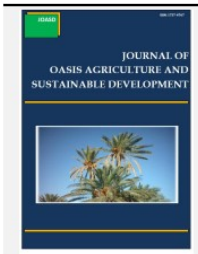

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Insights into the distribution of Freshwater Leeches (Annelida, Clitellata, Hirudinea) in Southwestern Tunisia

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Abstract

The study aims to highlight the diversity of leech species living in southwestern Tunisia. Our study highlights the prevalence of *Limnatis nilotica*, which represents 63.01% of the leech population, while the less frequently observed species include *Erpobdella johanssni* (25.31%) and *Batracobdella argila* (10.75%). These species thrive in watersheds, rivers, water sources and swamps. Interestingly, This study reveals a poverty of leech diversity in the south-west of Tunisia compared to the north, which favors conditions conducive to the development of leeches.

1. INTRODUCTION

In Tunisia, leeches play a significant role in freshwater ecosystems, contributing to biodiversity and ecosystem functioning. They are found in various habitats such as rivers, streams, ponds, and wetlands across the country. While there is limited specific research on leech species in Tunisia, general studies suggest the presence of several common species, including *Limnatis nilotica* and *Erpobdella johanssoni*. These leech species are known to colonize different water bodies, including rivers, streams, springs, and marshes, where they fulfill important ecological functions. They are often involved in the decomposition of organic matter, nutrient cycling, and maintaining ecosystem balance. The distribution, taxonomy, and ecological dynamics of leech populations in North Africa, particularly Tunisia, have been extensively explored by researchers such as Blanchard (1891, 1908), Seurat (1922), Ben Ahmed et al. (2008a, 2008b, 2008c), Ben Ahmed & Tekaya (2009), and Ben Ahmed et al. (2013). The study of leeches in Tunisia has revealed a diverse array of species, with significant research focusing on taxonomy, distribution, and ecological preferences. Research conducted by Ben Ahmed, Harrath, and Tekaya (2008a) has provided valuable insights into freshwater leech diversity in Tunisia, listing 13 species across 2 orders, 2 suborders, 4 families, and 11 genera. This research also delves into host associations, habitats, global and local distribution patterns, emphasizing the need for further exploration to enhance the current checklist of leech species in Tunisia. However, Research on leech distribution in southern Tunisia is relatively scarce, with few studies specifically focusing on this region. Most of the available literature on leeches in Tunisia has concentrated on general aspects of their taxonomy, ecology, and distribution across the country, without detailed exploration of their distribution patterns in southern regions. The southern part of Tunisia is characterized by diverse and fragile ecosystems, including desert environments, oases, and semi-arid regions. However, due to the limited accessibility and harsh environmental conditions of these areas,

scientific exploration and research activities have been relatively limited compared to other regions. Given the potential importance of leeches in freshwater ecosystems and their ecological significance, further studies are needed to assess their distribution and abundance in southern Tunisia. Hence, this study aims to establish an inventory of freshwater leech species currently present in the southwestern region of Tunisia. Such research could provide valuable insights into the biodiversity of these regions, as well as contribute to our understanding of the ecological roles and conservation needs of leech populations in southern Tunisia. Additionally, investigations into the potential impacts of environmental changes and human activities on leech habitats in these areas are warranted to guide effective conservation and management efforts.

2. MATERIALS AND METHODS

2.1 Sample collection

In this study, leech species were collected from rivers, drainage basins, water sources, and streams located in the governorates of Gafsa, Tozeur, and Gbelli during the period 2020-2023. Free-living species were discovered adhering to the underside of diverse objects, including rocks and submerged vegetation in watercourses. Specimens were collected manually and kept in containers filled with water.

2.2. Laboratory methods and identification

In the laboratory, the collected leech species were identified based on their external morphology. To examine both the external and internal features, the leeches were first anesthetized, then dissected and finally preserved in 70% ethanol solution. The photos were taken with a digital camera (Sony).

3. RESULTS

3.1. *Batracobdella algira*

Order: Rhynchobdelliformes (Blanchard, 1894).
Family: Glossiphonidae (Vaillant, 1894).
Genus: Batracobdella (Viguier, 1879).
Species : *Batracobdella algira* (Moquin-Tandon, 1849).
Batracobdella algira (Fig. 1) is a species of leech belonging to the order Rhynchobdelliformes, family Glossiphonidae, and genus Batracobdella. It was described by Moquin-Tandon in 1849. This species exhibits a dorso-ventrally flattened body with a brown coloration. The dorsal surface is covered with papillae arranged in three longitudinal rows, with larger papillae. It possesses two distinct eyes on the head and a larger posterior sucker. Genital pores are separated by two annuli. *Batracobdella algira* is an ectoparasitic species with a eurytopic habitat, commonly found in various freshwater environments, including drainage basins, rivers, water sources, and marshes. However, it is encountered relatively rarely in the region, with a frequency of 10.75%.

3.2. *Limnatis nilotica*

Order: Arhynchobdelliformes (Blanchard, 1894).
Family: Hirudinidae (Whitman, 1886).
Genus : Limnatis Moquin (Tandon, 1826).
Species : *Limnatis nilotica* (Savigny, 1822).

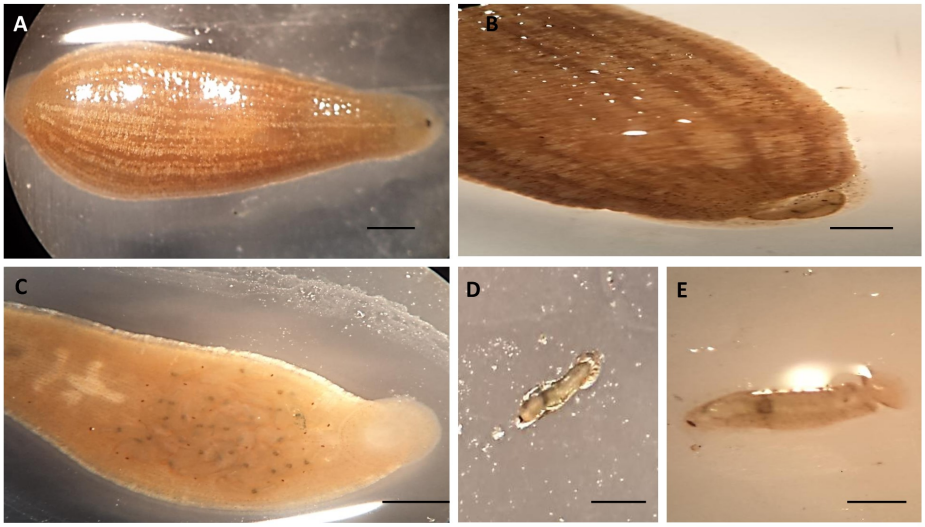

Fig. 1. External morphology of the leech: *Batracobdella algira*. **(A):** Dorsal view, **(B):** Posterior part of the dorsal view showing a powerful sucker. **(C):** Ventral view showing juveniles attached to the ventral surface of the leech (scale = 1.2 cm). **(D-E):** Juveniles of B. algira observed under a light microscope (scale = 0.5 mm).

This species exhibits an elongated, cylindrical, and vermiform body shape, measuring between 10 to 15 cm in length and 1 to 5 cm in width. It possesses five eyes arranged in an arc. The dorsal surface is typically brownish-green in color, characterized by the presence of four longitudinal lines of dark spots on the sides. Additionally, *Limnatis nilotica* features two orange lateral bands. The annuli are well-defined, generally equal in size with slight narrowing towards both suckers. It is a hematophagous ectoparasite species (Fig. 2).

It is a eurytopic ectoparasitic species that colonizes various freshwater habitats, including watersheds, streams, water sources, and marshes. It is the most commonly encountered species, comprising 63.01% of all individuals collected in the visited regions.

3.3. *Erpobdella johanssoni*

Suborder: Erpobdelliformes (Sawyer, 1986).
Family: Erpobdellidae (Blanchard, 1894).
Genus: Erpobdella (De Blainville, 1818).
Species: *Erpobdella johanssoni* (Johansson, 1927).

Erpobdella johanssoni is characterized by its cylindrical body shape, ranging in color from reddish-brown in juveniles to dark brown-black in adults. It features a pair of paramedian dark bands and two rows of light dots on each annulus. The body is strongly compressed at its basal part with lateral expansions, measuring between 7 and 8.5 cm in length. The annuli are of equal size, with a "double" annulus between every four rings. This species has eight eyes: four frontal eyes and two pairs arranged on the sides in a lateral line. Notably, it is a macrophagous species of aquatic invertebrates, distinguished by the absence of jaws and the presence of a wide mouth at the anterior sucker (Fig. 3).

Erpobdella johanssoni primarily inhabits stagnant waters in lowlands. It ranks second in frequency, accounting for 25.31% of the observed specimens.

4. DISCUSSION

The inventory of leeches identified in this study includes three species, with the most dominant species found everywhere in the examined regions being *Limnatis nilotica* (63.01%), followed by *Erpobdella johanssni* (25.31%), and finally *Batracobdella argila* (10.75%). The species *Limnatis nilotica* has been identified as a Mediterranean species present in various regions including Egypt, Turkestan, the Middle East, Italy, and Spain (Ben Ahmed et al., 2015). *Erpobdella johanssoni,* another Mediterranean species, has been observed in Italy and Morocco, with reports of its presence in the Iberian Peninsula by Jueg (2008). *Batracobdella algira* was initially documented in Algeria and classified under the genus Batracobdella by Viguier (1879). Nesemann (1999) noted this uncommon species, primarily found in northwest Africa and the western Mediterranean (Lunghi et al., 2018). The species found in the southwestern region of Tunisia are considered common throughout the country, inhabiting various environments such as watersheds, water sources, and marshes. A comparison with the study by Raja et al. (2015), which explores freshwater leech species across Tunisia, reveals a notable diversity difference between specimens from the northern and southern regions. This comparison underscores significant variations in species composition based on the regions studied, indicating a lower species diversity in the southwestern region of Tunisia compared to the northern areas. The diversity of leech species in northern Tunisia showcases a richer variety compared to the southwestern region, with a total of 13 distinct species identified, including three that were specifically collected in the southwest. This study showed the notable absence of several leech species recorded in northern Tunisia from the current list. Noteworthy examples include *Alboglossiphonia Hyalina*, a species exclusively confined to the Nabeul governorate at Lebna Dam; *Helobdella stagnalis*, documented in regions spanning El Kef, Siliana, and Bizerte; *Placobdella costata*, observed in both Jendouba and El Kef regions; and *Theromyson tessulatum*, a species found solely within the Nabeul region. The distribution and abundance of leech species in Tunisia may vary depending on factors such as habitat availability, water quality, and human activities. Further research is needed to better understand the diversity, ecology, and conservation status of leech populations in Tunisia, as well as their potential interactions with other aquatic organisms and ecosystems. Such studies could contribute to the development of effective conservation strategies and management practices for freshwater habitats in Tunisia. The southwestern region of Tunisia presents a unique ecological niche characterized by its Saharan environment and semi-arid climate. In this challenging landscape, the distribution of leech species is intricately tied to the availability of water and the capacity to withstand extreme environmental conditions.

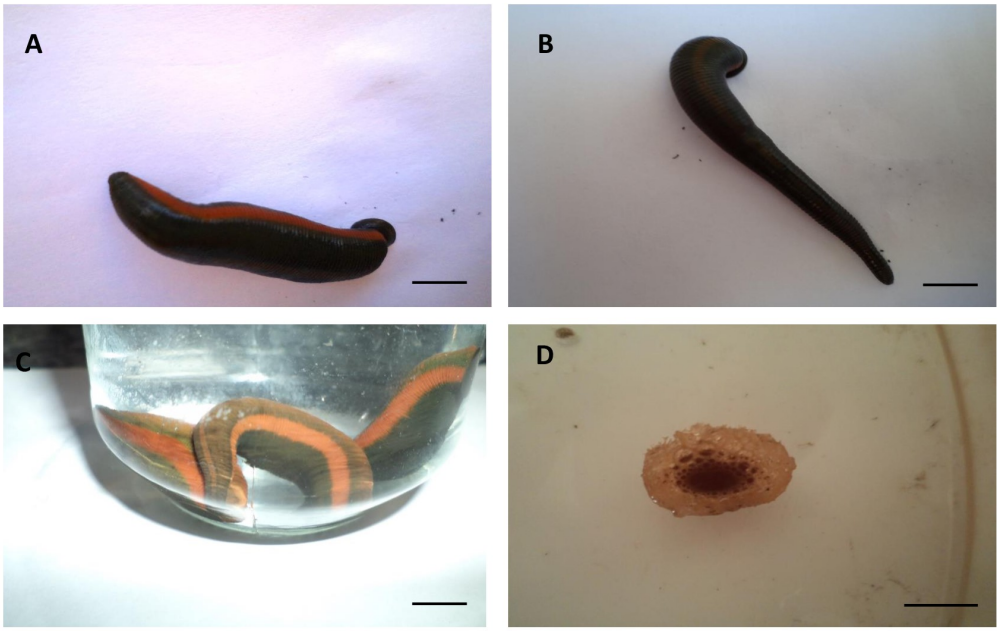

Fig. 2. External morphology of the leech: *Limnatis nilotica*. (A-B): Dorsal view. (C): Lateral and ventral view. (D): Recently laid cocoon of L. nilotica (scale = 1.2 cm).

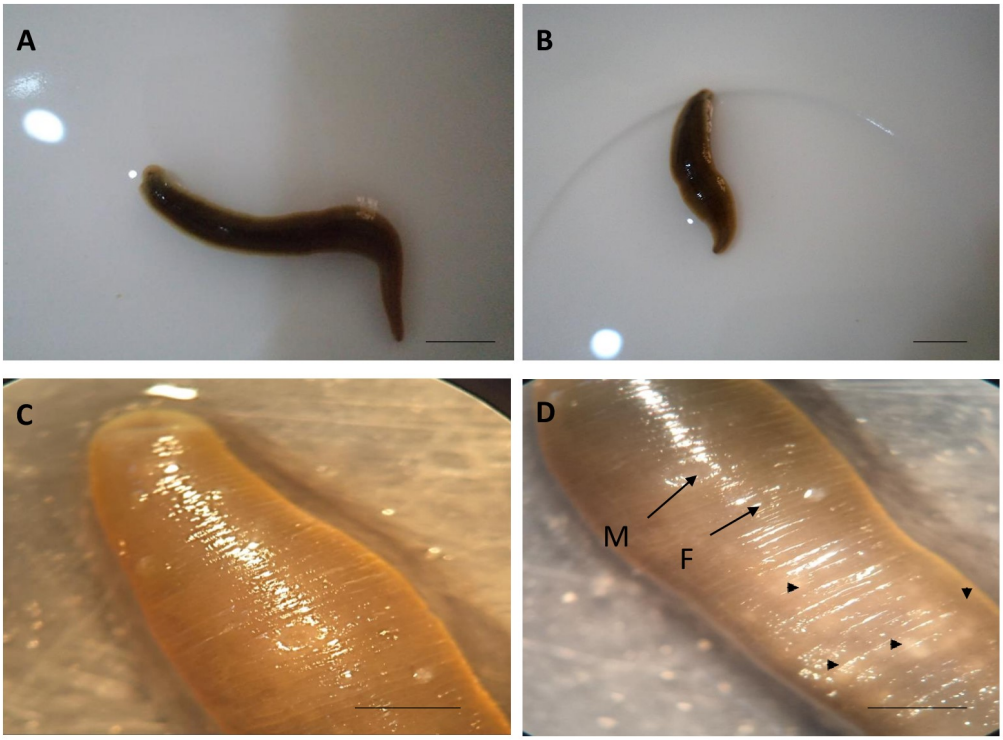

Fig. 3. External morphology of the leech: *Erpobdella johanssoni*. (A-B): Dorsal view. (C-D): Ventral view. Adult *Erpobdella johanssoni* showing the two gonopores (M: male and F: female) and the visible development of the vas deferens (arrowheads) (scale = 1.2 cm).

The arid nature of the region poses significant challenges for many organisms, yet certain leech species have managed to adapt and thrive in these harsh environments. Their presence in the area suggests a remarkable ability to withstand fluctuations in temperature and cope with limited water resources. These adaptations likely stem from a combination of physiological, behavioral, and morphological traits that enable them to survive in such demanding conditions. One key adaptation of leech species in southwestern Tunisia is their ability to endure prolonged periods of drought. These leeches have developed mechanisms to conserve moisture and reduce water loss during dry spells. Their highly efficient excretory systems minimize water loss, while specialized behaviors such as burrowing into moist substrates or

aestivating during periods of extreme aridity help them survive when water sources are scarce. Additionally, these leech species have likely evolved physiological adaptations to tolerate high temperatures prevalent in the region. Through adjustments in their metabolism and biochemical processes, they can maintain homeostasis even in environments where temperatures soar during the day and plummet at night. Such thermal tolerance enables them to remain active and forage for prey despite the challenging climate. Furthermore, the ability of these leech species to thrive in environments with potentially polluted water sources indicates a certain level of resilience to contaminants. They may possess detoxification mechanisms or symbiotic relationships with microorganisms that aid in the breakdown of pollutants, allowing them to persist in habitats affected by human activities such as agriculture or industrialization. Research into the specific ecological adaptations of these leech species in southwestern Tunisia would be invaluable for understanding their resilience and distribution patterns in arid regions. By elucidating the mechanisms behind their survival in such challenging environments, scientists can gain insights into broader questions related to species' responses to climate change and habitat degradation. Moreover, this knowledge could inform conservation efforts aimed at preserving biodiversity in arid ecosystems worldwide.

5. CONCLUSIONS

This study illustrates the different species of leeches present in the southwestern region of Tunisia, showing a dominance of the *Limnatis nilotica* species, followed by *Erpobdella johanssoni* and finally *Batrcobdella algira*. Unlike many species present in northern Tunisia, these species have been able to tolerate environmental fluctuations by adapting to changes in bioclimatic conditions. Thus, the impact of human activity, climate change, and environmental pollution can have direct deleterious consequences on the depletion of biological diversity.

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