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First molecularly supported detection of *Oochoristica* sp. (Cestoda: Anoplocephalidae) in a Yucatán spiny-tailed iguana (*Cachryx defensor*) from Italy: A case report

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ABSTRACT

During a parasitological investigation conducted in Italy on domestic reptiles, a motile proglottid was macroscopically observed in a fecal sample from a Yucatán spiny-tailed iguana (*Cachryx defensor*). Subsequent analysis using the quali-quantitative flotation technique (Mini-FLOTAC®) revealed the presence of cestode eggs. DNA was extracted from the sample and amplified using conventional PCR. Sequence comparison with those available in GenBank showed the highest similarity (98%) with *Oochoristica hemidactyli*, supporting identification at the genus level (*Oochoristica* sp.). To our knowledge, this represents the first molecularly supported detection of *Oochoristica* sp. in a captive reptile in Italy, highlighting the importance of parasitological surveillance in domestic reptile populations and the potential role of exotic pets in the introduction and spread of parasites.

1. Introduction

In recent years, the exotic pet trade has expanded, leading to an increasing presence of reptiles in private households. Reptiles are now commonly kept as companion animals due to their perceived low maintenance requirements and the wide availability of different species on the international market. However, this trend has raised considerable concerns for both animal and public health, highlighting the need for enhanced biosecurity in reptile management, particularly in relation to the emergence and spread of non-native parasites that may go undetected during routine clinical examinations [1–4]. Following their adoption as pets, scientific interest in these animals has grown, yet knowledge of their endoparasites remains limited [5]. In captive reptiles, parasites may be associated with non-specific clinical signs, such as weight loss, anorexia, lethargy and diarrhea. This underscores the importance of including coprological tests into routine veterinary surveillance protocols to ensure early detection and appropriate management [6].

Reptiles can act as definitive, intermediate, or paratenic hosts for the larval and adult stages of tapeworms belonging to various suborders, including Pseudophyllidea, Proteocephalidea, Trypanorhyncha, and Cyclophyllidea. Tapeworm infections are often subclinical; however, organ dysfunction may occur as a consequence of mechanical damage caused by larval stages. In cases of heavy parasite burdens, gastrointestinal obstruction or competition with the host for essential nutrients may also be observed [7].

Importantly, some parasites reported in lizards may have public health relevance [8,9]. Among cestodes, while *Oochoristica* spp. are not currently regarded as zoonotic parasites, lizards may harbor plerocercoid larvae (spargana) of *Spirometra* spp., the causative agents of sparganosis in humans [8].

The genus *Oochoristica* (Cyclophyllidea: Anoplocephalidae: Linstowiinae) comprises a taxonomically complex group of cestodes infecting reptiles and mammals as definitive hosts. Many species parasitizing lizards and snakes have been described from tropical and subtropical regions, whereas reports from other reptilian hosts and temperate countries, including Italy, remain scarce [1,10].

We report a case of *Oochoristica* sp. infection in a *Cachryx defensor*, supported by molecular characterization. To our knowledge, this is the first report of this cestode genus in this host species and in Italy.

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2. Case presentation

During a parasitological investigation conducted in Italy on pet reptiles, a cestode infection was identified in a Yucatán spiny-tailed iguana (*Cachryx defensor*). The animal was a captive-bred female acquired from a breeder at a reptile exhibition in Austria in 2020, at approximately six months of age.

Upon arrival at the household in northern Italy (Lombardy) in 2020, a complete coprological examination, including macroscopic inspection of the feces and microscopic screening for gastrointestinal parasites, revealed the presence of pinworm eggs only. No cestode eggs or proglottids were detected. The iguana was therefore treated with fenbendazole (Panacur®, 25 mg/kg PO every seven days for four treatments). No further veterinary or parasitological assessments were performed until 2025.

The animal was housed individually in a terrarium (90 × 50 × 50 cm) with a substrate composed of sterilized coconut fiber, clay, and river sand. Daytime temperatures in the terrarium reached 40 °C in the warm zone and decreased to 25 °C at night. From June to September, the animal had access to a garden that was not fully isolated, allowing occasional entry of wild animals. According to the owner, the animal maintained normal weight and behavior, remained alert and responsive, and exhibited normal ocular and oral mucosal coloration, with no shedding problems. The iguana was fed a mixed diet of fresh vegetables and live insects, which the owner purchased from a commercial website and subsequently bred at home in dedicated rearing boxes. In addition, the owner reported that the animal occasionally consumed arthropods collected from the garden during outdoor access.

In 2025, the iguana underwent a routine health assessment at a private veterinary clinic by a veterinarian experienced in reptile medicine. During the visit, the animal weighed 45 g and appeared clinically healthy. The sensorium was normal, the body condition index (BCI) was within the normal range [11], hydration status was adequate, mucous membranes were of normal color, and all clinical findings were unremarkable. No additional diagnostic procedures were carried out, as the animal was in good condition and further investigations were considered unnecessary and potentially stressful for this particularly sensitive species. A fecal examination was then conducted for the detection of gastrointestinal parasites. Macroscopic inspection revealed the presence of a single live motile proglottid that was examined under a stereomicroscope and appeared longer than wide (5 mm × 2.7 mm; length-to-width ratio: 1.85) and acraspedote. Subsequently, it was placed on a glass slide, compressed under a coverslip, and observed under a light microscope. Microscopic observation revealed the presence of numerous uterine capsules (> 1000), completely filling the gravid proglottid. Uterine capsules measured 49.65 ± 1.57 μm in length (range 46.4–52.9, $n = 26$) and 39.75 ± 1.56 μm in width (range 35.3–41.7; $n = 26$). Each uterine capsule contained an oncosphere measuring 36.02 ± 1.56 μm in length (range 31.7–40.3, $n = 26$) and 31.64 ± 1.31 μm in width (range 27.8–33.6, n = 26). Oncosphere hook length was 15.39 ± 1.03 μm (range 13.3–17.0, $n = 20$) (Fig. 1).

To assess whether the same cestode eggs were detectable in the fecal material, the sample was further analyzed using the quali-quantitative mini-FLOTAC® technique, employing two different flotation solutions, FS2 (sodium chloride, NaCl; s.g. = 1200) and FS7 (zinc sulphate, $ZnSO_4$; s.g. = 1350) [12]. Notably, cestode eggs were detected only when using the FS7 solution.

The morphology of the proglottid and the presence of numerous egg capsules were consistent with a cestode belonging to the family Anoplocephalidae, most likely the genus *Oochoristica* [13]. However, because the available material consisted of a single gravid proglottid densely filled with egg capsules, the internal genital structures were obscured and could not be reliably visualized or distinguished. As morphological examination alone was insufficient to achieve definitive identification at the genus or species level [10,14], the recovered proglottid was rinsed in sterile PBS and transferred into a sterile 1.5 mL Eppendorf tube containing 20 μL of Tris-HCl buffer (10 mM, pH 8). A crude DNA extraction protocol was selected to maximize template recovery for downstream PCR amplification and was performed as previously described [15]. The lysate was stored at −20 °C until further molecular analyses.

Amplification of partial 18S rRNA sequence (~1300 bp) was carried out using primers wormA (5′-GCGAATGGCTCATTAAATCAG-3′) and 1270R (5′-CCGTCAATTCCTTTAAGT-3′) [14] in 20 μL of reaction volume, containing 3 μL DNA, 4 μL 5× GoTaq® Reaction Buffer (Promega, Madison, WI, USA), 2 μL dNTPs mix (2 mM), 2 μL of each primer (10 μM), 0.2 μL GoTaq® DNA Polymerase (5 U/μL, Promega, Madison, WI, USA), 0.2 μL Mg^{2+} (50 mM) and DNase-free water up to the final volume. Undiluted DNA as well as serial dilutions of the sample were tested. Positive (previously sequenced *Mesocestoides* sp. DNA) and negative controls were included in the run. PCR amplification was performed under these conditions: initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 40 s, annealing at 53 °C for 1 min and extension at 72 °C for 75 s, with a final extension at 72 °C for 7 min. PCR products were detected by agarose gel electrophoresis and purified with NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany). Amplicons were then submitted to Sanger sequencing (Eurofins Genomics, Ebersberg, Germany) using primers described in Supplementary Table 1. Sequences were inspected and manually curated using BioEdit v7.1 [16] and compared with those available in GenBank using the BLAST algorithm (https://blast.ncbi.nlm.nih.gov). The resulting partial 18S rRNA sequence was deposited in GenBank under the accession number PZ053792. BLASTn analysis identified *Oochoristica hemidactyli* 18S rDNA (GenBank accession no. MK937577) as the closest match, showing 98% identity across 1446 aligned positions, 100% query coverage, 31 mismatches, and one gap, thereby supporting assignment of the isolate to the genus *Oochoristica*. Phylogenetic analysis of 18S rRNA sequences (Fig. 2) places the sequence generated in the present study within the *Oochoristica* clade, where it formed a sister relationship with *O. hemidactyli*, supported by high

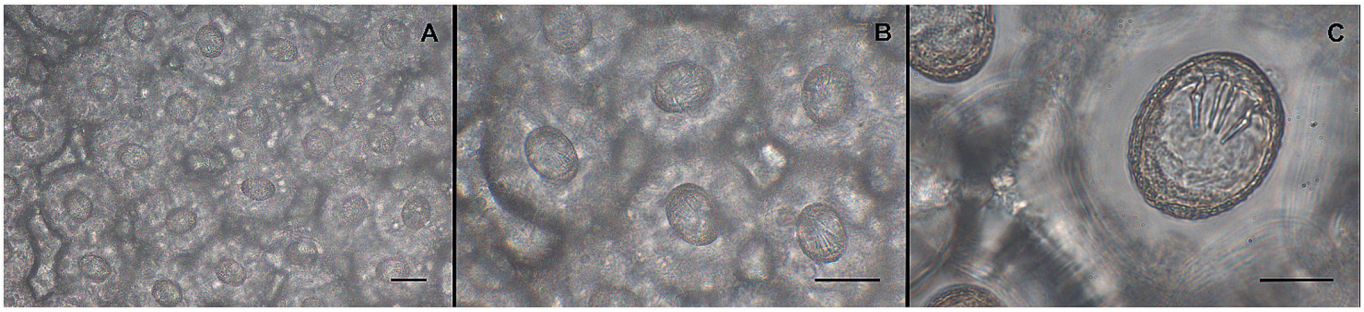

Fig. 1. Gravid proglottid recovered from the fecal sample of *Cachryx defensor*. A) Gravid proglottid containing numerous uterine capsules with oncospheres, observed at 20× magnification (scale bar = 50 μm). B) Uterine capsules containing oncospheres within the proglottid, observed at 40× magnification (scale bar = 50 μm). C) Detail of an oncosphere observed at 100× magnification, showing the characteristic hooks (scale bar = 20 μm).

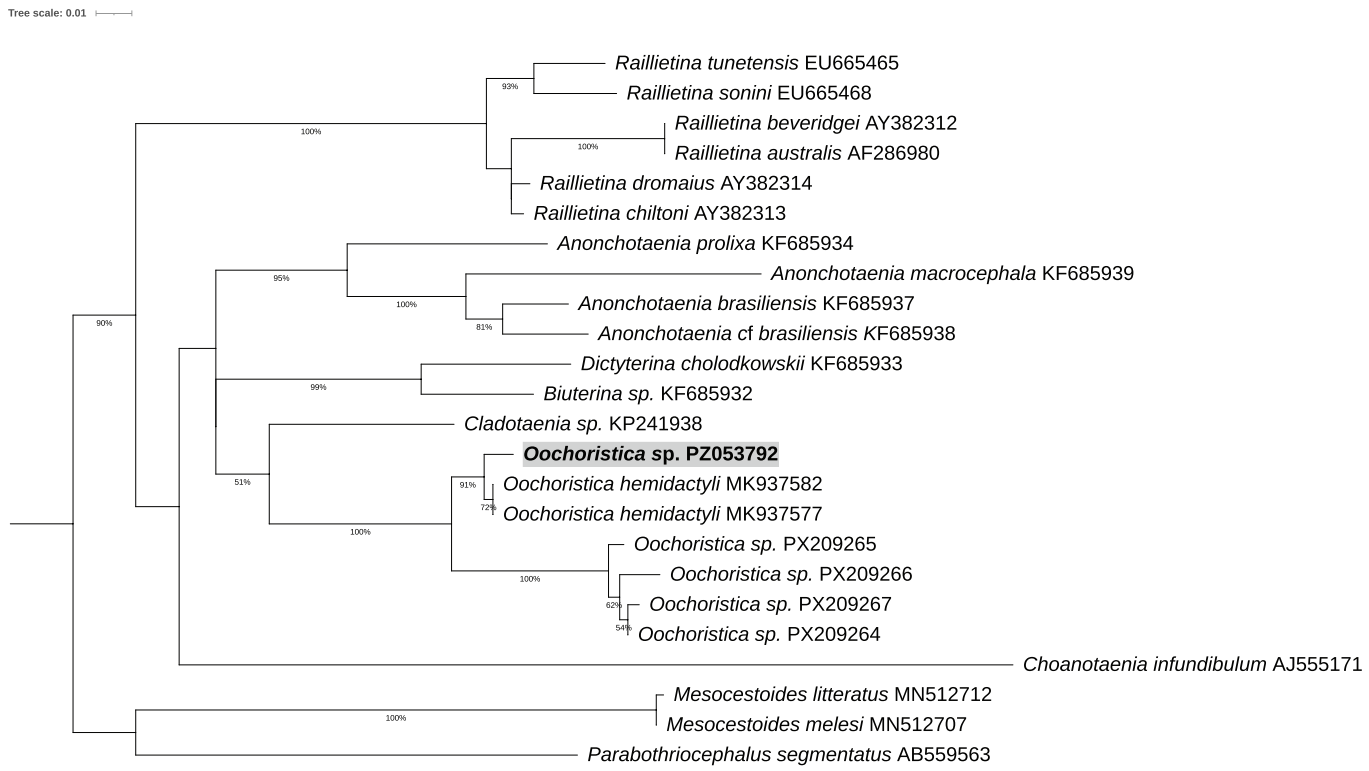

Fig. 2. Maximum Likelihood (ML) phylogeny inferred with 1000 bootstrap pseudo-replicates using W-IQ-TREE v3.1.1 [18]. The analysis included the 18S rRNA gene sequence obtained in this study, together with 18S rDNA sequences of several Cyclophyllidea and one representative of Pseudophyllidea retrieved from the GenBank database. Four unpublished *Oochoristica* sp. sequences retrieved from GenBank (PX209264–PX209267) were included as closely related taxa for comparison; no associated peer-reviewed publication was available at the time of manuscript preparation. Sequences were aligned using MUSCLE [19] and the multiple sequence alignment was refined using Gblocks v0.91b [20] to remove poorly aligned and ambiguous regions. Gblocks was run using default parameters, except for the minimum block length and gap settings (minimum block length = 5; gap positions allowed in up to half of the sequences). The initial alignment included 24 nucleotide sequences and comprised 1867 nucleotide positions, which were reduced to 1213 positions after Gblocks refinement. Species names and corresponding GenBank accession numbers (available at http://www.ncbi.nlm.nih.gov/nucleotide/) are indicated for each branch. The TN93 [21] model with gamma distribution (G) was selected as the best-fitting nucleotide substitution model using ModelFinder, as implemented in W-IQ-TREE, based on the Akaike Information Criterion (AIC). Bootstrap values above 50% are shown at the nodes. The sequence obtained in this study is indicated in bold and highlighted with a grey box.

bootstrap values.

Additional PCRs targeting fragments of the mitochondrial NADH dehydrogenase subunit 1 (*nad1*) and 12S rRNA genes were performed using the primers and conditions described in Supplementary Table 1. Although these primer sets were originally developed for *Echinococcus* spp. and related taeniid cestodes, their use has also been reported for other cestode taxa. Reference sequences for these loci are currently scarce in GenBank, limiting their utility for phylogenetic inference. Nevertheless, these amplifications were performed to generate novel sequence data and contribute to the expansion of available molecular resources for this taxon. The partial *nad1* and 12S rRNA gene sequences obtained in this study were deposited in GenBank under accession no. PZ055627 and PZ053795, respectively. Attempts to amplify additional gene targets commonly used for phylogenetic inference within this genus were unsuccessful, possibly due to partial DNA degradation. BLASTn analysis of the *nad1* sequence (PZ055627) showed the closest match with *Hymenolepis folkertsi* (KX792193), with 81% identity over 446 bp, and 100% query coverage. The 12S rRNA sequence (PZ053795) showed the closest match with *Cladotaenia* sp. (KT943417), with 83% identity over 298 bp, and 98% query coverage. These results confirmed that the amplified fragments were of cestode origin; however, the limited availability of closely related reference sequences prevented a more precise taxonomic assignment based on these markers alone.

Following laboratory analyses, the iguana was treated with praziquantel (Drontal®) administered orally at a dose of 8 mg/kg, with a second administration performed two weeks later, according to recommended treatment protocols for cestode infections in reptiles [17]. One month after treatment, follow-up fecal examination revealed no additional proglottids or cestode eggs.

3. Discussion

The genus *Oochoristica* comprises a large group of cyclophyllidean tapeworms mainly infecting reptiles and mammals. Most species have been identified in reptiles from the Americas and Asia [14,22–24]. In contrast, records from Europe are scarce and, to date, have been limited to Slovenia [1].

Although the complete life cycle of *Oochoristica* sp. has not been fully clarified, experimental studies on several species within the genus suggest an indirect life cycle, with larval development occurring in arthropod intermediate hosts and subsequent infection of reptile definitive hosts through ingestion of infected arthropods [25]. While species of the genus *Oochoristica* have been reported in various reptile hosts worldwide, this is the first documentation in *Cachryx defensor* from Italy. Molecular analysis showed that the generated 18S rRNA sequence clustered with *O. hemidactyli*; however, its position as a distinct lineage within the genus may reflect genetic divergence, suggesting the possibility of a closely related but distinct species. This interpretation should be considered in light of an important morphological limitation: the available material was limited to a single gravid proglottid, in which the dense packing of egg capsules prevented a detailed assessment of the internal reproductive anatomy. In particular, characters commonly used for species-level identification in this group, such as the number of testes, cirrus sac position and size, and ovarian morphology, could not

be evaluated. The absence of the scolex further reduced the morphological resolution of the identification. Thus, additional molecular and morphological data obtained from complete adult specimens would be required to achieve a definitive species-level identification.

The animal was purchased at a reptile exhibition and had not undergone parasitological screening prior to its importation into Italy, highlighting a potential gap in preventive health management. Furthermore, outdoor access to an unfenced garden, together with the ingestion of arthropods collected from the environment, may have facilitated exposure to intermediate hosts involved in the life cycle of *Oochoristica* sp. [25]. No cestode eggs or proglottids were detected during the complete coprological examination performed upon the animal's arrival in Italy in 2020; however, as no further parasitological assessments were conducted before 2025, the timing of infection acquisition remains uncertain. These findings highlight the importance of appropriate health screening in traded reptiles and support concerns regarding the introduction of non-native parasites through the international reptile trade [1,22,26,27].

With regard to diagnosis, the use of Mini-FLOTAC® in combination with an appropriate flotation solution allowed the detection of cestode eggs and facilitated post-treatment monitoring [12]. Although the iguana showed no overt clinical signs at the time of diagnosis, subclinical parasitic infections may negatively affect host health, particularly under conditions of stress, concurrent disease, or inadequate husbandry. Therefore, routine parasitological surveillance should be considered an important component of preventive health care in captive reptiles [4,28,29]. Moreover, treatment with praziquantel resulted in the resolution of the parasitological findings, as evidenced by negative post-treatment coprological examinations and the absence of further proglottid shedding during follow-up. These observations are consistent with the documented efficacy of praziquantel against cestode infections in reptiles [17].

To reduce the risk of parasite introduction and transmission in captive reptile populations, veterinarians should encourage owners to avoid the use of wild-caught animals as pets or live prey items. In addition, clinicians should emphasize the value of preventive health care, including regular veterinary examinations, routine body weight monitoring, periodic coprological screening, proper husbandry practices, and quarantine procedures for newly introduced animals.

4. Conclusions

The present case expands the known host and geographic range of *Oochoristica* spp. and contributes to the limited knowledge currently available on cestodes infecting iguanid lizards, highlighting the importance of routine parasitological surveillance in captive reptiles. Moreover, molecular data were generated to support taxonomic identification within this genus.

These findings suggest the need for increased awareness among veterinarians, pet owners, and regulatory authorities regarding the parasitological risks associated with the exotic pet trade and underscore the importance of implementing appropriate surveillance and control measures in captive reptile populations.

Further investigations into the occurrence and diversity of *Oochoristica* spp. would contribute to a better understanding of their epidemiology, life cycle, and transmission dynamics.

CRediT authorship contribution statement

Carolina Allievi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alessandra Cafiso:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Nicholas De Filippo:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Emanuele Lubian:** Writing – review & editing, Investigation, Formal analysis. **Susanna Adelaide Sechi:** Writing – review & editing, Methodology, Data curation. **Alessandro Zanon:** Writing – review & editing, Data curation. **Luca Villa:** Writing – review & editing, Methodology, Data curation. **Maria Teresa Manfredi:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Data curation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parint.2026.103338.

Data availability

Sequences generated in this study were deposited in GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/) under accession numbers PZ053792, PZ055627 and PZ053795.

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