



The first record of *Dolops carvalhoi* (Crustacea: Branchiura) parasitizing three farmed fish species of the Peruvian Amazon

Anai Flores Gonzales¹ · Jorge Babilonia¹ · Marian Paredes¹ · Patrick Mathews Delgado² · Marcos Sidney Brito Oliveira³ · Christian Fernández-Méndez⁴

Received: 11 September 2023 / Accepted: 18 May 2024

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Abstract

Branchiura are a crustacean parasite widely known around the world as fish lice. In South America, they have been most studied in Brazil as with high levels of infestation, the parasites can be harmful to the health of fish and cause economic losses in fish farms. The objective of the present study is to provide, for the first time, significant information about the parasitic indices, infestation sites, and morphology of *Dolops carvalhoi*, and to report the appearance of a new host in the Amazon region of Peru. A total of 24 specimens of *Colossoma macropomum*, *Piaractus brachypomus*, and *Calophrys macropterus* from fish farms were individually analyzed to identify the presence of the ectoparasites. The Branchiura collected from the body surfaces of the fish were separated according to sex, the parasitic indices were calculated, and the parasites were clarified in 90% lactic acid for taxonomic identification. They were also preserved in 2.5% glutaraldehyde, and dehydration and critical point procedures were applied by scanning electron microscopy to visualize the detailed structures. The taxa identified was *D. carvalhoi*, which had a prevalence of 64.7%, a mean intensity of 1.6, and a mean abundance of 1 parasite/fish in *C. macropterus*; a prevalence of 100%, a mean intensity, and mean abundance of 2.8 parasites/fish in *P. brachypomus*; and a prevalence of 100%, but a mean intensity and mean abundance of 15 parasites/fish in *C. macropomum*. The pectoral and dorsal fins and the urogenital opening had the highest occurrence of *D. carvalhoi* in the host. *Dolops carvalhoi* has sexual dimorphism, and we reported for the first time the infestation of this parasite in leather fish *C. macropterus*.

Handling Editor: Amany Abbass

✉ Anai Flores Gonzales
agonzalesf@iia.gov.pe

¹ Institute of Research the Peruvian Amazon (IIAP, AQUAREC), Puerto Maldonado 17000, Peru

² Department of Biodiversity and Biostatistics, Institute of Biosciences, São Paulo State University, Botucatu 18618–689, Brazil

³ Programa de Pós-graduação em Biodiversidade Tropical, Universidade Federal do Amapá (UNIFAP), Macapá, AP 68903-419, Brazil

⁴ Institute of Research the Peruvian Amazon (IIAP, AQUAREC), Iquitos 16000, Peru

Keywords Aquaculture activity · Parasite · Amazonia · Catfish · Serrasalmidae

The subclass Branchiura Thorell, 1864, comprises small crustaceans popularly known as fish lice (Van As and Van As 2015) and contains a single family, the Argulidae Leach, 1819. This family consists of only four genera: *Dolops* Audouin, 1837, *Argulus* Müller, 1785, *Dipteropeltis* Calman, 1912, and *Chonopeltis* Thiele, 1900 (Hadfield 2019). They are widely distributed throughout the world and have mainly been identified as parasitizing freshwater fish. They have little host specificity, except the *Chonopeltis* which is endemic to Africa (Lemos de Castro 1985; Poly 2008; Møller and Olesen 2010; Alsarakibi et al. 2014; Neethling and Avenant-Oldewage 2020).

Dolops comprises 12 species identified parasitizing more than 15 families of wild and farmed fish in the Amazon regions of South American countries (Malta and Varella 1983; Malta 1984; Mamani et al. 2004; Fontana et al. 2012; Møller and Olesen 2012; Luque et al. 2013; Oliveira et al. 2017; Pereira et al. 2018; Morey and Arellano 2019). Of these, *Dolops carvalhoi* Lemos de Castro, 1949, was most frequently found, parasitizing more than 12 species of fish from the Siluriformes, Characiformes, and Clupeiformes (Malta and Varella 1983; Malta 1984; Carvalho et al. 2003; Mamani et al. 2004; Møller and Olesen 2012).

Female adult *D. carvalhoi* lay their ellipsoidal eggs in rows, one on top of the other on available surfaces. These develop over 16 days, producing small specimens with an adult form (Gomes and Malta 2002). In farming, transmission occurs as a result of high densities, the introduction of other infected fish into farming systems, poor tank disinfection practices, or through the water supply, and outbreaks are related to water quality factors, stress, and inadequate fish nutrition (Hecht and Endemann 1998; Alsarakibi et al. 2014; Morey and Arellano 2019). The parasites attach to the host using their first maxillae, which due to their hook-like shape and the movement of the host can cause skin lesions, which can be deep when infestation levels are high, erosions in the dermal layers, and significant mucus production (Tavares-Dias et al. 2007; Møller 2009; Morey and Arellano 2019), leaving an entry point for secondary pathogens such as bacteria and fungi (Bandilla et al. 2006; Shinn et al. 2023). They can also alter hematological parameters, such as reductions in hematocrit levels and high levels of glucose, protein, sodium, potassium, and magnesium (Tavares-Dias et al. 2007). These changes can damage the health of the fish, resulting in a decrease in production parameters, poor performance, low reproductive capacity, and low annual profitability (Taylor et al. 2006; Pekmezci et al. 2011).

Gamitana (*Colossoma macropomum* Cuvier, 1816) and Paco (*Piaractus brachipomus* Cuvier, 1818) are the most cultivated native freshwater species in Brazil, Colombia, Venezuela, Bolivia, Ecuador, and Peru (Poleo et al. 2011; Valladão et al. 2016; Lima et al. 2020). In the Peruvian Amazon, *C. macropomum* production is around 880.60 Tn, and that of *P. brachipomus* is 2165.68 Tn (Produce 2020). Cultivation is considered semi-intensive, mainly undertaken with the use of nurseries in earth tanks, resulting in an advantageous, potentially efficient production cycle (Avadí et al. 2015), capable of addressing the needs of government policies such as “zero hunger,” but which is also highly adaptable to other high-density cultivation systems that occupy small spaces (Sandoval-Vargas et al. 2020; Silva et al. 2021). Mota punteada (*Calophysus macropoterus* Lichtenstein, 1819), however, remains a promising species for aquaculture, and the recently achieved captivity-induced reproduction offers potential for diversification

and the production of mercury-free meat in Peru (Babilonia et al. 2021), where there is still a high index of consumption of fish from nature, despite this practice being prohibited in the Madre de Dios region (Supreme Decret No. 034–2016-PCM) (Martinez et al. 2018; Feingold et al. 2020).

Therefore, due to the commercial importance of these three fish species and the economic impact of *D. carvalhoi* on Amazonian fish farms in South American countries, the objective of this study is to provide, for the first time, relevant information on the parasitic indices, infestation sites, and morphology of *D. carvalhoi* and report a new occurrence of host-parasite interaction in a region of Peru, so that future specific therapeutic or phyto-therapeutic control measures for this parasite species can be implemented.

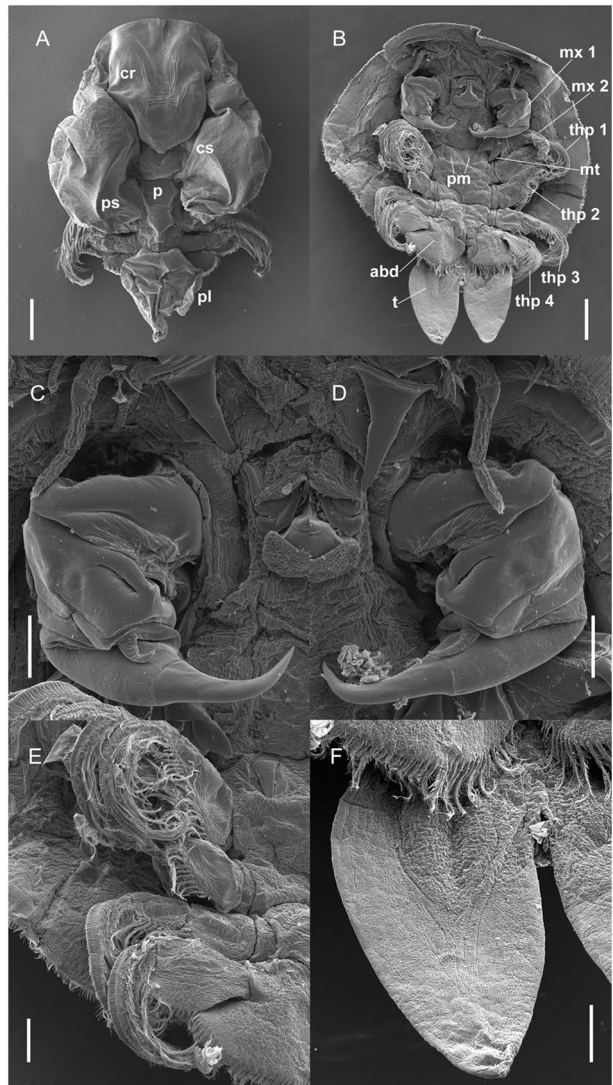
Twenty-four specimens of fish were analyzed: two *C. macropomum* (3660.62 ± 361.56 g and 60.68 ± 3.84 cm), five *P. brachypomus* (3660.62 ± 361.56 g and 56.56 ± 1.96 cm), and 17 *C. macropterus* (4650.0 ± 632.0 g and 57.64 ± 5.79 cm), which were kept in a 2033 m² earth tank, at a density of 1 fish per 84.7 m². The physical and chemical parameters of the water were monitored daily (08:00–16:00 h), and the temperature (26.2 ± 0.4 °C), dissolved oxygen (5.5 ± 6.6 mg/L), pH (5.6 ± 0.1), nitrite (0.05 ± 0.1 mg/L), and ammonia (1.5 ± 0.1 mg/L) levels were measured with a multiparameter (model HI98194, Hanna Instruments, USA) and a photoperiod of 12 h light and 12 h dark. The fish were fed a commercial extruded diet of Aquatech® (Naltech, Lima) containing 22% protein, 3.0% fat, 8.0% fiber, 10% ash, and 12% moisture, at a feed rate of 1.5%, and a frequency of once a day. All fish were evaluated individually for the presence of ectoparasites in the integument, oral cavity, fins, gills, and operculum.

The Branchiura were carefully collected from the fish surfaces with a #10 flat brush, placed in 60 × 15 mm Petri dishes with distilled water, and transported to the Aquatic Biology Laboratory of the Research Institute of the Peruvian Amazon (IIAP), Madre de Dios, Peru, for identification. The parasites were separated by sex with a binocular stereomicroscope (model Stemi 305, Carl Zeiss™, USA), carefully cleaned with a #4 flat brush to preserve their body structures, and counted to determine the parasitological indices: prevalence (P%), mean intensity (MI), and mean abundance (MA) were calculated according to Bush et al. (1997). Of the total number of parasite numbers found, 52 were preserved in 2-mL polypropylene microtubes containing 70% alcohol (Poly 2016) and clarified in 90% lactic acid (Eiras et al. 2000) for initial species identification with a binocular microscope (model Primo Star, Carl Zeiss™, USA). With images obtained through a camera incorporated in the microscope (model AxioCam ERc 5 s, Zeiss, USA).

For detailed visualization of the structures through the scanning electron microscope, 10 Branchiura were fixed at 2.5% glutaraldehyde solution buffered in 0.1 M sodium cacodylate buffer with a pH of 7.2. Subsequently, were then subjected to three washes at 30-min intervals in buffer solution and were incubated in 4% osmium tetroxide and 0.1 M sodium cacodylate buffer pH 7.2 three times. The parasites were incubated in 1% aqueous tannic acid solution for 45 min and gradually dehydrated in 50, 70, 90, and 100% ethanol for 20 min per solution. After dehydration, the samples were subjected to a drying process in a critical point chamber using carbon dioxide and coated with a thin layer of platinum (model Sputtering, Leica EM SCD 500, Germany). Samples were visualized with a scanning electron microscope (model DSM 940, Zeiss, Germany) operated at an acceleration of 15 kV (Gonzales et al. 2020). The taxonomic key used for the confirmation of the species was based on the taxonomy of Lemos de Castro (1985).

Branchiura was identified as *Dolops carvalhoi* Lemos de Castro, 1949, with a lobed body distributed in segments in the dorsal region, a pair of eyes, and small setas along the borders of the carapace (Figs. 1A and 2A). The ventral region has a mouth with a

Fig. 1 **A** Scanning electron microscopy micrograph in dorsal view of *Dolops carvalhoi* Lemos de Castro, 1949, showing the cephalic region (cr), the posterior cephalic sulcus (cs), the pereon (p), the posterior sinus of the carapace (ps), and the pleon (pl). **B** Scanned electron microscopy micrograph in ventral view of *Dolops carvalhoi* Lemos de Castro, 1949, showing the first maxillae (mx1), the second maxillae (mx2), maxillary teeth (mt), the post-maxillary teeth (pm), the testicle (t), and the first to fourth thoracopod (thp 1–thp 4). **C, D** The first maxillae ending in hooks. **E** The thoracopods. **F** The bilobed testis in males. Bars scale: 500 μ m (**A**), 400 μ m (**B**), and 100 μ m (**C–F**)



central jaw, a pair of antennae on the anterosuperior part, a pair of robust first maxillae ending in a hook, and a pair of second maxillae with the presence of maxillary three teeth in the base (Fig. 1B–E). Males have bilobed testicles and females have small spermathecae on the abdomen (Figs. 1F and 2A–D).

A total of 62 adult parasites were found male. *D. carvalhoi* were more abundant than females in the total number of fish examined. One hundred percent parasite prevalence was determined in the scaled fish *P. brachyomus* and *C. macropomum*, while the leather fish *C. macropomum* showed a prevalence of 64.7% (Table 1). The pectoral fin, the dorsal fin, and the urogenital opening were the preferred areas of the parasites. No lesions were observed with the naked eye in places where the parasites were fixed to the host, but great mobility was observed on the surface of the fish bodies during capture (Fig. 3).

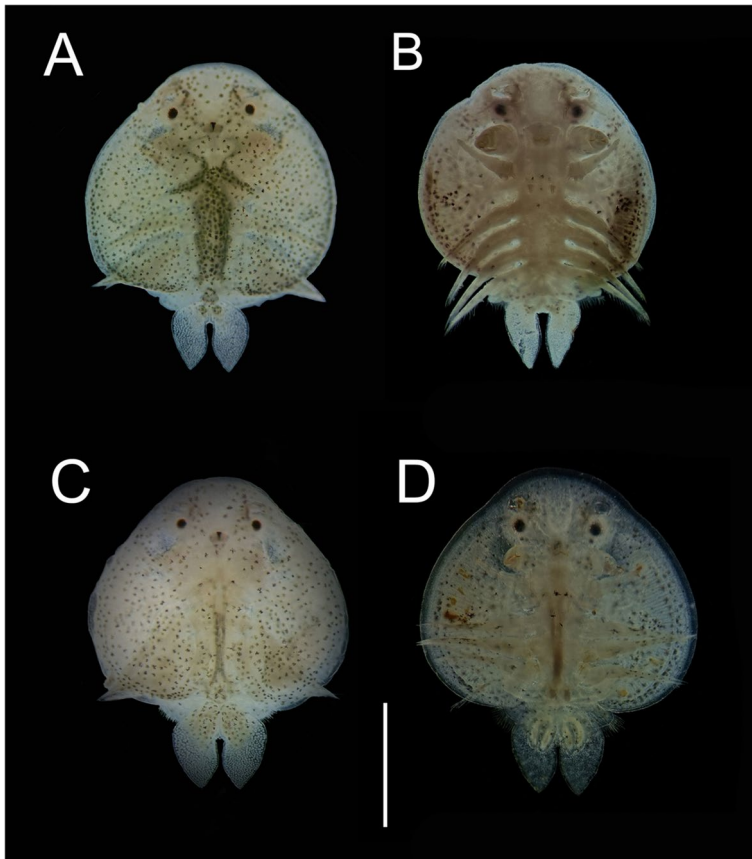


Fig. 2 Light microscopy of *Dolops carvalhoi* Lemos de Castro, 1949. **A** Dorsal view of female; **B** ventral view of female; **C** dorsal view of male; **D** ventral view of the male. Bar scale: 3 mm

Dolops is widely distributed in South America, including Brazil, Paraguay, Argentina, Venezuela, French Guiana, Bolivia, and Peru (Ringuelet 1943; Malta and Varella 1983; Malta 1984; Carvalho et al. 2003; Fontana et al. 2012; Møller and Olesen 2012; Luque et al. 2013; Pereira et al. 2018; Morey and Arellano 2019), but has also been found in Africa and Tasmania, with a largely tropical distribution (Fryer 1969). The *D. carvalhoi* identified in this study is characterized by a carapace wider than it is long, a pair of robust first maxillae ending in a hook and a pair of secondary maxillae with three teeth in the base.

In the present study, the area of greatest occurrence in the hosts was the base of the pectoral fin, the dorsal fin, and the urogenital opening, agreeing with other studies which reported that the regions of greatest infestation were the pectoral fin, the periopercular area, the pelvic region, the cephalad, and the dorsal and ventral surfaces of the body (Malta and Varella 1983; Fontana et al. 2012; Morey and Arellano 2019). The preference for the base of the pectoral fin may be related to the need for protection during swimming and water currents. *Dolops* attach to fish through the perforating hooks of the jaws (Fryer 1969), during fixation on the host; the parasites cause wounds in the integument, epidermal lesions,

Table 1 Parasitological indexes of *Dolops carvalhoi* Lemos de Castro, 1949, parasitizing *Calophrys macropterus* Lichtenstein, 1819, *Piaractus brachipomus* Cuvier, 1818, and *Colossoma macropomum* Cuvier, 1816

Host sex	P (%)	MI	MA	TTNFT/TNPF	Infestation site	No. parasite female	No. parasite male	No. total of parasites
<i>Calophrys macropterus</i>								
Female	33.3	1.5	0.5	6/2	PF, AF	0	3	3.0±0.8
Male	81.8	1.6	1.3	11/9	AF, UP, PF, ADF, CFA	2	13	15±0.9
Total fish	64.7	1.6	1.0	17/11	-	2	16	18±0.7
<i>Piaractus brachipomus</i>								
Female	100	3.3	3.3	3/3	PF, UP	5	5	10±3.0
Male	100	2.0	2.0	2/2	UP	1	3	4.0±0.0
Total fish	100	2.8	2.8	5/5	-	6	8	14±1.3
<i>Colossoma macropomum</i>								
Female	100	15.0	15.0	2/2	UP, PF, AF, CFA	12	18	30±7.8
Male	0.0	0.0	0.0	0/0	-	0	0	0.0±0.0
Total fish	100	15.0	15.0	2/2	-	12	18	30±7.8

P, prevalence; MI, mean intensity; MA, mean abundance; TTNFT, total number of fish tested; TNPF, total number of parasitized fish; PF, pectoral fin; AF, anal fin; UP, urogenital pore; ADF, adipose fin; CFA, caudal fin appendage

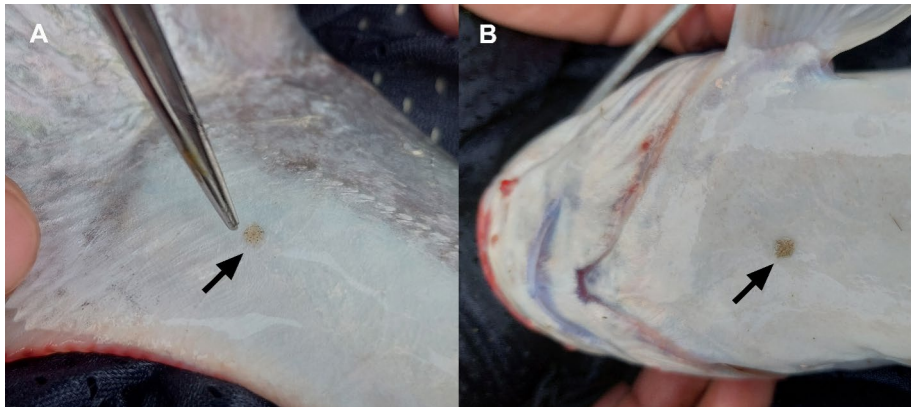


Fig. 3 *Dolops carvalhoi* Lemos de Castro, 1949, on the body surface (→) of *Calophysus macropterus* Lichtenstein, 1819 (A, B)

hemorrhages, changes in osmoregulation, and alter hematological parameters, as a result of the penetration and the action of the mandible hooks (Fryer 1969; Avenant-Oldewage 1994; Tavares-Dias et al. 2007). The intensity of the lesions is related to the parasites' ability to move around the host's body and attach to nearby regions (Fryer 1969; Avenant-Oldewage 1994).

D. carvalhoi has been reported parasitizing a large number of freshwater fish such as *Pseudoplatystoma tigrinum* Valenciennes, 1840, *Pseudoplatystoma fasciatum* Linnaeus, 1766, *Pseudoplatystoma corruscans* Spix & Agassiz, 1829, *Pseudoplatystoma reticulatum* Eigenmann & Eigenmann, 1889, *Phractocephalus hemiliopterus* Bloch & Schneider, 1801, *C. macropomum*, *Rhaphiodon vulpinus* Spix & Agassiz, 1829, *Pellona castelnaeana* Valenciennes, 1847, *Serrasalmus marginatus* Valenciennes, 1837, *Asterophysus batrachus* Kner, 1858, *Arapaima gigas* Schinz, 1822, *Pygocentrus nattereri* Kner, 1858, *P. brachypomus*, and *Piaractus mesopotamicus* Holmberg, 1887 (Malta and Varella 1983; Malta 1984; Carvalho et al. 2003; Mamani et al. 2004; Møller and Olesen 2012; Aguiar et al. 2017). The present study is the first record of *D. carvalhoi* in Peru, parasitizing *C. macropterus*, *C. macropomum*, and *P. brachypomus*. Other studies have identified *C. macropomum* and *P. brachypomus* as hosts of other Branchiura in Brazil (Malta 1984; Luque et al. 2013), but *D. carvalhoi* had not previously been reported parasitizing *C. macropterus*. The introduction of *C. macropterus* individuals into the same tank as *C. macropomum* and *P. brachypomus* has caused parasitism, demonstrating that this parasite is not specific to a particular group of fish, and has no preference for fish with or without scales, as it parasitizes whichever hosts are available (Fryer 1969; Malta 1982; Malta and Varella 1983).

Parasite load under natural conditions is often lower than in farmed fish, due to the higher density in culture, which favors parasite dissemination among fish (Ringuet 1943; Morey and Arellano 2019). In fish collected from the wild, the intensity of *D. carvalhoi*, *D. striata*, *D. discoidalis*, and *D. bidentata* was relatively low (Malta and Varella 1983; Malta 1984), and in captive *Brachyplatystoma tigrinum* Britski, 1981, reported the intensity of 12–30 parasites per fish from *Argulus pestifer* Ringuet, 1948, which caused skin lesions and erosions, up to mortality (Alcántara et al. 2008). However, there are also reports of cultured fish outside the rule as they in farmed *A. gigas* parasitized by *D. striata* reported a relatively low abundance of 10.03 (Pereira et al. 2018), in *P. punctifer* determined an average abundance of

D. discoidalis of 4.6 (Morey and Arellano 2019), and in tambacu (hybrid fish resulting from the crossing of the *P. mesopotamicus* with the *C. macropomum* fish) have also been reported with slight infestation by this Branchiura (Tavares-Dias et al. 2007).

Carvalho et al. (2003), on the other hand, reported that fish collected in the natural environment were more parasitized by Branchiura than farmed fish and that the parasites possibly distance themselves from their hosts for long periods to develop breeding processes in the culture tanks. Some fish can eliminate these parasites from their bodies by various mechanisms, such as predation, as reported by Gomes and Malta (2002), or by voluntary movements such as repetitive leaping during the day, scraping their bodies on the surfaces of the tank, or swimming in circles, as seen in Atlantic salmon, thus decreasing the rate of infestation (Furevik et al. 1993; Bui et al. 2018). In the present study, *C. macropomum* had a higher parasite load than *P. brachypomus* and *C. macropterus*, which was relatively low, with no skin lesions observed in any of the hosts. However, the appearance of parasites in cultivation should highlight the importance of preventive actions (drying and disinfection of tanks) and therapeutic or phytotherapeutic treatments, as in unfavorable conditions in high infestation, mortality, and irreversible economic losses can result (Pekmezci et al. 2011).

In conclusion, in this study, we reported for the first time the infestation of *D. carvalhoi* in leather fish *C. macropterus*, demonstrating that this parasite does not have taxonomically related host group specificity and is useful for proposing control solutions in fish farms. *D. carvalhoi* has a pair of robust first maxillae ending in a hook and a pair of second maxillae with the presence of maxillary three teeth in the base. Furthermore, *D. carvalhoi* did not cause apparent lesions in the hosts.

Acknowledgements The authors thank Noelia Estela and Alexandra Cornejo for obtaining light microscope images.

Author contribution AGF: conceptualization, methodology, validation, and drafting—preparation of the original draft; MSBO: taxonomic identification and review; JB: methodology and review; MP: methodology, data curation, and research; PM: preparation of samples; MEV: imaging and review; CFM: conceptualization, review, editing, and acquisition of funds.

Funding The present research was funded by Consejo Nacional de Ciencia, Tecnología e Innovación Tecnológica (Concytec) through its executing unit, PROCENCIA, for their work (Project 055–2021-Fondecyt).

Declarations

Ethics approval The authors declare that all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Competing interests The authors declare no competing interests.

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