MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *POLYSTOMOIDES OCELLATUM* IN *EMYS ORBICULARIS* FROM AN ALGERIAN LACUSTRINE ECOSYSTEM

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Received February 2026; Accepted March 2026; Published May 2026;

DOI: https://doi.org/10.31407/ijees16.239

ABSTRACT

The El Kala region, located in El Taref Province in northeastern Algeria, is a coastal wetland recognized as a Ramsar site since 1980 and designated as a national park in 1983. This area supports remarkable floristic and faunal diversity. Two freshwater turtle species occur in this ecosystem: *Mauremys leprosa* (Schweigger, 1812), an endemic species in Algeria, and *Emys orbicularis* (Linnaeus, 1758), which is highly sensitive to environmental disturbance and pollution, making it a valuable bioindicator of ecosystem health. Despite its ecological importance, this species remains poorly investigated from a parasitological perspective. The present study focuses on the morphological and molecular characterization of a monogenean parasite belonging to the family Polystomatidae, *Polystomoides ocellatum* (Rudolphi, 1819), infecting the oral cavity of *E. orbicularis*. A total of 19 turtles were examined from two lacustrine ecosystems, Lake Oubeira and Lake Tonga. Eight individuals were found to be infected, yielding a prevalence of 42.1%, a mean abundance of 0.53, and a mean intensity of 1.25. Comparative analysis between the Algerian population and previously described populations from Corsica, where the parasite infects *E. orbicularis*, and Morocco, where the host species is *M. leprosa*, revealed significant intraspecific variability, notably in the shape of the testis and hamuli. Phylogenetic analysis based on the mitochondrial cytochrome c oxidase subunit I (COI) gene, comparing the Algerian population with populations from France and Spain sharing the same host species, showed a genetic divergence of 2.7%. The Algerian specimens formed a distinct subclade within the *P. ocellatum* lineage. These findings highlight both genotypic and phenotypic intraspecific differentiation in *P. ocellatum* populations from Algeria. This differentiation likely reflects local adaptation to environmental conditions within the host habitat. The occurrence of this parasite further supports its potential role as a bioindicator of the ecological status of the lacustrine ecosystems of the El Kala wetland complex.

Keywords: Wetland ecosystem, parasite, Monogenea, *Polystomoides ocellatum*, *Emys orbicularis*, Algeria.

INTRODUCTION

Wetlands are globally important ecosystems that support exceptional biodiversity and provide essential ecosystem services, including hydrological regulation, carbon sequestration, and the maintenance of trophic dynamics (Mitsch and Gosselink, 2015). The El Kala wetland, located in northeastern Algeria, represents one of the best-preserved wetland systems in the Mediterranean Basin, despite increasing pressure from pollution and anthropogenic activities (Saadali et al., 2015). It is characterized by a mosaic of lacustrine, marsh, and coastal environments forming a heterogeneous ecological gradient of high biological value. This site was designated as a national park in 1983 and later recognized as a UNESCO Biosphere Reserve in 1990. It also includes several wetlands of international importance (Saadali et al., 2015).

Freshwater turtles play a key role in aquatic trophic networks. Two species coexist in the El Kala wetland: *Mauremys leprosa* (Schweigger, 1812) and *Emys orbicularis* (Linnaeus, 1758), both widely distributed across North Africa and southern Europe (Fritz and Havaš, 2007). *E. orbicularis* is particularly sensitive to pollution and anthropogenic disturbance and is affected by habitat degradation and fragmentation, as observed in both European and North African populations, which makes it especially vulnerable (Gherbi et al., 2023).

The study of the parasitofauna of these turtles provides valuable insight into host-parasite interactions in ecologically connected and biologically diverse environments (Combes, 2001; Santana et al., 2023). Monogeneans of the genus *Polystomoides* (Platyhelminthes: Polystomatidae Gamble, 1896) are exclusive parasites of freshwater chelonians and have a nearly worldwide distribution. They infect the buccopharyngeal and nasal cavities, urinary bladder, and conjunctival sacs. They have a direct life cycle: after hatching, a free-swimming larva (oncomiracidium) emerges from the egg and directly seeks a new host (Lambert et al., 1978).

These parasites are characterized by a high degree of host and site specificity, and their distribution within distinct microhabitats reflects morphological and functional adaptations as well as coevolution with their hosts (Chaabane et al., 2022). *Polystomoides ocellatum* (Rudolphi, 1819) has been reported in *E. orbicularis* and *M. leprosa* in Europe and Africa (Knoeppfler and Combes, 1977; Lambert et al., 1978; Combes and Thiéry, 1983). Its occurrence is closely linked to that of its host, but is also influenced by environmental factors such as climate, water temperature, pH, salinity, and the abundance of free-living stages in aquatic environments (Combes, 2001; Goedknegt et al., 2015).

The present study combines two complementary approaches, morphological examination and molecular analysis using the mitochondrial cytochrome c oxidase subunit I (COI) gene as a genetic marker. It aims to characterize *P. ocellatum* infecting the oral cavity of *E. orbicularis* in Algerian lacustrine ecosystems, a system that has not previously been investigated using both approaches simultaneously. It also compares the obtained sequences with those of populations from France and Spain and examines morphological variation relative to populations previously described in Corsica (Knoeppfler and Combes, 1977) and Morocco (Combes and Thiéry, 1983), with particular attention to the potential influence of environmental conditions and host geographical distribution.

MATERIALS AND METHODS

Study Area

Host Collection and Sampling (Emys orbicularis)

A total of 19 individuals of *Emys orbicularis* were collected in 2013, including 10 males and 9 females, from two sites within the El Kala region (El Taref Province, northeastern Algeria): Lake Tonga (36°51′24.89″N, 8°30′10.56″E) and Lake Oubeira (36°51′15.46″N, 8°23′47.45″E). Turtles were captured either manually or using baited traps containing sardines placed at different locations. Traps were checked every six hours to prevent drowning. Given the protected status of freshwater turtles in Algeria, a non-lethal sampling method was adopted. Monogeneans present in the buccopharyngeal cavity were collected using sterile swabs, following the protocol described by Valdeón and Iglesias (2010). All procedures were conducted in accordance with ethical guidelines and complied with Algerian regulations on animal welfare, specifically Executive Decrees No. 10-90 and No. 04-82. Capture permits were issued by the Ministry of Agriculture and Rural Development (General Directorate of Forests) under permit number 718/BOG/160/DPFF-SDAC/DGF/2013.

After capture, turtles were kept individually in containers filled with water for 24 h at room temperature. The following day, the water was filtered sequentially through sieves of 500 µm and 100 µm mesh size to recover monogenean eggs. The first sieve retained coarse debris, whereas the second allowed the collection of finer particles, including parasite eggs when present. The filtrate was then examined under a stereomicroscope. In the absence of detectable eggs, the procedure was repeated up to three times. After examination, turtles were released at their original capture sites. Infected individuals were manually inspected, and parasites were carefully removed from the oral cavity before release.

Morphological characterization

Live parasites (polystomes) were collected, placed in Petri dishes, flattened, and fixed in aqueous Bouin's solution. Specimens were stained with Semichon's acetic carmine, differentiated in ethanol acidified with a few drops of hydrochloric acid, and dehydrated through successive ethanol baths (70%, 96%, and 100%). They were then cleared in clove oil (eugenol) and mounted on slides in Canada balsam. To accelerate hardening, the slides were incubated at 60°C for 24 h. Observations were performed using a Carl Zeiss Axioskop 50 microscope at the University of Science and Technology Houari Boumediene, and microphotographs were obtained using a Euromex microscope equipped with a camera at the University of Tamanrasset. Measurements are expressed in micrometers (µm), unless otherwise stated, and are presented as ranges followed by the mean in parentheses. The number of examined specimens is indicated before the species name.

Molecular characterization and phylogenetic analysis

Two adult specimens of polystomes were preserved in ethanol and sent to the University of Perpignan Via Domitia for molecular analysis, following a protocol previously described by Héritier et al. (2017). DNA extraction, PCR amplification, and sequencing of the target fragments were carried out according to the methodology detailed by Héritier et al. (2017). The mitochondrial cytochrome c oxidase subunit I (COI) gene was used as the genetic marker for species identification. This marker is widely used in phylogenetic and systematic studies of monogeneans. The obtained sequences were compared with homologous sequences available in the GenBank database. Sequence alignment and phylogenetic tree reconstruction were performed using MEGA11 software. Phylogenetic trees were constructed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method based on a dataset of 26 sequences.

RESULTS

Morphological characteristics of P. ocellatum from Algeria and comparison with populations from France and Morocco

Parasitological examination of 19 *E. orbicularis* individuals revealed the presence of 10 polystomes in 8 hosts. Among the infected individuals, five males each harbored a single parasite, whereas three females were infected, one carrying two parasites and the other two carrying one parasite each (Table 1).

Table 1. Parasitological indices in turtles examined in the El Kala region in 2013.

Turtles examined number	Turtles infected number	Prevalence	Intensity	Abundance
19	8	0.42	1.25	0.53

Morpho-anatomical analysis was conducted on eight mature specimens. All measurements are presented in Table 2, together with data from populations described in Corsica (Knoeppfler and Combes, 1977) and Morocco (Combes and Thiéry, 1983). No comparison was possible with the specimens reported by Héritier et al. (2017), as that study was based exclusively on molecular data.

The body is elongated and dorsoventrally flattened, displaying the typical morphology of Polystomatidae. Prominent vaginal papillae are present in the anterior third of the body, with marked lateral expansion, and paired eye spots are visible on both sides (Figure 1a, b). Total body length (TBL) ranges from 1800 to 4700 (3062.50), and maximum body width (BW) at the level of the vagina ranges from 500 to 1300 (900), resulting in a TBL/BW ratio of 3.42. The haptor is slightly longer than wide, measuring 500 to 1500 in length (1000) and 600 to 1500 in width (1065), corresponding to an HL/HW ratio of 0.94 and an HL/BL ratio of 0.48. The subterminal oral sucker measures 72.48 to 190.04 in length (139.67) and 300.04 to 543.18 in width (429.54). It opens into a buccal funnel leading to a

muscular pharynx measuring 211.55 to 268.99 in length (246.34) and 240.28 to 366.21 in width (305.59), with a length-to-width ratio of 0.82. From the pharynx, two non-confluent intestinal caeca extend laterally toward the posterior extremity of the body.

Table 2. Morphometric data for the three populations of *Polystomoides ocellatum* (Rudolphi, 1819) studied.

Comparative data	*P.ocellatum* Corsica n=4	*P. ocellatum* Morocco n=2	*P. ocellatum* Algeria n=8
Total body length (TBL)	3130-4060 (3462.5)	4770-4980 (4875)	1800-4700 (3062.50)
Body length without haptor	1990-2860 (2337)	3510-3750 (3630)	1300-2600 (2050)
Body width (at vagina) (BW)	1100-1170 (1143)	1570-1740 (1655)	500-1300 (900)
TBL/BW	3.02	2.94	3.42
Haptor length	1000-1200 (1125)	1230-1260 (1245)	500-1500 (1000)
Haptor width	1230-1370 (1323)	1500-1530 (1515)	600-1500 (106.50)
Haptor length/haptor width ratio	0.85	0.82	0.94
Haptor length: body length ratio	0.49	0.34	0.48
Oral sucker length	/	/	72.48-190.04 (139.67)
Oral sucker width	318-350(333.25)	341-347 (344)	300.04-543.18 (429.54)
Pharynx length	193-248 (220.5)	460-476 (468)	211.55-268.99 (246.34)
Pharynx width	276-331 (318.5)	600-620 (610)	240.28-366.21 (305.59)
Pharynx length/pharynx width ratio	0.71	0.76	0.82
Ovary length	219-230 (234.75)	300-308 (304)	189.51-302.50 (236.84)
Ovary width	114-130 (122)	145-184 (164.5)	98.69-171.63 (140.77)
Testis length	400-660 (536)	710-840 (775)	77.49-475.40 (376.98)
Testis width	415-590 (501.25)	665-700 (682.5)	186.87-384.98 (271.66)
Testis ratio	1.05	1.13	1.26
Genital bulb	115-170 (138.75) Ø	196-212 (204) Ø	106.55-260.63 (167.81)
Number of genital spines	29-34	29-30	29-32
Number of eggs in utero	1	1	1
Egg length	230-260 (247.75)	285	193.04-285.9 (236.56) n=6
Egg width	172-200 (193)	215	155.09-190.9 (167.10) n=6
Haptoral sucker	318-350 (333.5)	341-347 (344)	248.26-374.26 (331.87)
Hamuli length: external pair	55-62 (58)	86-100 (93)	40.4 -77.6 (59.19)
Hamuli length: internal pair	43-68 (54.5)	50-53 (51.5)	45.6-59.35 (52.42)

A single ovary, either dextral or sinistral, is located laterally and measures 189.51 to 302.50 in length (236.84) and 98.69 to 171.63 in width (140.77). A single testis is situated posterior to the ovary in the median region of the body and measures 77.49 to 475.40 in length (376.98) and 186.87 to 384.98 in width (271.66), with a ratio of 1.26, indicating an oval to elongated shape. The genital atrium is armed with 29 to 32 spines arranged irregularly, with a mean diameter of 167.81. The longer spines measure 35.60 on average, whereas the shorter ones average 24.69 (Figure 1c). Eggs measure 193.04 to 285.90 in length (236.56) and 155.09 to 190.90 in width (167.10), with one rounded end and the other tapered (Figure 1d).

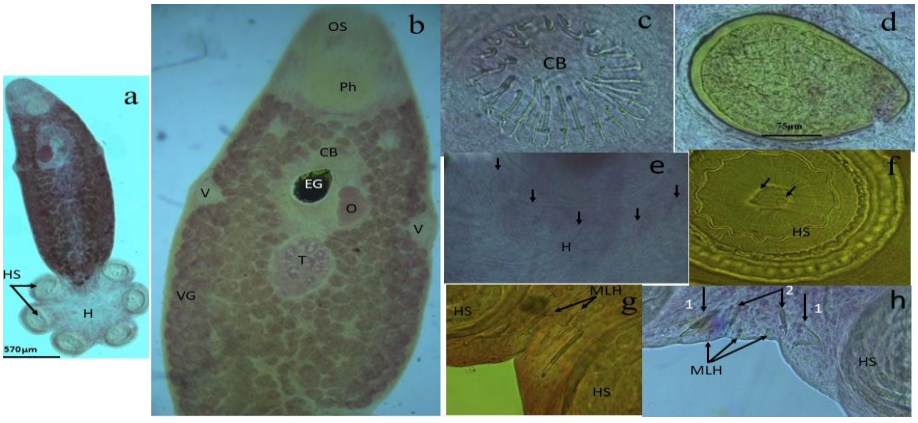

Figure 1. Morphology of the different body parts of *Polystomoides ocellatum* recorded in Algeria (personal photographs).

a and b : Ventral view of P. ocellatum H: Haptor with three pairs of haptoral suckers HS, OS: Oral sucker, Ph: Pharynx, CB: Copulatory bulb, O: Ovary, T: Testis, V: Vagina, VG: vitelline glands. c: Copulatory bulb surrounded by a genital corona of 29 spines. d: Egg. e: Anterior border of the haptor with marginal larval hooks. f: Haptoral sucker with a central larval hook. g: Posterior border of the haptor, MHL: marginal larval hooks. h: Posterior border of the haptor with two pairs of hamuli 1: external pair, 2: internal pair.

The haptor retains larval characteristics, including 16 sickle-shaped marginal hooks distributed in the anterior and posterior regions and laterally within the haptoral suckers. Six haptoral suckers are present, each with a diameter ranging from 248.26 to 374.26 (331.87), and each bears a central larval hook that may be either closely associated or separate (Figure 1e, f). Four additional hooklets are present in the posterior region, either retaining only the ogives or occurring alone (Figure 1g, h). The haptor is equipped with two pairs of hamuli: an external pair (Figure 1h-1) measuring 40.4 to 77.6 (59.19) and an internal pair (Figure 1h-2) measuring 45.6 to 59.35 (52.42).

Overall, the body morphology of the three populations corresponds to the typical characteristics of the genus *Polystomoides*, sharing the same microhabitat (oral cavity) and hosts (*E. orbicularis* and *M. leprosa*). The main differences between populations are as follows. The Algerian population exhibits a smaller body size, with a mean total length of 3062.5 compared with 3462.5 in Corsica and 4875 in Morocco. A similar pattern is observed for body width at the level of the vagina. The TBL/BW ratio is highest in the Algerian population (3.42), indicating a more slender body than in the Corsican (3.02) and Moroccan (2.94) populations. The oral sucker is wider in the Algerian population (429.54) than in the Corsican (333.25) and Moroccan (344) populations. Ovary dimensions are generally smaller in the Algerian specimens, with lower minimum values than those reported for Corsican and Moroccan populations, although mean values remain broadly comparable. Genital bulb diameter and spine size also vary among populations, with greater variability observed in the Algerian specimens. Egg size shows moderate interpopulation variation. The testis differs markedly among the three populations. In the Algerian population, mean testis length and width are 376.98 and 271.66, respectively, with particularly low values in some smaller individuals, compared with mean values of 536 and 501.25 in Corsica and 775 and 682.5 in Morocco.

The genital bulb has a mean diameter of 167.81 in the Algerian population, with values ranging from 106.55 to 260.63, compared with a mean of 138.75 in Corsica and 204 in Morocco. The number of genital spines ranges from 29 to 34 across the three populations and shows irregular alternation. Spine size is especially variable in the Algerian population, with large spines ranging from 31.79 to 49.33 and small spines from 14.63 to 37.34, compared with 25 to 37 and 27 to 28, respectively, in the Corsican population. No spine-size values were reported for the Moroccan specimens. Egg size varies moderately among populations. In the Algerian population, eggs measure 193.04 to 285.90 in length (236.56) and 155.09 to 190.90 in width (167.10), compared with a mean of 247.75 × 193 in Corsica and 285 × 215 in Morocco.

The external hamuli show similar values in the Algerian and Corsican populations but differ markedly from those of the Moroccan specimens. In contrast, the internal hamuli show relatively similar values across all populations. Shared features include the number of sclerotized elements in the copulatory bulb, similar haptoral sucker dimensions, and comparable haptor-to-body length ratios between the Algerian and Corsican populations, whereas the Moroccan population shows a lower ratio. The haptor length-to-width ratio is 0.94 in the Algerian population, 0.85 in the Corsican population, and 0.82 in the Moroccan population.

Molecular identification and phylogenetic analysis

The mitochondrial cytochrome c oxidase subunit I (COI) gene is a well-established marker for the study of genetic diversity and phylogenetic relationships in monogeneans. It was used to assess genetic variability within the species and to investigate potential population structuring. The COI gene from three sequences of *Polystomoides ocellatum* from Algeria was analyzed and compared with homologous sequences available in the GenBank database. These sequences were subsequently incorporated into a phylogenetic analysis including several species of the genus *Polystomoides*, with *Polystomoidella* (*P. whartoni*) used as the outgroup.

The resulting topology revealed a clear phylogenetic structure among the analyzed sequences (Figure.2), allowing the identification of several clades corresponding to different species within the genus *Polystomoides*. The three Algerian sequences (KY704672.1, KY704671.1, and KY704670.1), belonging to the same haplotype (H89), were grouped within a single subclade, indicating that they share an identical COI fragment sequence. This subclade forms part of a larger clade that includes sequences from France and Spain associated with a distinct haplotype (H67), forming a strongly supported monophyletic group (bootstrap = 100) corresponding to *P. ocellatum*. This phylogenetic placement indicates a close relationship between the Algerian specimens and European populations. Genetic similarity between Algerian and European populations was estimated at 97.34%, corresponding to a divergence of 2.7%.

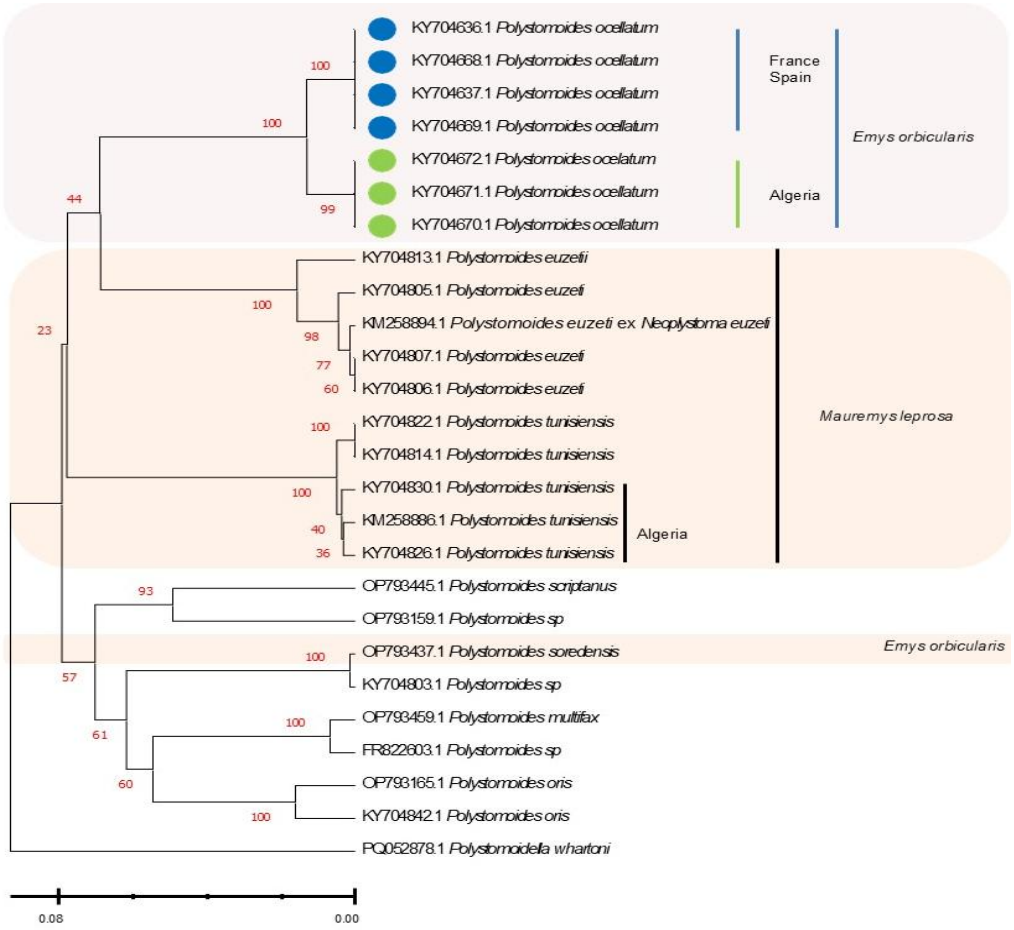

Figure 2. Molecular phylogenetic analysis using the UPGMA tree, conducted from the analysis of 26 COI sequences of polystomes from different countries, using MEGA11 software, ClustalW algorithm alignment (Gap Opening Penalty 15 and Gap Extension Penalty 6.66), 10,000 bootstrap values, and the Kimura 2-parameter model with gamma distribution. The *Polystomoides ocellatum* sequences from Algeria and the reference sequences from France and Spain are highlighted in green and blue, respectively. The numbers above the branches indicate bootstrap values, and the *Polystomoidella whartoni* sequence was used as an outgroup.

Comparative sequence analysis revealed nine nucleotide substitutions between the Algerian specimens and European populations. These substitutions were observed at positions 18 (A→G), 24 (T→G), 75 (T→G), 204 (A→T), 210 (T→C), 228 (T→C), 240 (T→C), 324 (A→G), and 327 (A→G) within the analyzed COI fragment (Figure 3).Outside the *P. ocellatum* clade, the phylogenetic tree includes 29 species belonging to the genus *Polystomoides*, of which only 10 are presented here based on available GenBank sequences. The tree shows several distinct clades corresponding to different species within the genus. Sequences assigned to *P. euzeti* form a distinct clade including specimens from Morocco, France, and Algeria, all associated with *M. leprosa*. Similarly, sequences attributed to *P. tunisensis* form another distinct clade including populations from the same regions and associated with the same host species (*M. leprosa*).

Additional species identified within the genus include *P. scriptanus*, described in the United States from *Pseudomys peninsularis*, *Polystomoides* sp. from *Trachemys scripta* in the United States, *P. multifalx*, *Polystomoides* sp. from *Pseudemys nelsoni*, and *P. oris*, reported from *Chrysemys picta* in the United States and *Trachemys scripta* in France. Each of these taxa forms a distinct clade, illustrating the phylogenetic diversity within the genus *Polystomoides*. Overall, the obtained topology confirms that the Algerian specimens cluster within *P. ocellatum* and reveals a genetic structuring within this species.

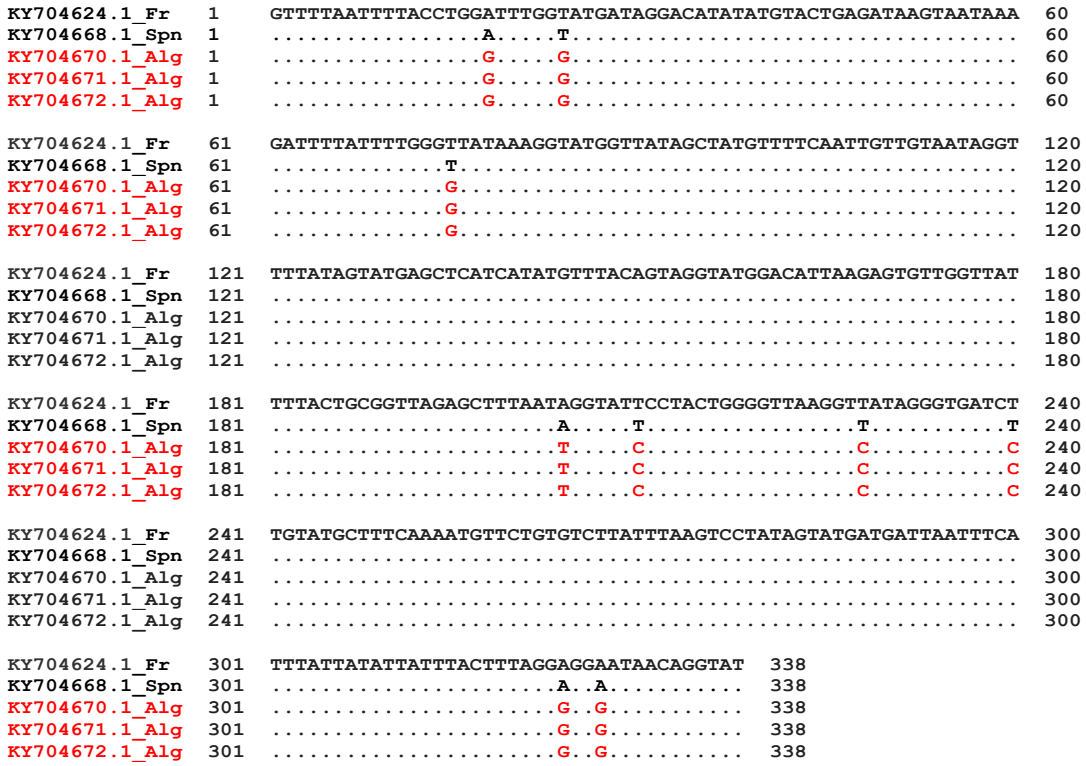

Figure 3. The multiple 5′ to 3′ alignment of COI gene sequences from the three populations of *Polystomoides ocellatum* (France, Spain, and Algeria) using the BLAST tool, the identical nucleotides are represented by (.), and the non-matching nucleotides are highlighted in red.

Discussion

The structure of parasitic populations is strongly influenced by the ecological characteristics of the biotope (Combes, 2001; Santana et al., 2023), while their evolutionary dynamics are closely linked to host biology (Hafner and Nadler, 1990; Hoberg and Brooks, 2005). In the present study, among the 19 examined turtles, only 8 individuals were infected, mostly by a single parasite, except for two hosts harboring two individuals each. This corresponds to a prevalence of 0.42, a mean abundance of 0.53, and a mean intensity of 1.25, indicating a low level of infestation in the studied population.

These low parasitological indices suggest limited transmission of *Polystomoides ocellatum* to its host *Emys orbicularis*, likely reflecting both environmental constraints and the high specificity of host-parasite interactions (Littlewood et al., 1997), as well as the apparent rarity of this parasite species. This observation is consistent with results from our previous unpublished study on *Mauremys leprosa*, in which *P. ocellatum* was not detected despite both hosts sharing the same biotope. Furthermore, studies by Meyer (2015) and Héritier et al. (2017) indicate that parasite transfer may occur among *M. leprosa*, *E. orbicularis*, and *Trachemys scripta*. However, in natural populations of *E. orbicularis* in southern Europe, *P. ocellatum* has been reported as a rare species, restricted to this host and less prevalent than other polystome species, which is consistent with our findings.

The morpho-anatomical differences observed in *P. ocellatum* from Algeria, particularly the reduced total body length and maximum body width, combined with a higher TBL/BW ratio (3.42), indicate a more elongated and slender morphology compared to populations from Corsica and Morocco (Knoepffler and Combes, 1977; Combes and Thiéry, 1983). Additionally, the relatively larger oral sucker and smaller ovary observed in Algerian specimens suggest functional adaptations to local environmental conditions and host-related constraints. Such variations may reflect spatial limitations within the microhabitat, which can influence body size evolution and favor smaller, more adaptable forms capable of exploiting specific ecological niches (Poulin, 2005).

A key distinguishing feature is the shape of the testis, which is elongated in Algerian specimens, whereas it is typically rounded in populations from Corsica, Morocco, and Spain (Valdeón and Iglesias, 2010). Reproductive organs in Polystomatidae are recognized as major taxonomic characters (Tinsley and Jackson, 1989; Du Preez and

al., 2017; Héritier et al., 2018; Chaabane and al., 2022). Previous studies emphasize the importance of male reproductive structures not only for species identification but also for revealing evolutionary divergence (Vieira et al., 2008; Sales et al., 2023). The relative stability of testicular morphology compared to other variable traits further supports its reliability in comparative taxonomy. Therefore, the observed variation in testis shape in Algerian specimens suggests a significant morphological divergence, likely resulting from geographic isolation and local adaptation.

The genital atrium and its associated sclerotized structures, including the number, size, and arrangement of spines, also represent key taxonomic features. In the Algerian population, these structures exhibit pronounced variability, with larger spine sizes (31.79-49.33 for large spines and 14.63-37.34 for small spines) compared to previously described populations. Such variability is consistent with the high interspecific diversity observed in these structures across Polystomatidae, ranging from a few spines to more than one hundred in some species (Pichelin, 1995; Héritier et al., 2017; Tinsley, 2017; Lignon et al., 2023).

Another important diagnostic character is the pair of hamuli located at the posterior region of the haptor, which plays a central role in species delimitation (Du Preez and Maritz, 2006). While the size of the hamuli in Algerian specimens is broadly comparable to that of Corsican populations, their morphology differs markedly. The external pair is characterized by a broad handle without a guard and a long, curved blade, whereas the internal pair presents a constriction between the handle and blade, with a V-shaped guard forming an angle of approximately 90°. Similar morphological differentiation is observed in larval hooks, which display elongated and slender guards. These structural differences further support the hypothesis of local morphological divergence.

The molecular analysis based on the mitochondrial cytochrome c oxidase subunit I (COI) gene revealed nine nucleotide substitutions between Algerian and European populations. These substitutions represent point mutations contributing to intraspecific genetic variability and indicate a divergence of approximately 2.7%. Such genetic differentiation is likely associated with geographic isolation and environmental heterogeneity across regions. The concordance between molecular and morphological data reinforces the robustness of species identification and highlights the importance of integrative taxonomy (Lafay et al., 1995; Littlewood, 2008; Héritier et al., 2016, 2018; Sales et al., 2023).

These results support the phenotypic variability observed in Algerian specimens and are consistent with the limited diversity of polystomes reported in *E. orbicularis* prior to 2017. Héritier et al. (2017) described a new species, *P. soredensis*, associated with both *M. leprosa* and *E. orbicularis*, with *Trachemys scripta* as the original host, further illustrating the complexity of host-parasite relationships within this group.

Overall, *P. ocellatum* exhibits a strong host specificity toward *E. orbicularis*, a pattern commonly observed in polystomes that display both host and microhabitat specialization (Verneau et al., 2022; Tinsley and Jackson, 1989). This dual specificity reflects a high degree of evolutionary adaptation. The restricted mobility of freshwater turtles, combined with geographic isolation, may limit gene flow and promote genetic structuring, potentially leading to speciation within the genus *Polystomoides* (Kulo, 1980). Consequently, the taxonomic status of the studied populations may warrant further investigation using additional molecular markers beyond COI, combined with detailed morphological and ecological analyses.

CONCLUSION

- This study demonstrated significant intraspecific variability in *Polystomoides ocellatum*, a monogenean parasite infecting the freshwater turtle *Emys orbicularis*. Genetic differentiation, evidenced by nucleotide substitutions, is associated with marked morphological variation, resulting in clear distinctions between populations from Algeria and those from Europe. These differences appear to be closely linked to environmental conditions within each aquatic biotope, highlighting the influence of habitat characteristics on both host and parasite dynamics. Environmental variability affects host populations, which in turn shapes the biology, distribution, and evolution of associated parasites.
- This work underscores the importance of an integrative approach combining morphological, molecular, and phylogenetic data to achieve reliable taxonomic identification. It also highlights the role of geographic and ecological factors in driving evolutionary processes in host-parasite systems.

Conflicts of interests. The authors declare no conflict of interests.

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