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LEECH PARASITISM IN *PELOPHYLAX SAHARICUS* AND *DISCOGLOSSUS PICTUS* (ANURA, RANIDAE, ALYTIDAE) IN NORTH-EASTERN ALGERIA

N. Cheribiri^{1,*}, O. Boughaba², N. Ziane¹, S. Benyacoub³ & R. Rouag⁴

¹ Laboratory of Environmental Bio-surveillance, Department of Biology, Faculty of Sciences, Badji Mokhtar — Annaba University 12 P.O. Box, 23000 Annaba, Algeria

² Laboratory of Biodiversity and Ecosystems Pollution.

Chadli Bendjedid University, El Tarf, Algeria

³ Department of Biology, Faculty of Science, Chadli Bendjedid University, El Tarf, Algeria

⁴ Laboratory of Environmental Bio-surveillance, Department of Biology, Faculty of Sciences, Badji Mokhtar — Annaba University 12 P.O. Box, 23000 Annaba, Algeria

* Corresponding author

E-mail: nabila.cheribiri@univ-annaba.dz

N. Cheribiri (<https://orcid.org/0009-0006-7343-1030>)

O. Boughaba (<https://orcid.org/0000-0001-5861-680X>)

N. Ziane (<https://orcid.org/0000-0002-9999-2040>)

R. Rouag (<https://orcid.org/0000-0003-1145-0165>)

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Leech Parasitism in *Pelophylax saharicus* and *Discoglossus pictus* (Anura, Ranidae, Alytidae) in North-Eastern Algeria. Cheribiri, N., Boughaba, O., Ziane, N., Benyacoub, S. & Rouag, R. — Populations of two amphibian species, *Pelophylax saharicus* (Boulenger in Hartert, 1913) and *Discoglossus pictus* Otth, 1837, widely distributed in North-eastern Algeria, face multiple ecological threats, including parasitism by freshwater leeches. This study investigates the infestation of ectoparasites in the Edough Massif and Tonga Lake, analysing the prevalence and intensity of leech infestations. A total of 187 leeches, identified as *Batrachobdella algira* (Mouquin-Tandon, 1846), were collected and examined from 93 host individuals. Parasitism was more prevalent in Edough, though intensity was higher in Tonga Lake. Statistical analyses revealed no significant correlation between parasite load and host morphology, suggesting that infestation is influenced by environmental and ecological factors rather than host size.

Key words: *Pelophylax saharicus*, *Discoglossus pictus*, leeches, prevalence, infestation, Tonga Lake, Edough Massif, Algeria.

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Introduction

Algeria is strategically located as a biogeographical contact zone between the Maghreb and Europe. It is home to several endemic amphibian species or those with limited distribution. However, amphibians have been relatively understudied here compared to other countries in the Maghreb region (Ben Hassine & Escoriza, 2017).

Discoglossus pictus Otth, 1837, the painted frog, and *Pelophylax saharicus* (Boulenger in Hartert, 1913), the North African green frog, are widespread in Algeria. The painted frog is commonly found in the northern part of the country, where it inhabits cultivated and semi-arid steppes; it can also be found in humid mountainous forest areas. On the other hand, *P. saharicus* is known to occur in humid to arid environments. It primarily breeds in large bodies of water, including temporary, semi-permanent, or permanent rivers, springs, rock pools, and reservoirs. The presence or absence of aquatic emergent vegetation does not significantly impact its breeding behaviour (Ben Hassine & Escoriza, 2017; Hassine & Nouira, 2012).

All 14 amphibian species in Algeria are considered to be in danger due to natural climatic changes, over-exploitation of aquifers, and eutrophication or pollution (Mateo et al., 2013). Amphibians are more threatened and declining more rapidly than birds or mammals, with 48% of rapidly declining species facing extinction due to unidentified processes (Stuart et al., 2004).

Frogs and other amphibians are frequent hosts of leeches (Hirudinea), which are common ectoparasites in freshwater environments. Their attachment to hosts for blood feeding may affect amphibian health through increased stress, disease transfer, and direct blood loss (Sauer et al., 2020). Predation and parasitism by leeches can lead to population declines, especially in vulnerable amphibian species with fragmented populations, as observed in North Africa (Merabet, 2021).

Leeches pose a multifaceted threat to frogs, acting as both ectoparasites and predators, and their impact can be severe, especially under conditions of high infestation or environmental stress. The primary threats include direct blood loss, impaired locomotion, increased mortality, reduced growth and survival of tadpoles, and the potential for disease transmission. High leech loads have been observed to cause significant difficulty in frog movement and can be fatal, particularly when more than two leeches are present on a single frog (Benitez et al., 2023; Conan et al., 2023). In addition to direct parasitism, leeches prey on frog eggs and larvae, sometimes inducing early hatching as a defensive response, which may affect developmental success (Chivers et al., 2001; Ireland et al., 2007; Schalk et al., 2002). Some leech species can even act as endoparasites, living internally within frogs (Mann & Tyler, 1963). Furthermore, leeches may serve as vectors for pathogens, contributing to the spread of diseases among amphibian populations (Raffel et al., 2006; Siddall & Dessler, 1992).

The impact of leech parasitism is influenced by environmental factors such as anthropogenic changes, pollution, and habitat disturbance, which can exacerbate the prevalence and severity of infestations (Benitez et al., 2023; Conan et al., 2023; Schmeller et al., 2018). Collectively, these findings highlight the significant and sometimes underappreciated role of leeches in amphibian morbidity, mortality,

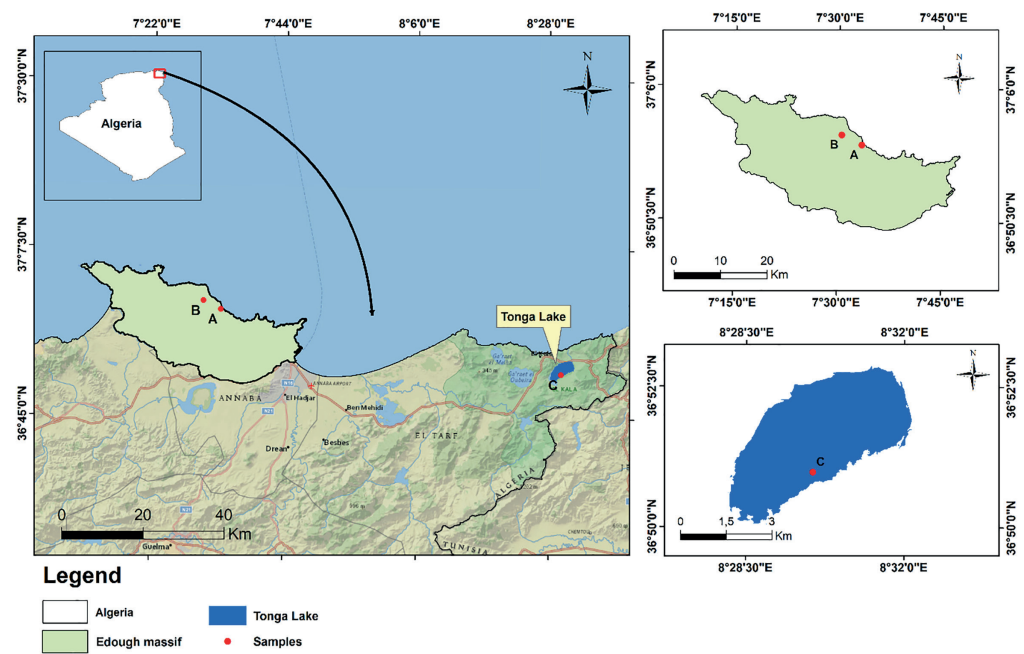


Fig. 1. Location of the study site and sampling areas

and population declines (Berven & Boltz, 2001; Comesaña Iglesias & Ayres, 2008; Merabet, 2021; Merilä & Sterner, 2002; Stead & Pope, 2010). Further research is needed to understand the full impact of leech predation on amphibian populations and to develop conservation strategies to mitigate these effects (Conan et al., 2023; Merabet, 2021).

Leech predation on amphibians is a widespread occurrence; however, in North Africa, it has only been recorded in a limited number of brief reports (Beukema et al., 2010; Merabet, 2021; Merabet et al., 2017). This study aims to characterise the morphological parameters of *P. saharicus* and *D. pictus* populations in the Edough Massif and Tonga Lake. Additionally, it seeks to assess the prevalence of leech parasitism within these populations, providing valua-

Table 1. The two sites environmental characteristics

Site	Coordinate	Area, m ²	Depth, m	Elevation, m	Plant species
El-Golla	36°59'22.7" N, 7°33'23.7" E	15	1.50	412	<i>Ranunculus aquatilis</i> L., 1753; <i>Ceratophyllum submersum</i> L., 1753; <i>Convolvulus arvensis</i> L., 1753; <i>Juncus acutus</i> L., 1753
Daouret Eldib	37°00'30.9" N, 7°30'28.4" E	15	2.5	350	<i>Pistacia lentiscus</i> L., 1753; <i>Lotus corniculatus</i> L., 1753; <i>Rubus ulmifolius</i> Schott, 1818; <i>Callitriche obtusangula</i> Le Gall, 1852; <i>Chara vulgaris</i> L., 1753; <i>Myriophyllum alterniflorum</i> DC., 1813



Fig. 2. El-Golla



Fig. 3. Daouret Eldib

ble insights into host-parasite interactions and potential ecological impacts on amphibian health in these unique habitats.

Material and Methods

Study area

Sampling was conducted during the breeding season (March–May) of 2021 and 2022 at two localities: the Edough Massif and Tonga Lake (Fig. 1).

The Edough Massif sampling sites are located at high altitudes within a cork oak forest. This massif is a Mediterranean coastal region in northeastern Algeria, bordered to the north by the Mediterranean Sea, to the west by the Oued El Kebir valley, to the south by Fetzara Lake, and to the east by the Kherraza plain (Oularbi & Zeghiche, 2009). Two

sites were sampled: El-Golla (Fig. 2), a buttercup pond, and Daouret Eldib (Fig. 3), a temporary artificial pond.

Tonga Lake is located in the El Kala National Park in northeastern Algeria. It is a sizeable shallow lake with a mean altitude of 2.20 m, a surface area of 2200 ha, and a maximum depth of 1.5 m. Its two tributaries are Oued El Eurg from the east and Oued El Hout from the southeast. The constructed Messida canal in the northern part of Tonga Lake connects it to the Mediterranean Sea (Boughaba et al., 2024).

Environmental parameters

Edough experiences a humid Mediterranean climate, with an average annual temperature of 16 °C and substantial precipitation often exceeding 1200 mm (Hadj-Zobir, 2012). At Tonga Lake, the mean seasonal temperatures were recorded at 16.23 °C in winter and 25.24 °C in summer. Water temperatures varied between 15.11 °C and 17.11 °C during winter, while summer temperatures ranged from 20.42 °C to 29.73 °C. Dissolved oxygen levels in surface water averaged between 8.80 and 9.27 mg/L. Salinity remained low, ranging from 0.02% to 0.07%, while the water pH fluctuated between neutral and alkaline levels (Naili et al., 2021).

Sampling

Frogs were visually surveyed and captured by hand. For *P. saharicus*, the Edough sample comprised 70 individuals (40 males and 30 females), while the Tonga sample included 32 individuals (23 males and 9 females). In both sites, males outnumbered females. For *D. pictus*, the Edough population included 23 individuals (9 males and

14 females), whereas in Tonga, there were 11 individuals (6 males and 5 females). Unlike *P. saharicus*, *D. pictus* showed a more balanced or female-skewed ratio, particularly in Edough.

Frogs were temporarily kept in moist cloth bags for a few hours (maximum 24 hours) to maintain hydration before being transported to the laboratory for analysis. In the laboratory, each frog was weighed using a digital scale (± 0.1 g), and morphometric measurements were taken with a digital caliper (± 0.01 mm): Snout-Vent Length (SVL), Foot Length Right (FOLR), Foot Length Left (FOLL), Femur Length Right (FLR), Forelimb Length Left (FLL), Head Length (HL), Head Width (HW), Eye-Snout Distance (ES), and the distance between the anterior corners of the eyes (EAD). The sex of individuals was determined by macroscopic examination of the gonads during dissections performed within the framework of a parallel investigation on endoparasites of the two species.

Statistical analyses

Leech prevalence (%) and intensity were analysed using descriptive statistics (mean \pm standard deviation, range). For *P. saharicus*, group comparisons were performed using Pearson's correlation coefficient, the Mann-Whitney test, the Kruskal-Wallis test, and independent-sample t-tests, with significance set at $p \leq 0.05$.

For *D. pictus*, where sample sizes (particularly for females in some sites) were small and unbalanced, non-parametric exact tests (Wilcoxon-Mann-Whitney with exact p-values) were used to compare groups, as these methods do not assume normality and are robust for small datasets. Effect sizes for these tests were calculated as rank-biserial correlations to provide a measure of the magnitude of differences regardless of statistical significance. All analyses were conducted in R (version 4.3.2).

Results

Prevalence and intensity of leeches infection

A total of 187 leeches were collected and carefully examined. The identification revealed the presence of a single leech species, *Batrachobdella algira* (Moquin-Tandon, 1846) (Hirudinida: Glossiphoniidae) (Fig. 4).

Leeches parasitising *P. saharicus* were smaller in both length and width compared to those on *D. pictus*, with mean lengths of 8.05 mm in Edough and 7.38 mm



Fig. 4. *B. algira* attached to *P. saharicus* and *D. pictus*

in Tonga Lake, while leeches on *D. pictus* reached 24.64 mm in Edough but only 9.33 mm in Tonga Lake. Two-way ANOVA revealed highly significant effects of Host Species (Length: $F = 351.29$, $p < 0.001$; Width: $F = 331.03$, $p < 0.001$), Site (Length: $F = 57.47$, $p < 0.001$; Width: $F = 51.71$, $p < 0.001$), and their interaction (Length: $F = 181.00$, $p < 0.001$; Width: $F = 174.72$, $p < 0.001$), indicating that both host species and environmental conditions significantly influenced leech size. Post-hoc Tukey tests confirmed that leeches on *D. pictus* in Edough were significantly larger in both length and width than leeches in the other groups ($p < 0.001$), whereas differences among *P. saharicus* across sites were smaller and mostly non-significant. Non-parametric Kruskal-Wallis tests supported these findings (Length: $\chi^2 = 35.68$, $p < 0.001$; Width: $\chi^2 = 36.62$, $p < 0.001$) (Table 2).

For *P. saharicus*, the Mann-Whitney test comparing parasite intensity between males and females yielded $W = 1381$, $p = 0.2996$, indicating no significant difference in parasite load between sexes. However, the Kruskal-Wallis test for site comparison showed a significant difference ($\chi^2 = 7.39$, $df = 1$, $p = 0.0065$), suggesting that parasite intensity varied between Edough and Tonga Lake. Total prevalence was higher in Edough (60.00%) than in Tonga Lake (25.00%), indicating that individuals from Edough were more frequently infected. Total mean parasite intensity was higher in Tonga Lake (4.00 ± 2.67 , range 1–10) compared to Edough ($3.21 \pm$

Table 2. Measurements of leeches

Species	Site	N	Length, mm		Width, mm	
			Mean \pm SD	Min–Max	Mean \pm SD	Min–Max
<i>P. saharicus</i>	Edough	135	8.05 \pm 2.34	4–12	4.41 \pm 1.32	2–7
	Tonga	32	7.38 \pm 1.86	5–10	4.09 \pm 1.03	3–6
<i>D. pictus</i>	Edough	11	24.64 \pm 1.21	23–26	13.64 \pm 1.29	11–15
	Tonga	9	9.33 \pm 2.29	7–13	5.11 \pm 1.05	4–7

Table 3. Prevalence and Intensity by Site and Sex in *P. saharicus* and *D. pictus*

Species	Site	Sex	N	Prevalence, %	Intensity	
					Mean \pm SD	Range
<i>P. saharicus</i>	Edough	Female	30	63.33	3.26 \pm 2.26	1–9
		Male	40	57.50	3.17 \pm 2.64	1–10
		Total	70	60.00	3.21 \pm 2.44	1–10
	Tonga	Female	10	30.00	3.33 \pm 1.15	2–4
		Male	22	22.73	4.40 \pm 3.36	1–10
		Total	32	25.00	4.00 \pm 2.67	1–10
<i>D. pictus</i>	Edough	Female	14	28.57	1.75 \pm 0.96	1–3
		Male	9	33.33	1.33 \pm 0.58	1–2
		Total	23	30.43	1.57 \pm 0.79	1–3
	Tonga	Female	4	50.00	2.50 \pm 0.71	2–3
		Male	7	42.86	1.33 \pm 0.58	1–2
		Total	11	45.45	1.80 \pm 0.84	1–3

2.44, range 1–10), suggesting that while fewer individuals in Tonga Lake were infected, they tended to carry heavier parasite loads. These results indicate that parasite intensity did not significantly differ between males and females ($W = 289.0$, $p = 0.2996$), but differed significantly between sites ($\chi^2 = 9.41$, $df = 1$, $p = 0.0065$). The higher prevalence in Edough (60.00%) and higher intensity in Tonga Lake (4.00) suggest that environmental or host-related factors influenced both the likelihood and severity of infestation.

For *D. pictus* There was no significant difference in the prevalence of parasites between sites ($\chi^2 = 0.85$, $df = 3$, $p = 0.8384$) or in parasite intensity between males and females ($W = 26$, $p = 0.1899$). However, prevalence was higher in Tonga (45.45%) than in Edough (30.43%), and *Discoglossus pictus* females in Tonga had the highest prevalence (50%). Parasite intensity was slightly higher in females (2.50 in Tonga, 1.75 in Edough) compared to males (1.33 in both sites), but the difference was not statistically significant. In *D. pictus*, parasite intensity did not differ significantly between sexes (Wilcoxon–Mann–Whitney exact test: $Z = 0.081$, $p = 0.954$), with a very small effect size (rank-biserial correlation = 0.0139). The small sample size, especially in some groups (e. g., Tonga females, $n = 4$), may have limited statistical power to detect significant differences. (Table 3).

The relation between parasite intensity and morphometric parameters in the populations of Edough and Tonga
The correlations between parasite load and morphological parameters of *P. saharicus* were generally weak, with p-values often exceeding 0.05, suggesting no significant relationship in most cases. However, slight correlations were observed in the Tonga Lake population, notably with SVL ($r = 0.28$, $p = 0.032$), FOLR ($r = 0.27$, $p = 0.029$), and HW ($r = 0.26$, $p = 0.021$). Overall, morphological structure appeared to be more influenced by internal factors than by parasite load (Fig. 5).

For *D. pictus*, the results also indicated no positive correlations between parasite load and morphological parameters; all correlations remained statistically non-significant ($p > 0.05$), suggesting that parasite intensity did not strongly in-

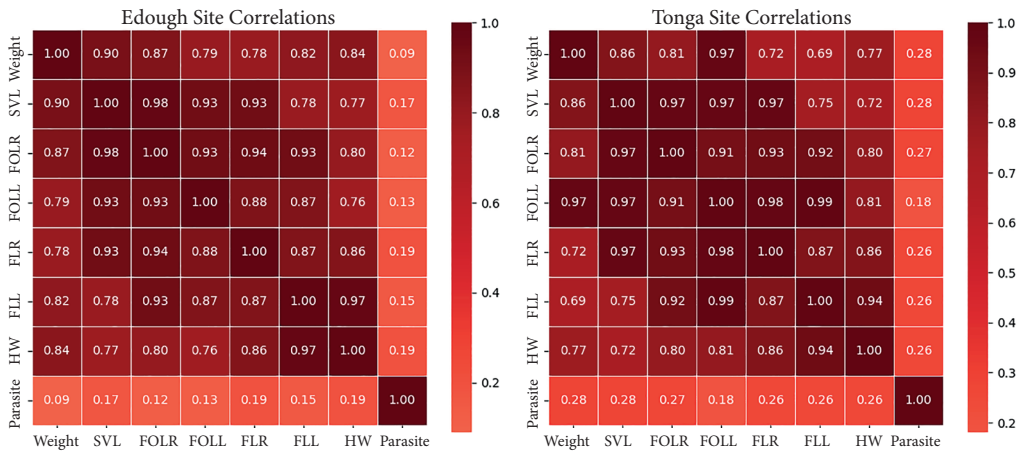


Fig. 5. Correlations between mean parasite intensity and *P. saharicus* morphometric parameters

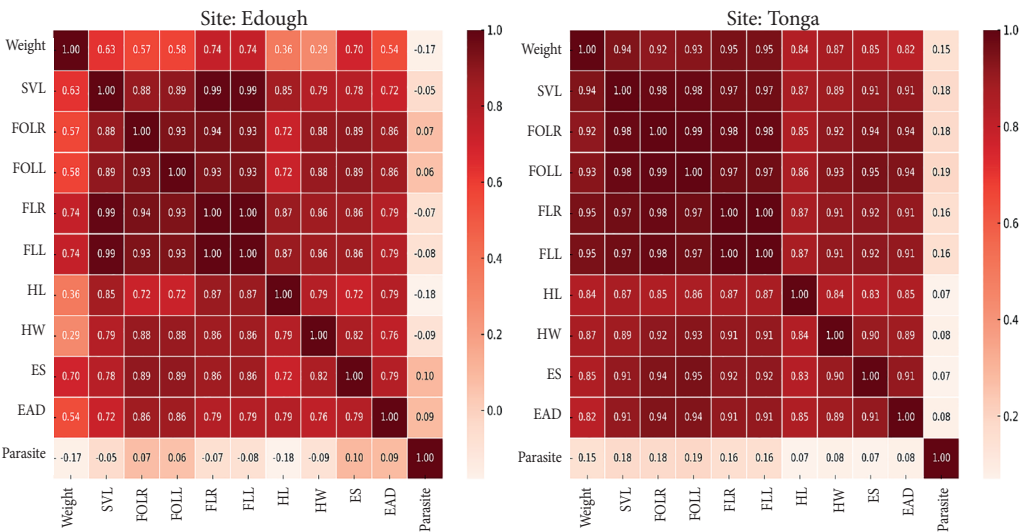


Fig. 6. Correlations between mean parasite intensity and *D. pictus* morphometric parameters

fluence the measured morphometric traits. These findings imply that other ecological or physiological factors may play a more significant role in shaping the morphology of *D. pictus* across these sites (Fig. 6).

Attachment sites

Figure 7 provides an overview of parasite attachment prevalence across different body sites, sampling locations, and sexes in *P. saharicus*. The highest prevalence (approximately 35%) was observed at the tympanum of males from Edough, suggesting that this anatomical site may be particularly favourable for parasite attachment in this population. In contrast, males from Tonga Lake exhibited lower tympanum infestation, with prevalence below 15%. The trunk and tympanum of females from Tonga Lake also showed relatively high infestation rates, reaching around 20–25%, while females from Edough displayed a more balanced distribution of parasites across multiple body sites. The forelimbs and head of females from Tonga Lake showed prevalence levels of around 15%, whereas hindlimbs generally had low infestation rates across all groups,

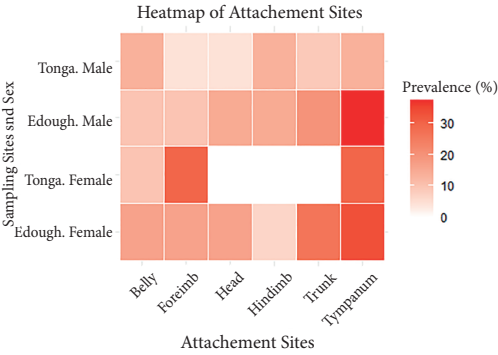


Fig. 7. Prevalence of attachment sites on *P. saharicus*

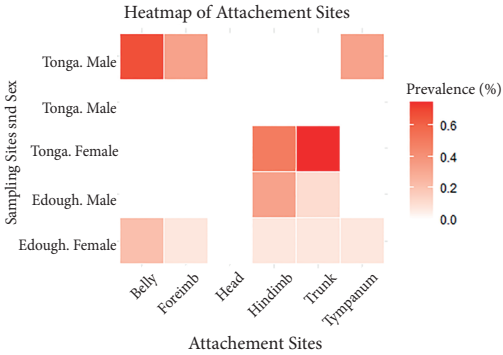


Fig. 8. Prevalence of attachment sites on *D. pictus*

rarely exceeding 5–10%. Interestingly, some body sites, such as the belly in certain groups, showed 0% prevalence, indicating that these areas might be less suitable for parasite attachment or that environmental factors played a role in infestation patterns.

For *D. pictus*, the highest prevalence (around 70%) was observed in the trunk and hindlimb regions for males from Tonga Lake, while other significant values included approximately 50% on the belly and 40 % on the forelimb for the same group. Males from Edough showed moderate prevalence, with values ranging between 30–50% on the hindlimb, trunk, and forelimb. In contrast, the female group from Edough exhibited the lowest prevalence, mostly below 20% across all attachment sites (Fig. 8).

Discussion

Only one species of leech was found parasitising the studied populations: *Batrachodella algira* (Moquin-Tandon, 1846). This species has been reported in North Africa, particularly in Algeria and Tunisia, where it parasitises local amphibians such as *Bufo spinosus* Daudin, 1803 and *P. saharicus* (Ahmed et al., 2009; Merabet, 2021). It has also been found in Sardinia, Italy, parasitising terrestrial salamanders of the genus *Hydromantes* (Lunghi et al., 2018; Manenti et al., 2016). The leech was identified in southern England, affecting common toads and frogs; its presence in the UK is likely due to introduction, as genetic data matches specimens from Tunisia (Seilern-Macpherson et al., 2024).

The findings of this study provide valuable insights into the size variations of leeches parasitising *P. saharicus* and *D. pictus*, highlighting the influence of host species and geographic location on parasite growth. The general size distribution indicates that most examined leeches fall within the medium-size category (10–15 mm), which aligns with previous observations on glossiphoniid leeches, such as *Batrachodellodes moogi* (Bielecki et al., 2011), where distinct size classes have been reported in relation to host and environmental factors.

A more detailed comparison reveals that leeches from *P. saharicus* are consistently smaller in both length and width than those found on *D. pictus*. Additionally, site-dependent differences are evident, particularly in *D. pictus*, where leeches from Edough reach significantly larger sizes (24.64 mm) compared to those from Tonga Lake (9.33 mm). This suggests that *D. pictus* in Edough provides more favourable conditions for leech growth, possibly due to factors such as higher infestation rates or prolonged feeding periods.

The correlation analysis reveals strong relationships between the morphological variables of *P. saharicus*, but weak and non-significant correlations ($r < 0.2$, $p > 0.05$) between parasite intensity and these traits. Only slight correlations with SVL ($r = 0.28$, $p = 0.032$), FOLR ($r = 0.27$, $p = 0.029$), and HW ($r = 0.26$, $p = 0.021$) suggest a moderate effect of parasites. Similarly, in *D. pictus*, the correlation between parasite intensity and morphometric parameters across the two study sites (Edough and Tonga Lake) remains weak and statistically non-significant ($p > 0.05$). The strongest positive associations were found in Tonga Lake for FLR ($r = 0.27$) and FLL ($r = 0.26$),

while the strongest negative correlation appeared in Edough for ES ($r = -0.18$). These findings indicate that parasite intensity does not strongly influence the measured morphometric traits in *D. pictus*.

In comparison, the study on *B. algira* parasitising *Hydromantes* salamanders (Lunghi et al., 2018) also reported low prevalence (< 3%, except for *H. flavus* at 9%) and a host size influence, with no direct link to environmental conditions. Overall, parasite intensity does not appear to significantly shape the morphology of *P. saharicus* or *D. pictus*, suggesting that intrinsic factors such as genetics and growth play a more decisive role. However, host species and environmental conditions may still contribute to variations in parasite prevalence and intensity.

Leeches exhibit specific attachment preferences on amphibian hosts, targeting areas with abundant blood supply and thinner skin. *B. algira* has been observed infesting common toads and frogs in the UK, attaching to areas like the eyes, throat, and axillae (Seilern-Macpherson et al, 2024) These preferences align with findings in *P. saharicus*, where the tympanum region shows a high prevalence of leech attachment, particularly in males from Edough. Similarly, in *D. pictus*, males from Tonga exhibit significant leech attachment on the trunk and hindlimb regions. These patterns suggest that leeches select attachment sites based on host anatomical features that facilitate feeding, such as areas with thinner skin and rich blood supply.

Conclusion

This study provides key insights into the morphology and leech parasitism of *P. saharicus* and *D. pictus* populations from two ecologically distinct regions in Algeria. The identification of *B. algira* as the primary ectoparasite highlights the role of parasitism in amphibian population dynamics. While leech prevalence was higher in Edough, infestation intensity was greater in Tonga Lake, suggesting site-specific ecological pressures. The weak correlation between parasite load and host morphology indicates that factors such as habitat conditions, water quality, and host behaviour likely play a more significant role in infestation patterns. Given the ongoing environmental threats to amphibian populations in Algeria, including habitat degradation and climate change, further research is necessary to explore the broader ecological implications of parasitism and to develop effective conservation strategies.

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