Biodiversity and ecology of Hirudinea (Annelida) from the Natural Reserve of Isla Martín García, Río de la Plata, Argentina

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Received August 6, 2008 – Accepted December 22, 2008 – Distributed November 30, 2009

(With 3 figures)

Abstract

The Island of Martin Garcia lies at the confluence of the Uruguay and Paraná Rivers (upper Río de la Plata). This island is an outcrop of the crystalline basement. Due to basalt exploitation the island exhibits several ponds covered by carpets of free-floating macrophytes. Seven major environmental variables were measured: water and air temperature, percentage of oxygen saturation, dissolved oxygen, electrical conductivity, total dissolved solids and pH. Eleven leech species were found, *Helobdella striata*, *H. diploides*, *H. adiastola* and *H. hyalina* were new records. UPGMA clustering of species based on their occurrence in different ecological conditions revealed three main species groups. Canonical Correspondence Analysis (CCA) explained 97.2% of the correlation between species and environmental variables. *H. triserialis* shows the widest range of tolerance, *H. hyalina* shows positive relationships to conductivity; *H. diploides* shows a high correlation with dissolved oxygen, *H. striata*, *H. lineata*, and *S. similis* are negatively correlated with water temperature, and *H. simplex* is positively correlated with pH. Relationships between the species richness (S) and the sampling sites were negatively correlated with water temperature and positively correlated with dissolved oxygen. Leech biodiversity from the water bodies of Martín García Island, shows a great diversity of species and a wide plasticity regarding the characteristics of the environmental factors considered.

Keywords: biodiversity, CCA, cosmopolitan, ecology, endemics, leeches.

A Biodiversidade e a Ecologia de Hirudinea (Annelida) da Reserva Natural da Ilha Martín García, na Argentina

Resumo

A Ilha Martín García encontra-se na afluência dos Rios Uruguay e Paraná (no Rio de la Plata superior) e constitui um afloramento do maciço cristalino de Brasília. Por causa da exploração do basalto, formaram-se lacunas que apresentam tapetes de vegetação flutuantes e macrófitas enraizadas. Mediram-se sete variáveis físico-químicas da água: temperatura do ar e da água, porcentagem de saturação de oxigênio, oxigênio dissolvido, condutividade elétrica, sólidos totais dissolvidos e pH. Encontraram-se onze espécies de sanguessuga, como *Helobdella striata*, *H. diploides*, *H. adiastola* e *H. hyalina* que constituem novos registros para a ilha. A análise de agrupamento baseado na ocorrência das espécies em condições ecológicas diferentes revelou três grupos principais. A análise canônica da correspondência sugere que a distribuição das espécies relaciona-se às variáveis ambientais consideradas, 97,2% da correlação entre as espécies e as variáveis se distribui no eixo dois do diagrama de ordenamento. As variáveis com maior flutuação foram a condutividade e o pH. *H. triserialis* foi a espécie mais tolerante às variáveis ambientais; *H. hyalina* se relacionou positivamente com a condutividade; *H. diploides* teve alta correlação com o oxigênio dissolvido; *H. striata*, *H. lineata* e *Semiscolex similis* relacionaram-se negativamente com a temperatura da água e *H. simplex*, positivamente com o pH. As relações entre a riqueza de espécies (S) e os lugares de amostras foram negativas com a temperatura da água e positivas com o oxigênio dissolvido. Os corpos da água da Ilha Martín García mostram uma grande biodiversidade de espécies de sanguessuga, assim como uma ampla ductilidade com relação aos fatores ambientais considerados.

Palavras-chave: biodiversidade, CCA, cosmopolitas, ecologia, endêmicos, sanguessuga.

1. Introduction

The Island of Martín García lies at the confluence of the Uruguay and Paraná rivers (upper Río de la Plata), 34° 11' S and 58° 15' W. This island is an outcrop of the crystalline basement, unconformably overlain by Pleistocene and Holocene sediments (Dalla Salda, 1981; Ravizza, 1984; González and Ravizza, 1987). Due to basalt exploitation the island exhibits several ponds covered by carpets of free-floating macrophytes. At present, Martín García is a multiple use reserve administered by the government of the Buenos Aires province, and although it has a permanent population of ca. 100 people, a large number of tourists visit the island.

The island's biota includes particular species of arborescent trees and bushes (Lahitte and Hurrell, 1994) and vertebrates (Juárez, 1995; Lahitte et al., 1995; Lahitte and Hurrell, 1998). Available information on aquatic invertebrate fauna mostly refers to mollusks, annelids oligochaetes, hirudinea, platyhelminthes temnocephalids, some crustaceans and insects (Viana, 1937; Austin et al., 1981; Ringuelet, 1985; Rumi et al., 1996, 2004; Damborenea et al., 1997; Fernández and López Ruff, 1999; Armendáriz et al., 2000; Armendáriz and César, 2001; César et al., 2001; Martín and Negrete, 2006; Martín, 2008).

Five families of aquatic leeches are present in South America: Glossiphoniidae, Psicolidae, Ozobranchidae (Rhyncobdellida), Cyclobdellidae, Semiscolecidae and Macrobdellidae (Arhyncobdellida). Glossiphoniidae and Semiscolecidae were found on Martín García Island.

The Hirudinea of Argentina represent one of the best known fauna in the Neotropical region closely related to that of Brazil, Paraguay, Bolivia, Uruguay and Chile.

The Glossiphoniidae comprise the largest number of described species including freshwater leeches which are mainly macroinvertebrate predators or temporal ectoparasites of freshwater fish, turtles, amphibians and aquatic birds. The Cyclobdellidae and Semiscolecidae are freshwater macrophagous leeches (Ringuelet, 1985; Sawyer, 1986).

Knowledge on biodiversity of a natural reserve is essential for the conservation and sustainable use of natural resources, therefore the aim of this paper is to identify the leeches species found on the island and to examine the relationships among the species and the environmental factors registered.

2. Materials and Methods

Description of sites studied: Two types of aquatic ponds are considered: the first type is composed of ponds originated in the old basalt exploitations which have been conducted since 1791 over more than a hundred years (Lahitte and Hurrell, 1996; Kröpfl, 2006). The second type is composed of small ponds formed in natural depressions in the marginal forest or in zones close to the inland sand plains.

Nine of these water bodies were surveyed monthly (June 2005 to July 2006) (see Figure 1). From these, eight are ponds originated in the old exploitations of basalt, while the remaining one is a pond located at the bottom of a sand dune. Two out of 9 (2 and 5) had a steady state while the remaining ones (1, 3, 4, 6, 7, 8 and 9) underwent, throughout the sampling period, remarkable fluctuations in their water volume until their complete desiccation, being considered ephemeral wetlands.

Inland water bodies close to the river banks receive water from river floods, as well as from rainfall, while in the other inner ponds water comes basically from rainfall accumulation.

These ponds show floating vegetation (*Lemna gibba*, *L. minuscula*, *Salvinia biloba*, *S. minima*, *Azolla filiculoides*, *Limnobium laevigatum*, *Pistia stratiotes*, *Spirodella intermedia*, *Wolffiella oblonga* and *Wolffia colombiana*) that varies in species composition according to the season of the year and the amount and quality of nutrients (Lahitte and Hurrell, 1996).

Samples of floating vegetation were collected using a net (frame size: 40 × 40 cm, mesh size: 150 < mu > m). Material was collected in 500 mL plastic jars, and three replicates were collected per sampling pond. Samples were kept in plastic bags and fixed in 10% formalin. They were then washed using a sieve (150 < mu > m), stained with Rose Bengal and preserved in ethanol 70°. Separation and species identification of leeches were conducted under a stereo microscope.

Seven major environmental variables were measured: water temperature, air temperature, percentage of

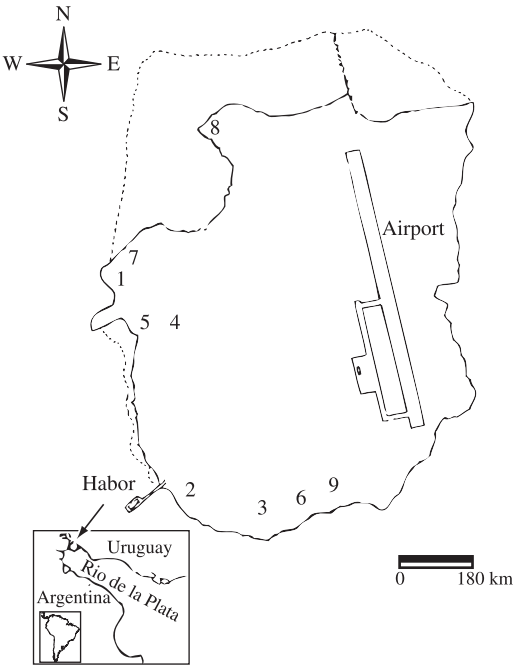

Figure 1. Samplings stations at Isla Martín García.

oxygen saturation, dissolved oxygen ($mg.L^{-1}$), electrical conductivity ($\mu S.cm^{-1}$), total dissolved solids and pH. All variables were measured using a digital Water Quality Meter (Sper Scientific Ltd).

Relationships between species and environmental variables were examined with Canonical Correspondence Analysis (CCA), considering the seven most abundant and most frequently occurring species and five environmental variables, the temperature of the air and the TDS contained redundant information (inflation factors > 20), therefore they were not considered in the analysis (Ter Braak, 1986; Ter Braak and Verdonschot, 1995; Külköylüoğlu, 2003; 2005; Külköylüoğlu and Dügel, 2004). The mean value of each environmental variable together with data on abundance species from seven sampling stations were used in CCA. Associations among species were analysed using clustering analysis (UPGMA) with a Jaccard Index (Crisci and López Armengol, 1983), the species analysed were the same as those used for CCA. Pearson correlation analysis was conducted and significance values of the correlations were tested using the "*t*-Student test".

3. Results

The leeches are important components of the benthos and pleuston of lotic and lenitic freshwater habitats. Lakes and ponds of Buenos Aires province and Martín García Island present dense - attached or unattached - floating vegetation and constitute adequate habitats for the development of their populations.

A total of eleven leech species were found, nine of them belonging to *Helobdella* Blanchard, 1896, one to *Semiscolex* Kinberg, 1867 and the other to *Orchibdella* Ringuelet, 1945.

The six most frequent and abundant species (more than 200 individuals in total) were *Helobdella triserialis* Blanchard, 1849 (n = 1632, 39.16%), *Helobdella hyalina* Ringuelet, 1942 (n = 1351, 25.77%), *Helobdella simplex* (Moore, 1911) (n = 635, 14.85%), *Semiscolex similis* (Weyenbergh, 1879) (n = 337, 8.38%), *Helobdella diploides* Ringuelet, 1948 (n = 315, 7.48%); while *Helobdella striata* (Ringuelet, 1943) (n = 137, 3.42%), *Helobdella lineata* (Moore, 1936) (n = 20, 0.50%), *Helobdella duplicata duplicata* (Moore, 1911) (n = 9, 0.23%), *Helobdella adiastola* Ringuelet, 1972 (n = 6, 0.15%), *Helobdella duplicata tuberculata* Ringuelet, 1958 (n = 1, 0.03%) and *Orchibdella pampeana* Ringuelet, 1945 (n = 1, 0.03%), showed lower frequency of occurrence and a lower number of individuals (as shown in Table 1). *H. striata*, *H. diploides*, *H. adiastola* and *H. hyalina* were new records for Martín García Island.

The UPGMA clustering was conducted with the same species used in the CCA. UPGMA clustering revealed clear distinctions among some species based on their phenology,

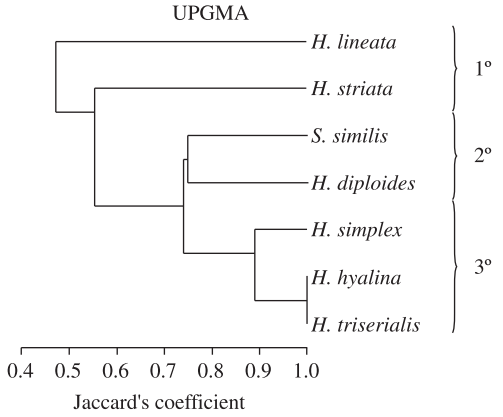

Figure 2. Dendrogram provided from binary (presence/absence). Based on their occurrence three main groups are clustered. Hlinea (*H. lineata*), Hstria (*H. striata*), *S. similis*, Hdiplo (*H. diploides*), Hsimpl (*H. simplex*), Hhya (*H. hyalina*), Htris (*H. triserialis*).

Table 1. Leeches species with their code and sites where they were collected on Isla Martín García during 12 months.

Species name	Code	Sites	%
Helobdella triserialis	Htris*	1, 2, 3, 4, 5, 6, 7, 8, 9	39.16
Helobdella striata	Hstria*	1, 3, 5, 6, 7, 8, 9	3.42
Helobdella hyalina	Hhya*	1, 2, 3, 4, 5, 6, 7, 8, 9	25.77
Helobdella lineata	Hlinea*	3, 5, 6, 8	0.50
Helobdella simplex	Hsimpl*	1, 2, 3, 4, 5, 6, 8, 9	14.85
Helobdella diploides	Hdiplo*	1, 2, 5, 6, 7, 8, 9	7.48
Helobdella adiastola	Hadias	1, 6	0.15
Helobdella duplicata tuberculata	Hdtuber	1	0.03
Helobdella duplicata duplicata	Hddupli	1, 7	0.23
Semiscolex similis	Ssimilis*	1, 3, 5, 6, 7, 8, 9	8.38
Orchibdella pampeana	Opamp	4	0.03

Frequency (%) indicates percentage of species occurrence (or the total numbers of occurrences by means of using presence-absence data) from samples (n = 55), (*) represents the seven most frequently occurring species explaining more than 90% of total species occurrence.

Figure 2 shows three main species groups. The first group included *H. triserialis*, *H. hyalina* and *H. simplex*, while the second group included *H. diploides* and *S. similis.* The third group consisted of *H. striata* and *H. lineata* with a lower frequency of occurrence. All species appeared to be eurychronal, being endemic to the Neotropical region, except for *H. triserialis* which is a cosmopolitan and eurychronal or semi eurychronal species.

In CCA (see Figure 3), the arrows depict the environmental variables and point out to the maximum variation of the factor. The length of the arrows is proportional to the importance of the variable in the ordination diagram. Electrical conductivity, pH, and dissolved oxygen showed the highest fluctuation during the sampling period. The dissolved oxygen was negatively related to pH (r = –0.53) and negatively related to water temperature (r = –0.48) (as shown in Table 3).

The analysis suggests that species distribution is related to the physico chemical conditions of water. Axis 2 of the ordination diagram displayed the 97.2% of the correlation between species and environmental variables (as shown in Figure 3 and Table 2). Generally, the analysed species were distributed around the mean values of the environmental variables. At the centre of the ordination diagram, *H. triserialis* showed the widest range of tolerance to the fluctuations of such variables. The occurrence of *H. hyalina* on the positive site of Axis 2 in CCA diagram may be explained by its positive relationships to conductivity (see Table 3) (r = 0.99). *H. diploides* shows a high correlation with dissolved oxygen (r = 0.77) but correlation with pH is negative (r = –0.53) *H. striata* (r = –0.72), *H. lineata* (r = –0.79) and *S. similis* (r = –0.82) are negatively correlated with water temperature, *H. simplex* is positively correlated with pH (r = 0.48).

From June 2005 to July 2006 relationships between the species richness (S) and the sampling sites, were negatively correlated with water temperature (r = –0.59) and positively correlated with dissolved oxygen (r = 0.58).

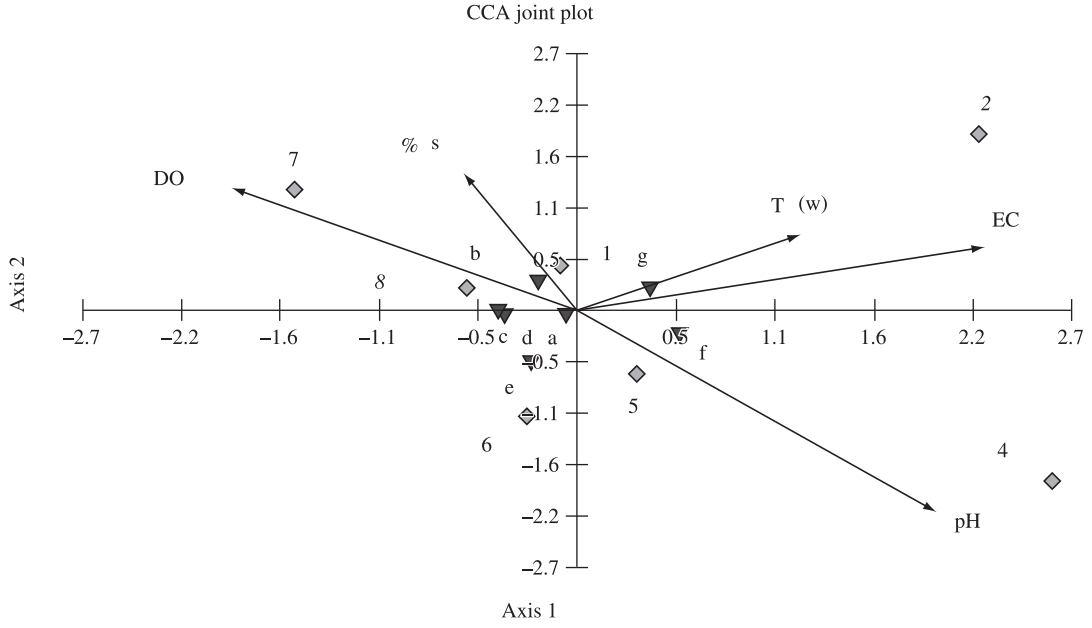

Figure 3. Canonical correspondence analysis diagram of sampling stations, seven species and five environmental variables. Abbreviations: a) *H triserialis*; b) *H. diploides;* c) *H. striata*; d) *S. similis*, e) *H. lineata*; f) *H. simplex*, g) *H. hyalina*. Sampling stations: 1, 2, 4, 5, 6, 7 and 8.

Table 2. Main results from the CCA.

Axes	1	2	3	4
Eigenvalues	0.133	0.048	0.030	0.01
Percentage	53.041	19.231	12.081	3.966
Cumulative percentage	53.041	72.272	84.353	88.318
Cumulative constrained percentage	59.995	81.747	95.412	99.898
Species environment correlations	0.925	0.972	0.991	0.918

Notice that about 97% of the correlations between five selected environmental variables and occurrence of seven species within 12 months were explained by the two axes of the ordination diagram.

Table 3. Pearson correlations matrix four five environmental variables and seven most abundant (S) species.

	T (W)	pH	DO	% S	EC	S	Htris	Hstria	Hhya	Hlinea	Hsimpl	Hdiplo	Ssimilis
T (W)	1	-	-	-	-	-	-	-	-	-	-	-	-
pH	0.07	1	-	-	-	-	-	-	-	-	-	-	-
DO	*–0.48	**–0.53	1	-	-	-	-	-	-	-	-	-	-
% S	*–0.40	*–0.42	**0.53	1	-	-	-	-	-	-	-	-	-
EC	–0.07	0.36	0.09	**0.53	1	-	-	-	-	-	-	-	-
S	**–0.59	0.07	**0.58	–0.02	–0.14	1	-	-	-	-	-	-	-
Htris	–0.06	0.28	*0.48	–0.09	0.04	**0.56	1	-	-	-	-	-	-
Hstria	**–0.72	–0.35	0.28	*0.49	–0.28	0.33	–0.13	1	-	-	-	-	-
Hhya	–0.06	0.31	0.09	**0.53	**0.99	–0.24	0.01	–0.30	1	-	-	-	-
Hlinea	**–0.79	*0.40	0.11	0.13	0.01	**0.56	0.30	**0.65	–0.03	1	-	-	-
Hsimpl	0.21	*0.48	0.16	–0.04	0.22	0.35	**0.85	–0.20	0.14	0.20	1	-	-
Hdiplo	–0.25	**–0.53	**0.77	0.01	–0.35	*0.40	*0.49	0.07	–0.29	–0.03	0.01	1	-
Ssimilis	**–0.82	–0.02	0.22	0.37	–0.16	*0.47	0.07	**0.93	–0.19	**0.88	–0.01	0.01	1

Numbers indicate strong correlations (*$p < 0.05$, **$p < 0.01$) where $n = 26$ for all variables. Abbreviations of the variables: T (W), water temperature; DO, dissolved oxygen ($mg.L^{-1}$); % S, oxygen saturation; EC, Electrical conductivity ($\mu S.cm^{-1}$); S, species richness. Abbreviation of each species is given in Table 1.

4. Discussion

The leech fauna of Martín García Island ponds does not differ markedly from that found on a beach of Río de la Plata river and in a steady pond near the coast of the same river (Gullo and Darrigran, 1991; Darrigran et al., 1998; Gullo, 2007).

As already mentioned in Material and Methods, the environments studied are of anthropic origin from the late XVIII century derived from the basalt exploitation with the exception of Station 7. These wetlands are mostly ephemeral and therefore the communities of invertebrates associated to the floating vegetation suffer a considerable physical stress. Some of them are also affected by refuse matter from human activity (Stations 1 and 6). According to Therriault and Kolasa (2000), human action produces environmental stress causing a reduction of biodiversity. However, our results indicated that in Stations 1 and 6 species richness (S) had its highest values, 9 and 8 species, respectively.

As pointed out by Davies and Govedich (2001) leeches show a strong physiological plasticity since they are able to live both in water with low salt concentration and higher salinities than marine water, despite the variations in the physico chemical conditions found in the freshwater aquatic ecosystems. In addition, some species can survive anoxic conditions for several days and hyperoxia for short periods of time. In relation to the opinion of these authors, the presence of epibiont species of *Epystilis* (Protozoa, Ciliophora) was observed in *H. simplex* in Station 4, where the lowest values of dissolved oxygen were recorded. Epibiont protozoan communities are very common in contaminated habitats or, as in this case, in aquatic habitats of low volume and high content of vegetal detritus (Gouda, 2006).

According to the clustering analysis, *H. triserialis* has a high association with *H. hyalina* and *H. simplex*, being these species the most frequently found and the most abundant in the ponds of Martín García Island: the first two species had a frequency of occurrence similar to that cited by Gullo (2007). The CCA results suggest that *H. triserialis*, at the centre of the ordination diagram, presented the greatest plasticity as far as environmental variables are concerned, being the most correlated with the rest of the species in relation to presence and abundance over the year, which is not surprising for a cosmopolitan species. Another species from this group, *H. hyalina,* on the positive side of the ordination diagram, was the only one with a high correlation with water conductivity, with the greatest abundance in Station 2. The highest values of conductivity over the sampling year were recorded in Station 2. *H. simplex* was the only species with a positive correlation with the pH. It is interesting to remark that the range of pH (6.9-7.8) and of dissolved oxygen (10-12.6 $mg.L^{-1}$) recorded by Gullo (2007) were higher than the ones measured in the island (5.30-7 and 0.90-5 $mg.L^{-1}$, respectively). Studies carried out in *H. stagnalis* (Linnaeus, 1758) in lentic environments and rivers of North America and Spain, mention that this species inhabits waters with a pH of 5.8-9.9, concentrations of dissolved oxygen of 5.9-8.5 $mg.L^{-1}$, and is found in any kind of waters (Herrmann, 1970; García Más and Jiménez, 1984), which evidences its high degree of plasticity within the environment as observed in *H. triserialis*.

In the ordination diagram *H. striata*, *S. similis* and *H. diploides*, constitute a group around the gradient of dissolved oxygen but in medium values. In the case of *H. lineata*, *H. diploides* and *H. striata* these species were not abundant throughout the year, as Gullo (2007) mentions for Los Patos pond and were negatively correlated with the water temperature as can be seen in the ordination diagram. Ringuelet (1985) mentions *S. similis* as the most common leech of Buenos Aires coast, inhabiting under stones, tuff blocks and loose submerged land or in humid places, contaminated waters and sewage waters. Its presence on the island has always been linked to the floating vegetation its total abundance during the sampling period was not very important maybe due to its preference for the benthic habitat.

Comparing data on conductivity obtained in the sampling periods from 1995 to 1997 (César et al., 2001) with those present in this paper, the current values are very similar. From our results we can conclude that biodiversity of leeches from the water bodies of Martín García Island, shows a great diversity of species in spite of its limited surface area (185 ha) and a wide plasticity regarding the characteristics of the environmental factors considered.

Acknowledgements — We wish to thank the Dirección de Islas, Ministerio de Gobierno, Provincia de Buenos Aires and Prefectura Naval Argentina Isla Martín García. Financial support was provided by the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires. We wish to thank also to Mr. José David Maciel and the island friends for their assistance during our samplings. César, I.I. and Martín, S.M.: Investigador CIC. Gullo, B.S., Cátedra Zoología Invertebrados I (FCNyM) and Liberto, R., Becaria CONICET

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