. [43]; in addition, *M. aradasi* presents characteristics such as the presence of eyes, soft cuticle, lunule and bands of the second and third teeth (Figure 6); characteristics that coincide with the description made in this paper for the species *M. aradasi*, indicating that the individuals studied are part of this species, which has only been reported in Antarctica, indicating that it is an endemic species.

These species were previously reported from other Antarctic regions, such as *M. arad*asi which was found on King George Island [43]; *D. rudnicki* and *H. conwentzii* on Ardley Island [41]; *D. victoriae* has been found in Victoria Land [40]; and finally, *H. dujardini* has been found in Maritime Antarctica [48] and is considered to have a cosmopolitan distribution. However, *H. conwentzii* and *Diphascon rudnicki* are considered new reports for the Antarctic Peninsula and Maritime Antarctica [41], [42].

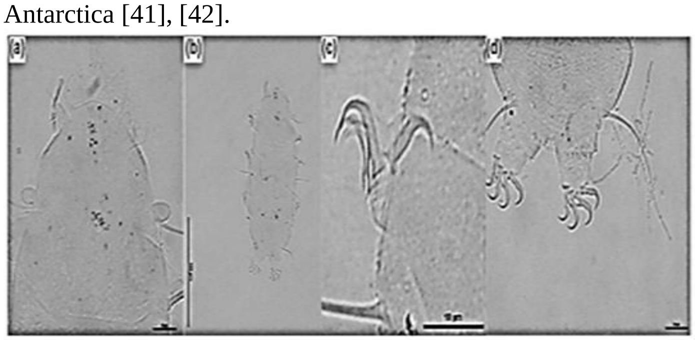

Figure 7. *Echiniscus* spp. a. Head,100X; b. Body, 20X; c. Leg II, 100X; d. Leg IV, 100X

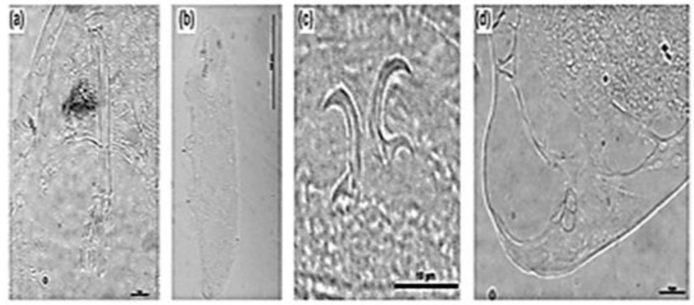

Figure 8. *Hexapodibius* spp. a. Bucco-pharyngeal apparatus, 100X. B. Body, 20X. C. Leg III, 100X. D. Leg IV, 100X

E. Echiniscus spp. (Schultze 1840) [49]

This specimen presented body length 156 µm; color yellow/orange, Dorsal and lateral cuticular plates are granulated with small granules, similar in size and evenly distributed, eyes absent or not visible after preparation. Cuticular armature consisting of single cephalic plate, scapular plate, 1st single median plate, 1st paired plate, 2nd median plate, 2nd paired plate, 3rd single median plate and terminal plate. Paired plates divided into two unequal anterior and posterior parts by a transverse stripe. Ventral plates not visible.

Scapular plate length could not be measured, cirrus internus 11.6 µm long, cephalic papilla 7.7 µm long, cirrus externus 14.3 µm, clava 7.1 µm long and cirrus A 48.2 µm long (cirrus A/body length ratio 31% and cirrus int/ext length ratio 81%). Length of body appendages: cirrus C 40.0 µm long, cirrus Cd 51.1 µm long, cirrus Dl 3.4 µm long, cirrus E 47.8 µm long and cirrus B, Bl, Bd, Cl, D, Dd, El and Ed are absent.

ELeg spines I, II, III and IV absent or not visible. Papillae present on legs IV. Serrated collar on legs IV with irregular and variable number of teeth. Outer claws of all legs smooth, inner claws with downward facing spurs. Serrated collar with approximately 6 sharp triangular teeth of unequal height. Leg II, branch 8.3 µm and spur 2.1 µm and leg IV, branch 9.2 µm and spur 2.2 µm long (Figure 7).

F. Hexapodibius spp. (Pilato 1969) [50]

Body length 549 µm; yellow, cuticle smooth, large eyes present. Buccopharyngeal apparatus of *Macrobiotus* type; terminal mouth surrounded by lamellae, armature of oral cavity absent. Ventral lamellae present. Buccal tube 52.7 µm long, with an internal width of 4.6 µm (pt = 8.7) and external width of 5.9 µm (pt = 11.1); stylet supports inserted into buccal tube at 72.7 of its length (pt = 72.7).

Pharyngeal bulb has apophysis, presents three rows of macroplacoids, presence of small microplacoid, second macroplacoid placed slightly closer to first than third macroplacoid, second macroplacoid is longer than the other and has central constriction, third macroplacoid has a contraction at the bottom. Granular microplacoids situated far from third macroplacoid; first macroplacoid 4.0 µm long (pt = 7.6) second 14.7 µm (pt = 27.9), third 7.2 µm (pt =13.7); row of macroplacoids 25.4 µm long (pt = 48.1).

Claws asymmetrical, hexapodibius type primary claws with small, short accessory tips; in addition, primary claws longer than secondary claws. Claws absent on the fourth pair of legs. Inner and outer claws of the third leg pair measure 11.2 µm (pt = 21.3) and 10.5 µm (pt = 19.9) respectively. Lunulae on leg IV and cuticular bar on legs I - IV absent (Figure 8).

G. Presence of tardigrade genera in relation to bryophyte genera.

Once the identifications of the different genera of mosses and lichens, as well as the genera of the different tardigrades, were made, it was possible to relate the presence of tardigrades as shown in Table 2.

Table 2. Number of individuals for each genus of tardigrades in each of the samples collected.

Moss or lichen species	Number of individuals found for each genus of tardigrades				
	Diphascon	*Hypsibius*	*Macrobiotus*	*Echiniscus*	*Hexapodibius*
Polytrichastrum alpinum	18	0	0	0	1
Sanionia uncinate	25	17	2	10	1
Hymenoloma grimmiaceum	4	6	0	0	0
Cladonia metacoralifera	5	21	2	0	0
Psoroma hypnorum	0	0	0	0	0
Sterocaulon alpinum	4	0	5	8	0
Ramalina terebata	10	0	3	4	0
Bryum pseudotriquetum	3	3	0	9	0
Andrea regularis	0	0	0	4	0
Bartramia patens	0	0	0	3	0
Total	69	47	12	38	2

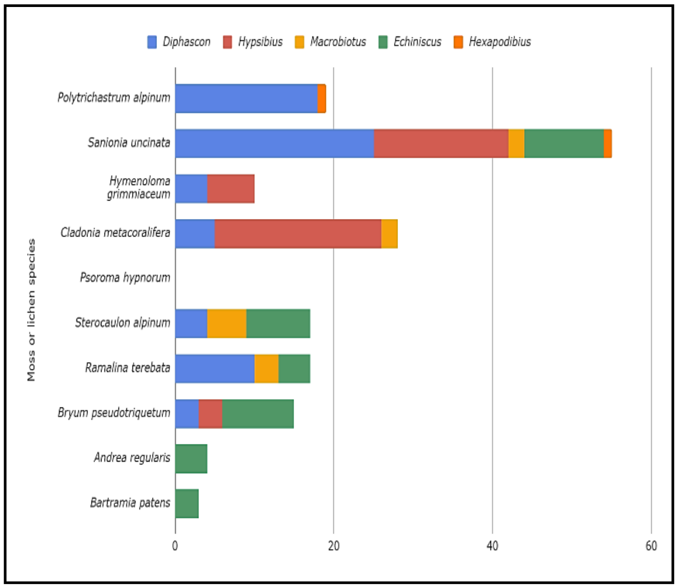

Figure 9. Number of individuals per genus of tardigrades in relation to the species of moss or lichen sampled.

H. Ecological indexes

The evaluation of species richness and specific abundance for all the samples analyzed showed a value of 1.17 for the Margalef diversity index. The result of the Shannon index was 1.76.

IV. Discussion

A. Habitat and diversity

As shown in Table 2 and Figure 9, all tardigrade genera were detected in the sample collected from *Sanionia uncinata*, which is one of the most abundant mosses in maritime Antarctica [51]. However, one of the most relevant characteristics of this moss corresponds to the ability to tolerate desiccation due to the accumulation of osmolytes and dehydrins, as well as the control of redox homeostasis [52].

This aspect is relevant if it is considered that in the South Shetland Islands, including Half Moon Island, there are times when ice cover rises above hundreds of meters [53], which would generate difficulties for plants to tolerate seasonal changes. For this reason, the plant species itself can provide substrate for tardigrades throughout the year. This is a relevant aspect for most tardigrade species, as some studies have shown that not many tardigrades are able to tolerate freezing immediately [54], but require some stages of adaptation. Freezing, it is worth clarifying, behaves at the physiological level as an absence of water.

It was also evident that 63% of the tardigrade individuals were found associated with moss species, while only 37% were found on lichens. This could be due to the fact that the species found feed mostly on plant cells [55], which coincides with the morphological characteristics found in the different species. On the other hand, this could be due to the relationship with the presence of soil, since according to Lindsay [53], the ecological succession in the South Shetland Islands, presenting first the lichens that help the formation of soil, to later allow the colonization of mosses. Several of the tardigrade species found in the study area correspond to terrestrial tardigrade species, which can inhabit the interstitial spaces of the soil [56].

It was also found that no tardigrades were present in the samples of *Psoroma hypnorum*. Since no studies have been carried out to detect the presence of secondary metabolites, one of the aspects that could influence the absence of tardigrades could be related to the morphology of the lichen, which presents a scaly thallus with scales that can become granular due to the presence of Nostoc [57]. This could hinder colonization by tardigrades and other species, in addition to being strongly adhered and flattened on the rock substrate, which could hinder the transit of different organisms.

In relation to what was obtained in the analysis of the diversity indexes, taking into account that regions with a Margalef index of less than two are considered low biodiversity regions, Half Moon Island, in the Cerro Capa Negra Region, could definitely be considered a low biodiversity region by having an index of 1.17. This situation is per se related to Antarctic conditions [58]. For its part, the Shannon index yielded a value of 1.76, which similarly corresponds to regions with low biodiversity [59]. Although these conditions are not surprising, much of the work reported for the South Shetland Islands corresponds to work related to fungal and bacterial diversity, as well as plant diversity. However, a record of tardigrade biodiversity allows us to begin explorations in this direction.

V. Conclusions

Tardigrades, or water bears, are a group of microscopic animals considered Extremophiles, capable of withstanding extreme conditions such as those of Antarctica, outer space, desiccation, osmotic changes, oxygen depletion, toxic chemicals, pressure and even ionizing radiation. from space. This study represents the first report of tardigrades on Half Moon Island, Antarctica; Therefore, this study adds important information about the diversity of tardigrades present on this Island in Antarctica; this continent; It is a strategic ecosystem to isolate extremephilic microorganisms; such as bacteria and tardigrades, with high biotechnological potential, due to their adaptation to the extreme conditions of Antarctica by Acevedo-Barrios [60], [61], [62], [63].

Regarding the Margalef index, Arrojo gave a value of 1.17 and the Shannon index gave a value of 1.76; Both indices have values less than 2, which indicates that Half Moon Island is considered a region with low biodiversity; which is related to the extreme conditions of Antarctica. The tardigrade species identified on Half Moon Island were: *Diphascon victoriae*, *Diphascon rudnicki*, *Hypsibius conwentzii*, *Hypsibius dujardini*, *Macrobiotus aradasi*, *Echiniscus* spp. and *Hexapodibius* spp; which were isolated in different species of mosses and lichens. The above allows us to establish that due to the extreme conditions of the southern Sherland Islands and the Antarctic Peninsula; These house a small variety of genera and species of Antarctic tardigrades.

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