This is a “preproof” accepted article for *Parasitology*.
This version may be subject to change during the production process
10.1017/S0031182025100267

Elucidation of the life cycle of the trematode *Curtuteria arguinae* (Digenea: Himasthlidae), using environmental DNA detection methods

Leslie Stout[1], Guillemine Daffe[2], Aurélie Chambouvet[3], Adrien de Montaudouin[4], Flore Daramy[1], Xavier de Montaudouin[1]

[1]University of Bordeaux, CNRS, Bordeaux INP, EPOC, UMR 5805, Station Marine, Arcachon, France

[2]University of Bordeaux, CNRS, OASU, UAR 2567 POREA, Pessac, France

[3]Sorbonne Université, CNRS, UMR7144 AD2M, ECOMAP, Station Biologique de Roscoff, Roscoff, France

[4]SEPANSO Aquitaine, Bordeaux, France

Corresponding author: Leslie Stout, Email: leslie.stout@u-bordeaux.fr

Abstract

Detection approaches based on environmental DNA (eDNA) are widely used for free-living species but remain underutilized for parasite species. This study applies eDNA detection methods to elucidate the life cycle of the trematode *Curtuteria arguinae*, which infects the socio-economically and ecologically important edible cockle (*Cerastoderma edule*) as its second intermediate host along the northeastern Atlantic coast, including Arcachon Bay, France. The first intermediate and definitive hosts remained unknown. To identify these hosts – presumed to be a gastropod and a shorebird – we developed a quantitative PCR (qPCR)-based eDNA approach targeting partial cox1 and SSU gene regions of *C. arguinae*. We tested for *C. arguinae* eDNA presence in water samples containing separately five dominant gastropod species and faecal samples from known cockle predators, the European oystercatcher (*Haematopus ostralegus*) and gulls (*Larus* spp.), collected in Arcachon Bay. *C. arguinae* eDNA was only detected in water containing the needle snail (*Bittium reticulatum*), with cercarial emergence confirming infection in 1.6% of individual hosts. Morphological analysis of the cercarial and metacercarial stages revealed variability in collar spine visibility. Additionally, *C. arguinae* was detected by qPCR in 42% of oystercatcher faeces and no gull faeces, suggesting oystercatchers are the definitive host. This study is the first to elucidate the complete life cycle of *C. arguinae*, identifying *B. reticulatum* as its first intermediate host and *H. ostralegus* as its definitive host. Our findings highlight the potential of eDNA approaches for resolving parasite life cycles and enable advances in ecological research on *C. arguinae*.

Keywords: eDNA; cox1; SSU (18S); Parasite; Molecular diagnosis; *Cerastoderma edule;* Morphology; Gastropods; Birds; Trematoda biology.

Introduction

Integrating parasites into biodiversity and ecosystem functioning frameworks is increasingly recognized as essential, given their vital roles in ecological processes (Preston *et al.*, 2016; Frainer *et al.*, 2018). A critical step toward this integration involves understanding a parasite's host range and ecological impacts, especially when a host species, such as the edible cockle, *Cerastoderma edule* Linnaeus, 1758 (Bivalvia: Cardiidae), plays a key role in ecosystem functioning. This marine bivalve is considered an ecosystem engineer that supports an economically significant shellfishery along the northeastern Atlantic coast. It provides numerous ecosystem services (Carss *et al.*, 2020) and hosts a rich community of digenean trematodes with over 15 species in Europe and northwest Africa (de Montaudouin *et al.*, 2021a; Stout *et al.*, 2024). Among these parasites is the echinostome *Curtuteria arguinae* (Desclaux, de Montaudouin, Freitas and Bachelet, 2006). *C. arguinae* (Digenea: Echinostomatoidea: Himasthlidae) was first described in Arcachon Bay (SW France) by Desclaux *et al*. (2006a) as metacercariae infecting cockles as their second intermediate host. Subsequent observations have recorded its presence in Portugal, Morocco, and sporadically in Brittany (NW France) (de Montaudouin *et al.*, 2009). In particular, cockle infections on Banc d'Arguin (Arcachon Bay, France) are characterized by persistently high prevalence and intensity. Stout *et al.* (2022) reported that 88% of adult cockles were infected by *C. arguinae* overall on Banc d'Arguin in 2021, with some individuals harboring up to 766 metacercariae. These heavy infection loads, with metacercariae lodged in the base of the cockle's foot, visceral mass and mantle tissue (excluding its thick margin), likely have substantial but as yet largely understudied impacts on cockle fitness and population dynamics (Desclaux *et al.*, 2006).

Such effects are particularly concerning given the ongoing decline in cockle populations in Arcachon Bay (de Montaudouin *et al.*, 2021b). In this context, it is interesting

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

to explore *C. arguinae*'s effects at individual, population, and ecosystem levels, for which it is first of all essential to understand its complete life cycle. The life cycle of a himasthlid trematode is complex, typically involving three hosts. Adult himasthlids sexually reproduce within a vertebrate definitive host, a bird, releasing their eggs into the environment via the host's faeces. These eggs develop into free-living miracidiae larvae, which infect a first intermediate host, a mollusk, typically a gastropod. Within this host, the parasite transforms into sporocysts or rediae, asexually producing numerous cercariae larvae. Cercariae are released into the environment and rapidly infect a second intermediate host, an invertebrate, where they settle in specific tissues as metacercariae. Finally, the parasite is transmitted trophically when the definitive host preys upon the second intermediate host, completing the life cycle (Niewiadomska and Pojmańska, 2011).

Despite the initial description of *C. arguinae* nearly two decades ago in its second intermediate host, *C. edule*, the identities of the first intermediate and definitive hosts remained unknown, largely due to methodological challenges. Indeed, morphological observation of endoparasites can be complicated due to conservational constraints restraining the possibility of dissecting potential host species (mostly vertebrates), particularly in protected areas like Banc d'Arguin, a national natural reserve, or due to ecological phenomena (e.g. low infection prevalence or strong seasonality of infection), which constitute barriers for the observation of infections and the elucidation of life cycles using traditional morphological methods. Efforts to identify the first intermediate host of *C. arguinae* have, so far, been inconclusive. A survey of 6,500 individuals belonging to nine gastropod species collected on Banc d'Arguin failed to identify the host (Desclaux, 2003). This may reflect a low prevalence of infection in gastropods or seasonal variation rather than host rarity, as first intermediate hosts of himasthlid species are typically abundant gastropods, such as *Peringia ulvae* for *Himasthla continua* and *H. interrupta*, *Littorina littorea* for *H. elongata*, and *Tritia*

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

reticulata for *H. quissetensis*. The first intermediate host of *C. arguinae* was thus suspected to also be a common gastropod species, whose abundance compensates for low infection prevalence and supports the high prevalence observed in downstream hosts, i.e. cockles. As with most himasthlid trematodes, *C. arguinae* is presumed to use shorebirds as definitive hosts. For example, the life cycle of its New Zealand congener, *C. australis* (Allison, 1979), implicates oystercatchers (*Haematopus finschi* and *H. unicolor*) as definitive hosts (Allison, 1979; Bennett *et al.*, 2023). In Europe, the European oystercatcher (*H. ostralegus*), which is abundant on Banc d'Arguin and known to prey on cockles, was thus a plausible candidate as a definitive host of *C. arguinae* (e.g. Sutherland, 1982; Triplet, 1996). Banc d'Arguin also hosts a substantial colony of gulls (*Larus* spp.), known as predators of cockles as well (Norris *et al.*, 2000), representing other plausible definitive hosts for the parasite. To address the challenges hampering host identification, molecular tools such as environmental DNA (eDNA) provide a powerful approach for detecting elusive parasite life stages under complex conditions. Widely used for studying free-living aquatic organisms, eDNA approaches are increasingly being applied to trematodes (Huver *et al.*, 2015; Bass *et al.*, 2015, 2023; Rusch *et al.*, 2018; Thomas *et al.*, 2022). In parasitology, the scope of eDNA is large, including DNA extracted from environmental samples such as water, soil, sediment or air, but also biological material from host species, such as faeces, blood or tissue. (Bass *et al.*, 2023). It thus represents a sensitive and adaptable tool that enables the detection of parasite DNA in host tissue or non-intrusively in water, sediment or faecal samples (Honma *et al.*, 2011; Bass *et al.*, 2015, 2023; Cabodevilla *et al.*, 2024).

In this study, we developed *C. arguinae*-specific primers to detect *C. arguinae* eDNA by quantitative PCR (qPCR) in water and bird faecal samples, aiming to identify both its first intermediate and definitive hosts. We described the morphological features of *C. arguinae* cercariae, with a special focus on the circumoral collar spines. Indeed, the presence and the

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

number of spines are key for identifying echinostome species, *C. arguinae* displaying 33 spines. However, the existence of a delayed appearance of collar spines has been reported in an another echinostome, *Isthmiophora hortensis* (Sohn *et al.*, 2024), prompting us to investigate whether a similar process occurs in *C. arguinae*. Finally, this study elucidates the complete life cycle of *C. arguinae* and demonstrates the efficacy of an eDNA-based approach in achieving this goal.

Materials and methods

Study area

Gastropods, cockles and bird faeces were sampled on Banc d'Arguin (44.60°N, 1.25°W), Arcachon Bay, France. Arcachon Bay is a 182-km² semi-enclosed macrotidal lagoon on the southwestern Atlantic coast of France, connected to the Atlantic Ocean by a 24-km² wide channel where Banc d'Arguin is located. This 4 km x 2 km sand bank (at low tide) is a national nature reserve comprising sand dunes and semi-sheltered sandflats where various marine bird species winter, nest or rest during migrations. Secondarily, cockles were collected on Ile aux Oiseaux (44.70°N, 1.17°W, Arcachon Bay, France), an island encompassing 17 km² of predominantly muddy and sandy intertidal flats at low tide.

Design of Curtuteria arguinae *specific primers*

Two primer pairs were designed to specifically amplify *C. arguinae* DNA: one targeting a fragment of the mitochondrial cytochrome *c* oxidase I gene (cox1), and the other a fragment of the small sub-unit (18S) ribosomal RNA gene (SSU). Suitable sequences were identified through two multiple sequence alignments of 94 cox1 sequences and 126 SSU sequences, respectively, from previous studies (de Montaudouin *et al.*, 2021a; Stout *et al.*, 2024). The dataset included 17 genetic lineages of trematodes infecting cockles, incorporating sequences

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

from *C. arguinae* and closely related species of the superfamily Echinostomatoidea (Stout *et al.*, 2024). Candidate primers specific to *C. arguinae* were identified in variable regions within the alignment. Primer specificity was first verified *in silico* using Primer-BLAST (NCBI). Second, experimental validation followed by testing with mock communities including 1) a mix containing genomic DNA (gDNA) from 12 trematode species infecting cockles (included in the multiple sequence alignments), including *C. arguinae* and 2) the same mix excluding *C. arguinae* DNA. All gDNA samples were obtained as described by Stout *et al.* (2024). Thermocycling conditions were optimized to ensure specific amplification of *C. arguinae g*DNA, whether from pure monospecific gDNA samples, the mock community 1 (as described above) or whole individuals of *C. edule* previously identified as infected by *C. arguinae* (under a stereomicroscope Nikon SMZ1500). Initial primer validation was performed via PCR, followed by quantitative PCR (qPCR) using a LightCycler 480 II (Roche). Two primer pairs yielding exclusive amplification of *C. arguinae* DNA were retained. For cox1, CACOIdetF (5'-CGGGAGTCGTGCTCGTTTAT-3') and CACOIdetR (5'-TGCGCTACCACAAACCAAGT-3') amplified a fragment of 147 bp. For SSU, CA18SdetF (5'-TTACGGCCGGGTCAAACTC-3') and CA18SdetR (5'-CCATACAAATGCCCCCGTCT-3') amplified a fragment of 302 bp. To verify the sensitivity of the primers for detecting *C. arguinae* eDNA, the limit of detection (LOD) was determined for both the cox and SSU primer sets. LOD testing followed recommendations of Hou *et al.* (2010) and was based on 10-fold serial dilutions of a linear DNA standard (purified PCR amplicons of plasmid DNA), which were amplified in triplicate using qPCR. The estimated LOD for the cox1 primers was 2.5×10^3 copies/µL, with a mean Ct value of 33. For the SSU primers, the LOD was lower, at 2.5×10^1 copies/µL, with a mean Ct value of 35. Additionally, gDNA extracted from a pool of ten *C. arguinae* cercariae was diluted 1:100 and amplified by qPCR in triplicate. The assays were able to detect DNA equivalent to 0.1

cercaria, with mean Ct values of 30 (cox) and 26 (SSU), confirming that both primer sets presented high sensitivity.

Identification of the first intermediate host

Sample collection and experimental setting

Marine gastropods were collected at low tide in and around *Zostera noltei* seagrass patches on Banc d'Arguin, in an area where cockle infection by *C. arguinae* is high (median: 102 metacercariae per cockle, Stout *et al.*, 2022). Five dominant gastropod species were collected: *Tritia reticulata* (Linnaeus, 1758, Nassariidae), *T. neritea* (Linnaeus, 1758, Nassariidae), *Peringia ulvae* (Pennant, 1777, Hydrobiidae), *Bittium reticulatum* (da Costa, 1778, Cerithiidae), *Steromphala umbilicalis* (da Costa, 1778, Trochidae) (Figure 1). The 250 largest individuals of each species were placed in decontaminated plastic containers with 50 individuals per container (5 containers per species). Exceptionally, due to its patchy distribution influenced by hydrodynamics (Armonies and Hartke, 1995), only 130 individuals of *P. ulvae* could be collected, and were distributed among four containers. One additional decontaminated container, free of gastropods, was set up as a negative control. All containers were filled with 1 L of open-ocean surface seawater, unlikely to contain *C. arguinae* DNA, and maintained at room temperature (23.5°C) under natural light conditions.

Water filtration and eDNA extraction

After 24 hours, water was filtered through a 3.0-µm pore size polycarbonate filter of 142 mm diameter (Isopore membrane filter TSTP14250, Merck) using a peristaltic pump to capture potential extracellular DNA or cercariae of *C. arguinae* that were shed into the water. Filter funnels, tubes and forceps used to handle the filters were decontaminated by UV before use, washed with 10% bleach and rinsed with ultrapure water (Milli-Q, Merck) before and

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

between samples. After filtration, each filter was cut in half with a sterile razor blade. One half was stored at -20°C as a backup and the other was processed for DNA extraction, stored overnight in 360 µL of Buffer ATL (from the Qiagen DNA extraction kit) at room temperature.

Molecular analyses

Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen), with doubled reagent volumes from step 1 to step 3 due to the high sample load and a 2h-incubation for the lysis. Therefore, step 4 was repeated 2-3 times to allow all of the mixture to be centrifuged through the spin column. Subsequent steps were performed following the manufacturer's instructions. Finally, a few filtration replicates containing *T. reticulata* and *S. umbilicalis* were particularly loaded and thus subdivided into two separate tubes for DNA extraction, after which their resulting DNA samples were pooled. Molecular detection was performed via qPCR using CACOIdetF/CACOIdetR and CA18SdetF/CA18SdetR (Table 1). qPCR reactions were performed in a LightCycler 480 II (Roche) with optical settings for SYBR Green I. Each 20-µL reaction contained 10 µL of 2x GoTaq qPCR Master Mix (Promega), 3 µL nuclease-free water and 5 µL template DNA. A negative control (nuclease-free water) and a positive control (*C. arguinae* DNA) was included for every qPCR assay. Cycling conditions for each primer set are detailed in Table 2. Melt curves were produced after the amplification cycles to check the melting temperature (Tm) of the qPCR products. Only samples with a cycle threshold (Ct) value below 35 were considered positive.

Collection of cercariae and rediae for morphological and molecular description

Following the qPCR results, individuals of the putative first intermediate host, were collected in October and November 2023 in the same sampling area on Banc d'Arguin using a 2-mm

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

mesh sieve. In the laboratory, 991 individuals were placed individually in plastic boxes (dimensions: 5.5 cm x 5.5 cm x 2.5 cm) filled with seawater (salinity 30) and kept at a temperature of approximately 22°C under light conditions for 24 hours to induce cercarial shedding. Each box was examined under a stereomicroscope (Wild Heerbrugg 1985 Inv. 2380). Cercariae were collected in sterile 1.5 mL tubes and stored in 96% ethanol at -20°C until further analysis. Ethanol-fixed specimens were examined under a Nikon SMZ25 stereomicroscope and a Nikon Eclipse Ci microscope, and photographed with a Nikon DS-Ri 2 camera. Measurements were performed on 15 ethanol-fixed cercariae under coverslip pressure using the NIS-Elements Analysis software. The drawing of the cercaria was made using the vector graphics softwares InkScape (1.2.2, retrieved from https://inkscape.org) and Affinity Designer 2 (v2.4.2, https://affinity.serif.com/fr/designer/). Dehydrated specimens used for examination by scanning electronic microscopy (SEM) were prepared by critical point drying, coated with gold, and examined and photographed with a Hitachi TM3030.

DNA sequences were generated for three samples containing a pool of 10 cercariae shed by the first intermediate individual host, conserved in 96% ethanol. Genomic DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen), following the manufacturer's instructions. A fragment of the cox1 gene was amplified by PCR using the primers TremCOI2S/TremCOI2AS (Magalhães *et al.*, 2020). A negative control (nuclease-free water) was included for every PCR reaction. All PCR amplification reactions were performed in a 50 µL total volume using GoTaq G2 Flexi DNA polymerase (Promega), following the manufacturer's protocol with 1 µL diluted template DNA. Cycling conditions are detailed in Table 1. Amplified PCR products were checked on a 1% agarose gel stained with ethidium bromide. PCR amplifications were sent for Sanger sequencing to Macrogen Europe B.V. Consensus sequences were assembled and manually edited using the MEGA v11.0.13 software (Tamura *et al.*, 2021). Three sequences were deposited in GenBank under accession

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

numbers PV216840-PV216842. Sequences were compared in MEGA v11.0.13 using the *p*-distance for genetic divergence calculations. Reference sequences included previously published partial cox1 and SSU sequences of *C. arguinae* obtained from metacercariae infecting cockles (Genbank accession numbers PP987234-PP987239, MT002920).

Experimental infection of cockles by cercariae

Ten infected snails were placed individually in plastic containers containing seawater at 24°C under light conditions to stimulate cercarial emergence. Small i.e. young cockles (10-14 mm i.e. about a year old) harboring little to no *C. arguinae* metacercariae were collected at Ile aux Oiseaux (Arcachon Bay, France), where the prevalence and parasite abundance is almost null (de Montaudouin *et al.*, 2012 and pers. obs.). Once *C. arguinae* cercariae had emerged (after approximately 7 hours), four cockles were placed in each box and kept under light conditions at 24°C. One cockle per box was checked for *C. arguinae* metacercariae after 1 day, 2 days, 3 days and 6 days. Metacercariae were examined for the presence of circumoral collar spines under a stereomicroscope (Nikon SMZ25) and/or a light microscope (Nikon Eclipse Ci).

Definitive host

Sample collection

Fresh faeces from European oystercatchers (*Haematopus ostralegus*) and gulls (*Larus* spp.) were collected in winter and spring from December 2022 to February 2024 on Banc d'Arguin. Oystercatcher faeces were sampled on the beach, while gull faeces were collected near nests. Samples were stored in 5-mL tubes at 4°C.

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

Molecular analyses

Genomic DNA was extracted from approximately half of each sample using the DNeasy PowerSoil Pro kit (Qiagen) following the manufacturer's instructions. *C. arguinae* DNA detection was performed using the primers CA18SdetF/CA18SdetR via qPCR as previously described (Table 1). Positive qPCR results prompted further examination of the remaining part of the faecal samples for the presence of trematode eggs under a stereomicroscope (Nikon SMZ1500), using a pipette and diluting the sample in seawater, following Born-Torrijos *et al*. (2017). Potential eggs were collected and stored individually in 1.5 mL tubes at -20°C. DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen), with a slightly increased amount of Proteinase K (25 µL, 600 mAU/ml) to facilitate eggshell lysis.

Results

First intermediate host

Identification of the first intermediate host by eDNA detection

Out of 25 water samples tested for the presence of *Curtuteria arguinae*, five tested positive with both primer pairs targeting partial sequence of the cox1 and SSU encoding genes by qPCR (Table 2). All positive samples were from gDNA recovered from filtered water containing needle snails, *Bittium reticulatum*. These samples exhibited significant fluorescence with cycle threshold (Ct) values ranging from 25 to 32 using the cox1 primers and 21 to 28 using the SSU primers, with mean melting temperatures of 83.0°C and 89.5°C, respectively (Table 2). Additionally, one water sample containing *Tritia reticulata* (replicate 5) showed fluorescence with a Ct value of 34 but remained entirely negative with the cox1 primers. Therefore, it was not considered as a reliable positive result, but rather a false positive due to late non-specific amplification or potential contamination. The control sample, along with the remaining samples associated with the gastropods *T. reticulata*, *T. neritea*,

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

Peringia ulvae and *Steromphala umbilicalis*, showed no presence of *C. arguinae* eDNA. Taken together, these results identified, for the first time and using eDNA detection, the needle snail, *B. reticulatum*, as the putative first intermediate host of the trematode *C. arguinae*.

Morphological features of rediae and cercariae

Out of 991 individuals collected on Banc d'Arguin using a 2-mm mesh sieve, a total of 16 needle snails were identified as infected through cercarial emergence. This corresponded to a prevalence of 1.6%, for this shell size class and considering cercariae-shedding snails. The shell height of infected snails ranged from 9.8 to 11.9 mm, with a mean of 10.7 mm.

Optical observation of these samples under a stereomicroscope revealed that rediae were located toward the posterior end of the gastropod's body, around the digestive glands and gonads (Figure 2). Rediae had a terminal mouth, a well distinguishable, rounded pharynx, and were elongated, varying in length. They contained cercariae at various stages of maturity, some of which were also found free outside the rediae. Emerged cercariae were highly active, moving rapidly with their tails and performing contracting movements with their body upon reaching the bottom. The cercarial body was elongated, slender and slightly flattened dorsoventrally, with its region just above the ventral sucker. Body length ranged from 250 to 400 µm, with a width of 110 to 130 µm at its largest point (Figure 3, Table 3). The tail was approximately the same length as the body, measuring between 270 and 370 µm. The body tegument bore folds along its entire surface. The oral sucker measured 50-80 µm in length and 40-70 µm in width, featuring an oval aperture surrounding the mouth, which lead to an oval pharynx. The oesophagus was elongated, bifurcating close to or above the large ventral sucker. The latter was 50-80 µm in length and 50-70 µm in width and protuberant. The excretory system was clearly visible, extending from the oral sucker to the base of the tail

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

without lateral diverticula. It was filled with dense, dark excretory granules, obscuring the intestines. Interestingly, circumoral collar spines were not constantly visible by stereo- and light microscopy and were absent in SEM, where only external features could be observed. Some cercariae exhibited circumoral collar spines, while others with the identical morphology lacked them. When present, a total of 33 spines surrounded the cephalic region. These included an uninterrupted main dorsal row of 27 spines, along with three additional, slightly shorter angular spines on each collar corner located on the ventral side, arranged identically to the metacercarial stage.

Collar spines

Cercariae of *C. arguinae* emerged from needle snails did not all exhibit the 33-spine circumoral collar observed in metacercariae. For a same cercariae-shedding needle snail, both cercariae with 33-spine collars (Figure 4B) and cercariae without spines (Figure 4A) were observed. Experimental infection of cockles with *C. arguinae* cercariae allowed for the observation of spine development at the metacercarial stage. While 0-3% of metacercariae bore collar spines up to three days post-infection (Figure 4D), 68% bore spines after six days post-infection (Figure 4C) (Table 4). These results showed a clear increase of spine-bearing metacercariae over time post-infection.

Molecular results

Partial sequencing of the cox1 gene marker from emerged cercariae generated three sequences of 253 nucleotides (Table 5). Two sequences were highly similar if not identical with the five reference sequences obtained from metacercariae infecting cockles, with pairwise genetic divergences (*p*-distances) close to zero (0-0.4%, 0-2 nt) (Table 6). This confirmed that *C. arguinae* infects the needle snail as its first intermediate host in the form of rediae which

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

produce cercariae. However, a third sequence obtained from cercariae shed by a needle snail presented significant genetic divergence (12.0-13.5%) (Table 6). A BLASTn search revealed no closer sequences than the *C. arguinae* reference sequences.

Definitive host

A total of 160 bird faeces was collected and analyzed, including 108 samples from oystercatchers and 52 samples from gulls (*Larus* spp.). *C. arguinae* molecular detection resulted in 46 (43%) positive oystercatcher faecal samples and no positive gull faecal sample (0%). PCR amplification of DNA from isolated eggs was not successful due to DNA extraction failure and scarceness of potential eggs. Consequently, molecular sequences from the eggs could not be compared to existing sequences of *C. arguinae*.

Discussion

According to the molecular and morphological results, *Curtuteria arguinae* infects needle snails *Bittium reticulatum* as its first intermediate host. The genetic signature of *C. arguinae* exclusively retrieved in oystercatcher faeces strongly suggests that adult worms use this shorebird as a definitive host. Our results provide, for the first time, a clearer understanding of the parasite's life cycle. The proposed life cycle for *C. arguinae* is represented in Figure 5. Interestingly, the cox1 partial sequences retrieved from cercariae shed by needle snails also revealed cercariae with greater genetic divergence (12.0-13.5%) (sequence B3_trem_COI_TremCOI_S2) than that observed among currently available *C. arguinae* reference sequences. This level of divergence exceeds the intraspecific variation reported for the same cox1 fragment in this species (Stout *et al.*, 2024), and also exceeds that of its congener *C. australis* (Donald and Spencer, 2016). *C. australis* has previously been shown to encompass a cryptic species, *Curtuteria* sp. A (Leung *et al.*, 2009), which presents 22% of

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

genetic divergence from *C. australis* based on cox1 sequences (Leung *et al.*, 2009; Donald and Spencer, 2016). In the case of *C. arguinae*, the genetic divergence of 12.0-13.5% is lower than for *C. australis*, keeping in mind that the cox1 fragment sequenced here is much shorter (259 bp vs. 920 bp). Also, no morphological differences were observed among *Curtuteria* cercariae emerged from needle snails in our study. Thus, while current data do not allow for conclusive statements about the presence of a cryptic species within *C. arguinae*, the observed genetic divergence - paired with a lack of corresponding morphological differentiation - warrants further investigation using longer gene regions and much broader sampling.

First intermediate host: **Bittium reticulatum**

Free-living miracidia hatched from eggs infect the parasite's first intermediate host, the needle snail (*Bittium reticulatum*). Cercarial emergence was used as a proxy for needle snail infection. Prevalence in needle snails was low (1.6%) yet expected. Indeed, the prevalence was comparable to what is typically observed (by cercarial emission or dissection) in first intermediate hosts for various trematode species, such as 2.6-3.2% for *C. australis* in *Cominella glandiformis* (Allison, 1979; Donald and Spencer, 2016), 2.4% for *Himasthla elongata* in *Littorina littorea*, or 1.9% for *H. continua* in *Peringia ulvae* (Thieltges *et al.*, 2006). However, cercarial emergence (as performed in the present study) notoriously underestimates infection rates (Born-Torrijos *et al.*, 2014). A strong seasonality of cercarial emergence is also suspected. We therefore assume that the real prevalence in needle snails is higher. Given the high host specificity typically exhibited by digenean trematodes at this stage (Poulin, 2007), we hypothesize that needle snails are the preferred, if not the exclusive first intermediate host of *C. arguinae* within the Banc d'Arguin ecosystem, similar to *C. australis* infecting *C. glandiformis* (Table 7). The morphological features of cercariae were consistent with those of the Himasthlidae family, such as a body not subdivided into regions of different

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

shapes, a smaller oral sucker than the large ventral sucker that is muscular and, when apparent, a reniform head collar bearing spines with angle spine-groups (Kostadinova, 2005). Cercariae also shared general characteristics with the metacercarial stage of *C. arguinae* (Desclaux *et al.*, 2006). There are few morphometric data available overall in the *Curtuteria* genus. Cercariae appeared to be slightly smaller than those of *C. australis* (Table 7). Comparison with the other three *Curtuteria* species, namely *C. numenii*, *C. grummti* and *C. haematopodis*, is not possible as they have only been described at the adult stage extracted from their definitive host, leaving their life-cycle and cercariae unknown (Table 7). Interestingly, we observed both spine-bearing and spine-lacking cercariae of *C. arguinae*. The number and arrangement of collar spines in echinostome trematodes serve as an important morphological character for species identification (Table 7). The presence of 33 spines in *C. arguinae* metacercariae is a key feature that differentiates it from other himasthlid metacercariae infecting cockles, such as *H. continua*, *H. quissetensis* and *H. elongata*, which display 29-31 collar spines (de Montaudouin *et al.*, 2009). However, experimental infection of cockles with cercariae revealed that the majority of metacercariae lacked visible collar spines during the initial days post-infection, with spines appearing after six days in over two-thirds of metacercariae. These results indicate that the collar spines develop as the larval stages mature. Interestingly, 3% of metacercariae exhibited spines as early as one to three days post-infection. We hypothesize that these originated from a minority of cercariae more mature at the time of shedding from the needle snail, underlining the variability in the maturity of cercariae within the first intermediate host. Some cercariae have developed visible spines already and transformed to metacercariae with spines immediately, while others need to mature further after infecting cockles before presenting spines. These observations align with reports documenting the absence of collar spines in the cercarial and early metacercarial stages of other echinostome species. The review from Fried *et al.* (2009) highlighted

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

contrasting observations regarding the presence of collar spines in conspecific cercariae, although the authors suggest that these discrepancies might stem from methodological inaccuracies in light and SEM microscopy. More recently, Sohn *et al*. (2024) demonstrated that the collar spines of the freshwater echinostome *Isthmiophora hortensis* only develop in 24-hour-old metacercariae, while earlier studies were inconsistent, observing cercariae of the same species both with and without spines. Here, we produce more evidence supporting this phenomenon.

Second intermediate host: **Cerastoderma edule**

Released cercariae will most likely survive 24 to 48 hours during which they swim actively the first hours to infect the second intermediate host (de Montaudouin *et al.*, 2016; Bommarito *et al.*, 2020), where they encyst to form metacercariae. Infections of *C. arguinae* metacercariae have been reported in cockles at several occasions and in multiple sites in France, Portugal and Morocco (de Montaudouin *et al.*, 2009). Interestingly, there are also a few reports of infections in other bivalves (Table 7). Dang *et al*. (2009) found three clam species (*Ruditapes decussatus*, *R. phillipinarum* and *Polititapes aureus*) infected by 0.1 to 1.2 metacercariae of *C. arguinae* per host overall in Arcachon Bay. On Banc d'Arguin specifically, only *R. decussatus* was reported infected by a mean of 0.7 metacercariae, whilst cockles were infected by a mean of 8.1 metacercariae. Furthermore, in the Oualidia lagoon (Morocco), *C. arguinae* metacercariae were found in *R. decussatus* with a prevalence of 60% and a mean abundance of 5.5 metacercariae per clam in the intertidal area in 2012 (X. de Montaudouin unpubl. data). At the same site, cockles are infected by up to 100 metacercariae per host (Alfeddy *et al.*, 2024), with 100% of prevalence (Correia *et al.*, 2020). To sum up, *C. arguinae* has been observed in four different second intermediate host species, all of which are bivalves. This relatively broad host range indicates that the parasite is not highly host-

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

specific at this stage, a common trait among digeneans (Poulin, 2007) and similar to what is observed for *C. australis* (Table 7). However, the general prevalence and abundance being remarkably lower in the clam species compared to cockles, the status of these clams appears as rather accidental yet compatible hosts, as cercariae transformed into metacercariae, but may also be dead-ends if the definitive host cannot predate them due to their deep-burrowing behaviour. We hypothesize that they serve as facultative second intermediate hosts capable of transmitting the parasite to its definitive host, while cockles serve as the main second intermediate host due to their higher prevalence and intensity of *C. arguinae* infections. Indeed, from an ecological and evolutionary point of view, cockles appear more interesting for the parasite's life cycle, constituting easier prey for birds (located just below the sediment surface and often at high densities) and more heavily parasitized, promising higher successful transmission rates than the clams.

Definitive host: **Haematopus ostralegus**

Finally, the European oystercatcher (*Haematopus ostralegus*) becomes infected by consuming cockles (and optionally clams) that harbor metacercariae of *C. arguinae*. Indeed, 43% of oystercatcher faeces were positive for the genetic signature of *C. arguinae* by qPCR. Similar prevalence has been observed for other trematodes infecting oystercatchers, such as *C. australis* in New Zealand (Allison, 1979) or *P. brevicolle* in the Wadden Sea (Borgsteede *et al.*, 1988). Contrastingly, our analyses revealed no infections of gulls. Digenean trematodes being commonly less host-specific when it comes to their definitive host (Poulin, 2007), gulls could however serve as secondary definitive hosts, with much lower infection prevalence, as *C. australis* also occurs in gulls (*Larus* spp.) (McFarland *et al.*, 2003; Bennett *et al.*, 2023) (Table 7), but that the fewer infections that occur were missed in our study. It is indeed possible that the prevalence in all faecal samples was underestimated due the applied method

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

that is not free of biases. Faecal samples represent a difficult matrix that contains many inhibitors that may have led to reduced genetic detection of *C. arguinae* by qPCR. Also, the number of eggs shed through the bird varies seasonally (according to worm maturity) and even daily (Goater, 1993; Presswell and Lagrue, 2016). As we sampled faeces at different times of the day (according to the high tide) and in different months, some faecal samples may have not contained eggs though the bird was infected by the parasite. Nevertheless, our findings suggest that oystercatchers represent the main definitive host with high prevalence.

Adult *C. arguinae* most likely reside in the bird's gastrointestinal tract, as it is generally the case for himasthlids, such as its congener *C. australis* in oystercatchers in New Zealand (Allison, 1979). This is a commonly infected organ for trematodes with avian definitive hosts, as it provides access to many nutrients and allows the worms to release eggs that are emitted into the environment with the bird's faeces. *C. arguinae* most likely lives in sympatry with other gastrointestinal trematodes (and other helminths). Indeed, oystercatchers (as well as gulls) also serve as definitive hosts to several co-occurring species, such as *Gymnophallus minutus* or *P. brevicolle* (Borgsteede *et al.*, 1988) which also utilize cockles as a second intermediate host.

As few potential eggs were observed in oystercatcher faeces, experimentation with different DNA extraction protocols to allow successful molecular identification of eggs was not possible. Trematodes of the genus *Curtuteria* are described as producing few eggs (10-80 per individual) (Odening, 1963; Reimer, 1963; Allison, 1979), which was reported for *C. australis* (Allison, 1979) and seems also to be the case for *C. arguinae*. Though eggs could not be molecularly matched with other life cycle stages directly, the drastically different detection rates in gull *vs*. oystercatcher faeces support that the molecular detection was not due to a diet based on cockles infected by *C. arguinae* (as it is also the case for gulls), but rather due to real infection of oystercatchers by adult worms. However, further attempts to

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

optically and molecularly identify eggs of *C. arguinae* are necessary to enable the study of the trematode's infection phenology at all stages of its life cycle.

Conclusion

Our study elucidated the complete life cycle of the trematode *Curtuteria arguinae*, until now only known to infect cockles as its second intermediate host. The needle snail (*Bittium reticulatum*) and the European oystercatcher (*Haematopus ostralegus*) were revealed to respectively be the first intermediate and definitive hosts of *C. arguinae*. The new identification of these hosts clearly explains why the specific sampling area on Banc d'Arguin was a hotspot of *C. arguinae* infection in cockles. Indeed, the site exhibits favorable conditions for all three hosts. Seagrass patches harbour many needle snails which are dominant grazers in these habitats, cockles are found buried in and around the seagrass patches in sandy sediments, while oystercatchers visit the area around low tide to prey on various macroinvertebrates. Tidal currents and winds can transport miracidia and cercariae from one host to another throughout the site, facilitating parasite transmission to upstream hosts, most of all cercariae shed by needle snails intensely infecting cockles, as it is observed. These findings open the path to investigating the infection phenology at each life cycle stage according to environmental constraints, the degree of the parasite's pathogenicity for the different hosts and its ecological implications within the marine ecosystem. Our study also highlights the powerful usefulness of eDNA approaches to help detect particularly inconspicuous life cycle stages under challenging conditions. We believe such methods to be particularly well suited for identifying first intermediate hosts which exhibit particularly low parasite prevalence.

Competing interests. The authors declare there are no conflicts of interest.

Ethical Standards. Not applicable.

Acknowledgements. Sampling was performed thanks to *Planula 4* vessels (CNRS-INSU, Flotte Océanographique Française) and its staff, and in collaboration with the SEPANSO Aquitaine in charge of the management of the Banc d'Arguin National Natural Reserve. We also thank Cécile Massé for help with water filtration, Ana Born-Torrijos for help with trematode egg observation and Nicolas Lavesque for help with SEM imaging.

Author's contribution. LS, GD, AC and XM designed the study. LS, AM and FG performed the analyses. LS, GD, AC and XM interpreted the data. AC and XM provided the funds. All authors drafted the manuscript and approved the final version of the article.

Financial support. This work is part of Leslie Stout's doctoral thesis (University of Bordeaux – 2022-RJ-114) financed by a doctoral grant of the French "Ministère de l'Enseignement Supérieur et de la Recherche".

References

Alfeddy, N, Bazairi, H, Gam, M and de Montaudouin, X (2024) Dynamics of “trematode – edible cockle (*Cerastoderma edule*)” parasite – host systems in three coastal ecosystems along a North-Eastern Atlantic gradient. *Biologia* **79**, 3611–3623. doi: 10.1007/s11756-024-01809-z.

Allison, FR (1979) Life cycle of *Curtuteria australis* n.sp. (Digenea: Echinostomatidae: Himasthlinae), intestinal parasite of the South Island pied oystercatcher. *New Zealand Journal of Zoology* **6**, 13–20. doi: 10.1080/03014223.1979.10428344.

Armonies, W and Hartke, D (1995) Floating of mud snails *Hydrobia ulvae* in tidal waters of the Wadden Sea, and its implications in distribution patterns. *Helgoländer Meeresuntersuchungen* **49**, 529–538. doi: 10.1007/BF02368380.

Bass, D, Stentiford, GD, Littlewood, DTJ and Hartikainen, H (2015) Diverse Applications of Environmental DNA Methods in Parasitology. *Trends in Parasitology* **31**, 499–513. doi: 10.1016/j.pt.2015.06.013.

Bass, D, Christison, KW, Stentiford, GD, Cook, LSJ and Hartikainen, H (2023) Environmental DNA/RNA for pathogen and parasite detection, surveillance, and ecology. *Trends in Parasitology* **39**, 285–304. doi: 10.1016/j.pt.2022.12.010.

Bennett, J, Presswell, B and Poulin, R (2023) Tracking life cycles of parasites across a broad taxonomic scale in a marine ecosystem. *International Journal for Parasitology* **53**, 285–303. doi: 10.1016/j.ijpara.2023.02.004.

Bommarito, C, Pansch, C, Khosravi, M, Pranovi, F, Wahl, M and Thieltges, D (2020) Freshening rather than warming drives trematode transmission from periwinkles to mussels. *Marine Biology* **167**, 46. doi: 10.1007/s00227-020-3657-3.

Borgsteede, FHM, Van Den Broek, E and Swennen, C (1988) Helminth parasites of the digestive tract of the oystercatcher, *Haematopus ostralegus*, in the Wadden Sea, The

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

Netherlands. *Netherlands Journal of Sea Research* **22**, 171–174. doi: 10.1016/0077-7579(88)90020-8.

Born-Torrijos, A, Poulin, R, Raga, J and Holzer, A (2014) Estimating trematode prevalence in snail hosts using a single-step duplex PCR: how badly does cercarial shedding underestimate infection rates? *Parasites & Vectors* **7**, 243. doi: 10.1186/1756-3305-7-243.

Born-Torrijos, A, Holzer, AS, Raga, JA, van Beest, GS and Yoneva, A (2017) Description of embryonic development and ultrastructure in miracidia of *Cardiocephaloides longicollis* (Digenea, Strigeidae) in relation to active host finding strategy in a marine environment. *Journal of Morphology* **278**, 1137–1148. doi: 10.1002/jmor.20700.

Cabodevilla, X, Malo, JE, Aguirre de Carcer, D, Zurdo, J, Chaboy-Cansado, R, Rastrojo, A and Traba, J (2024) DNA Prevalence of Eukaryotic Parasites with Zoonotic Potential in Urban-Associated Birds. *Birds* **5**, 375–387. doi: 10.3390/birds5030025.

Carss, DN, Brito, AC, Chainho, P, Ciutat, A, de Montaudouin, X, Fernández Otero, RM, Filgueira, MI, Garbutt, A, Goedknegt, MA, Lynch, SA, Mahony, KE, Maire, O, Malham, SK, Orvain, F, Van Der Schatte Olivier, A and Jones, L (2020) Ecosystem services provided by a non-cultured shellfish species: The common cockle *Cerastoderma edule*. *Marine Environmental Research* **158**, 104931. doi: 10.1016/j.marenvres.2020.104931.

Correia, S, Magalhães, L, Freitas, R, Bazairi, H, Gam, M and de Montaudouin, X (2020) Large scale patterns of trematode parasite communities infecting *Cerastoderma edule* along the Atlantic coast from Portugal to Morocco. *Estuarine, Coastal and Shelf Science* **233**, 106546. doi: 10.1016/j.ecss.2019.106546.

Dang, C, de Montaudouin, X, Bald, J, Jude, F, Raymond, N, Lanceleur, L, Paul-Pont, I and Caill-Milly, N (2009) Testing the enemy release hypothesis: trematode parasites in the non-indigenous Manila clam *Ruditapes philippinarum*. *Hydrobiologia* **630**, 139–148. doi: 10.1007/s10750-009-9786-9.

de Montaudouin, X, Thieltges, DW, Gam, M, Krakau, M, Pina, S, Bazairi, H, Dabouineau, L, Russell-Pinto, F and Jensen, KT (2009) Digenean trematode species in the cockle *Cerastoderma edule*: identification key and distribution along the north-eastern Atlantic shoreline. *Journal of the Marine Biological Association of the United Kingdom* **89**, 543–556. doi: 10.1017/S0025315409003130.

de Montaudouin, X, Binias, C and Lassalle, G (2012) Assessing parasite community structure in cockles *Cerastoderma edule* at various spatio-temporal scales. *Estuarine, Coastal and Shelf Science* **110**, 54–60. doi: 10.1016/j.ecss.2012.02.005.

de Montaudouin, X, Blanchet, H, Desclaux-Marchand, C, Lavesque, N and Bachelet, G (2016) Cockle infection by *Himasthla quissetensis* – I. From cercariae emergence to metacercariae infection. *Journal of Sea Research* **113**, 99–107. doi: 10.1016/j.seares.2015.02.008.

de Montaudouin, X, Arzul, I, Cao, A, Carballal, MJ, Chollet, B, Correia, S, Cuesta, J, Culloty, S, Daffe, G, Darriba, S, Diaz, S, Engelsma, M, Freitas, R, Garcia, C, Goedknegt, A, Gonzalez, P, Grade, A, Groves, A, Iglesias, D, Jensen, K, Joaquim, S, Lynch, S, Magalhães, L, Mahony, K, Maia, F, Malham, S, Matias, D, Nowaczyk, A, Ruano, F, Thieltges, D and Villalba, A (2021a) *Catalogue of parasites and diseases of the common cockle* Cerastoderma edule, 1st Edn. Aveiro, Portugal: UA Editora Universidade de Aveiro.

de Montaudouin, X, Grimault, S, Grandpierre, M and Garenne, A (2021b) Juvenile growth deficit as an early alert of cockle *Cerastoderma edule* mortality. *Marine Ecology Progress Series* **679**, 85–99. doi: 10.3354/meps13892.

Desclaux, C (2003) *Interactions hôtes-parasites: diversité, mécanismes d'infestation et impact des trématodes digènes sur les coques* Cerastoderma edule *(mollusque bivalve) en milieu lagunaire macrotidal*, PhD thesis, Bordeaux I, Bordeaux, France.

Desclaux, C, Russell-Pinto, F, de Montaudouin, X and Bachelet, G (2006) First record and description of metacercariae of *Curtuteria arguinae* n. sp. (Digenea: Echinostomatidae), parasite of cockles *Cerastoderma edule* (Mollusca: Bivalvia) in Arcachon Bay, France. *The Journal of Parasitology* **92**, 578–587. doi: 10.1645/GE-3512.1.

Donald, KM and Spencer, HG (2016) Host and ecology both play a role in shaping distribution of digenean parasites of New Zealand whelks (Gastropoda: Buccinidae: *Cominella*). *Parasitology* **143**, 1143–1156. doi: 10.1017/S0031182016000494.

Frainer, A, McKie, BG, Amundsen, P-A, Knudsen, R and Lafferty, KD (2018) Parasitism and the Biodiversity-Functioning Relationship. *Trends in Ecology & Evolution* **33**, 260–268. doi: 10.1016/j.tree.2018.01.011.

Fried, B, Kanev, I and Reddy, A (2009) Studies on collar spines of echinostomatid trematodes. *Parasitology Research* **105**, 605–608. doi: 10.1007/s00436-009-1519-5.

Goater, C (1993) Population biology of *Meiogymnophallus minutus* (Trematoda: Gymnophallidae) in cockles from the Exe Estuary. *Journal of the Marine Biological Association of the United Kingdom* **73**, 163–177. doi: 10.1017/S0025315400032707.

Honma, H, Suyama, Y and Nakai, Y (2011) Detection of parasitizing coccidia and determination of host crane species, sex and genotype by faecal DNA analysis. *Molecular Ecology Resources* **11**, 1033–1044. doi: 10.1111/j.1755-0998.2011.03048.x.

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

Hou, Y, Zhang, H, Miranda, L and Lin, S (2010) Serious Overestimation in Quantitative PCR by Circular (Supercoiled) Plasmid Standard: Microalgal pcna as the Model Gene. *PLoS ONE* **5**, e9545. doi: 10.1371/journal.pone.0009545.

Huver, JR, Koprivnikar, J, Johnson, PTJ and Whyard, S (2015) Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. *Ecological Applications* **25**, 991–1002. doi: 10.1890/14-1530.1.

Kostadinova, A (2005) Family Echinostomatidae. In: Jones, A., Bray, R.A., Gibson, D.I. (Eds). In *Keys to the Trematoda*. London, UK: CABI Publishing, Wallingford and The Natural History Museum, pp. 9–64.

Leung, TLF and Poulin, R (2008) Size-dependent pattern of metacercariae accumulation in *Macomona liliana*: the threshold for infection in a dead-end host. *Parasitology Research* **104**, 177–180. doi: 10.1007/s00436-008-1166-2.

Leung, TLF, Keeney, DB and Poulin, R (2009) Cryptic species complexes in manipulative echinostomatid trematodes: when two become six. *Parasitology* **136**, 241–252. doi: 10.1017/S0031182008005374.

Magalhães, L, Daffe, G, Freitas, R and de Montaudouin, X (2020) *Monorchis parvus* and *Gymnophallus choledochus*: two trematode species infecting cockles as first and second intermediate host. *Parasitology* **147**, 643–658. doi: 10.1017/S0031182020000402.

McFarland, LH, Mouritsen, KN and Poulin, R (2003) From first to second and back to first intermediate host: the unusual transmission route of *Curtuteria australis* (Digenea: Echinostomatidae). *The Journal of Parasitology* **89**, 625–628. doi: https://doi.org/10.1645/0022-3395(2003)089[0625:FFTSAB]2.0.CO;2.

Niewiadomska, K and Pojmańska, T (2011) Multiple strategies of digenean trematodes to complete their life cycles. *Wiadomoœci Parazytologiczne* **57**, 233–241.

Norris, K, Freeman, A and Vincent, JFV (2000) The economics of getting high: decisions made by common gulls dropping cockles to open them. *Behaviour* **137**, 783–807. doi: 10.1163/156853900502349.

Odening, K (1963) Echinostomatoidea, Notocotylata und Cyclocoelida (Trematoda, Digenea, Redioinei) aus Vögeln des Berliner Tierparks. *Bijdragen tot de dierkunde* **33**, 37–60.

Poulin, R (2007) *Evolutionary ecology of parasites*, 2nd Edn. Princeton, USA: Princeton University Press.

Presswell, B and Lagrue, C (2016) Assessing parasite infections from avian faecal samples: The old methods are still the best. *Notornis* **63**, 32–36. doi: 10.63172/426700ukrvop.

Preston, DL, Mischler, JA, Townsend, AR and Johnson, PTJ (2016) Disease ecology meets ecosystem science. *Ecosystems* **19**, 737–748. doi: 10.1007/s10021-016-9965-2.

Reimer, L (1963) *Curtuteria numenii* nov. gen., nov. sp. aus *Numenius Phaeopus* (L.) (Trematoda, Echinostomatidae, Himasthlinae). *Zeitschrift für Parasitenkunde* **23**, 249–252. doi: 10.1007/BF00259376.

Rusch, JC, Hansen, H, Strand, DA, Markussen, T, Hytterød, S and Vrålstad, T (2018) Catching the fish with the worm: a case study on eDNA detection of the monogenean parasite *Gyrodactylus salaris* and two of its hosts, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). *Parasites & Vectors* **11**, 333. doi: 10.1186/s13071-018-2916-3.

Smogorzhevskaya, LA and Iskova, NI (1966) *Curtuteria haematopodis* sp.nov. (Trematoidea, Echinostomatidae, Himasthlinae) new species from the oystercatcher. *Problemy Parazitologii* **5**, 108–111.

Sohn, W-M, Jung, W-J, Shin, E-H and Chai, J-Y (2024) Development of the head collar and collar spines during the larval stages of *Isthmiophora hortensis* (Digenea:

Echinostomatidae). *Parasites, Hosts and Diseases* **62**, 145–150. doi: 10.3347/PHD.23122.

Stout, L, Garenne, A and de Montaudouin, X (2022) Marine trematode parasites as indicators of environmental changes. *Ecological Indicators* **141**, 109089. doi: 10.1016/j.ecolind.2022.109089.

Stout, L, Daffe, G, Chambouvet, A, Correia, S, Culloty, S, Freitas, R, Iglesias, D, Jensen, KT, Joaquim, S, Lynch, S, Magalhães, L, Mahony, K, Malham, SK, Matias, D, Rocroy, M, Thieltges, DW and de Montaudouin, X (2024) Morphological *vs*. molecular identification of trematode species infecting the edible cockle *Cerastoderma edule* across Europe. *International Journal for Parasitology: Parasites and Wildlife* **25**, 101019. doi: 10.1016/j.ijppaw.2024.101019.

Sutherland, WJ (1982) Do oystercatchers select the most profitable cockles? *Animal Behaviour* **30**, 857–861. doi: 10.1016/S0003-3472(82)80159-0.

Tamura, K, Stecher, G and Kumar, S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* **38**, 3022–3027. doi: 10.1093/molbev/msab120.

Thieltges, DW, Krakau, M, Andresen, H, Fottner, S and Reise, K (2006) Macroparasite community in molluscs of a tidal basin in the Wadden Sea. *Helgoland Marine Research* **60**, 307–316. doi: 10.1007/s10152-006-0046-3.

Thomas, LJ, Milotic, M, Vaux, F and Poulin, R (2022) Lurking in the water: testing eDNA metabarcoding as a tool for ecosystem-wide parasite detection. *Parasitology* **149**, 261–269. doi: 10.1017/S0031182021001840.

Triplet, P (1996) Comment les Huîtriers-pies *Haematopus ostralegus* consommateurs de Coques *Cerastoderma edule* évitent les relations intraspécifiques. *Alauda* **64**, 1–6.

Table 1. Primers used for the detection or sequencing of *Curtuteria arguinae* and their specific cycling conditions.

Name	Sequence (5'→ 3')	Gene	Specificity	PCR/qPCR cycling conditions	Reference
CACOIdetF CACOIdetR	CGGGAGTCGTGCTCGTTTAT TGCGCTACCACAAACCAAGT	cox1	*Curtuteria arguinae*	94 ◦C/2min - (94 ◦C/15s - 66 ◦C/1min30s)× 40 cycles – 94°C	Present study
CA18SdetF CA18SdetR	TTACGGCCGGGTCAAACTC CCATACAAATGCCCCCGTCT	SSU	*Curtuteria arguinae*	94 ◦C/2min - (94 ◦C/15s - 66 ◦C/1min30s)× 40 cycles – 94°C	Present study
TremCOIS2 TremCOIAS2	TGTTYTTTAGKTCTGTKAC AATGCATMGGRAAAAAACA	cox1	Trematodes	95 ◦C/10min - (95 ◦C/60s - 43 ◦C/30s - 72 ◦C/60s) × 40 cycles - 72 ◦C/10min - 16 ◦C	Magalhães *et al.*, (2020)

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

Table 2. Detection of *Curtuteria arguinae* by quantitative PCR from the filtered water samples in which candidate first intermediate host species (gastropods) were held. Results (cycle threshold value (Ct)/melting temperature (°C)) are shown for the two gene markers (cox1 and SSU).

Candidate host species	*Peringia ulvae*		*Tritia neritea*		*Tritia reticulata*		*Bittium reticulatum*		*Steromphala umbilicalis*	
Gene	cox1	SSU	cox1	SSU	cox1	SSU	cox1	SSU	cox1	SSU
Replicate 1	-	-	-	-	-	-	28/82.7	23/89.4	-	-
Replicate 2	-	-	-	-	-	-	25/82.6	21/89.5	-	-
Replicate 3	-	-	-	-	-	-	28/83.4	25/89.5	-	-
Replicate 4	-	-	-	-	-	-	27/83.6	25/89.7	-	-
Replicate 5	/	/	-	-	-	34/89.5	32/82.9	28/89.2	-	-

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

Table 3. Dimensions of the main morphological features of *Curtuteria arguinae* cercariae (measurements based on 15 ethanol-fixed cercariae under coverslip pressure). Values represent the min-max (mean) measurements in µm.

Morphological structure	**Size (µm)**
Body length	250-400 (331)
Body width	110-130 (124)
Oral sucker diameter	50x40-80x70 (53x48)
Ventral sucker diameter	50x50-80x70 (60x89)
Tail length	270-370 (345)
Number of collar spines (when visible)	33

Table 4. Total number of metacercariae of *Curtuteria arguinae* encysted in cockles by experimental infestation and percentage of metacercariae for which a collar spine was visible 1, 2, 3 and 6 days after infection.

	1 day	**2 days**	**3 days**	**6 days**
Total number of metacercariae	61	57	104	74
Metacercariae with collar spines	3%	0%	3%	68%

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

Table 5. Cox1 sequences deposited in GenBank with corresponding accession number

Sequence ID	Primers	Host	GenBank accession numbers
B2_trem_COI_TremCOI_S2	TremCOI2S/TremCOI2AS	*Bittium reticulatum*	PV216840
B3_trem_COI_TremCOI_S2		*B. reticulatum*	PV216841
B4_trem_COI_TremCOI_S2		*B. reticulatum*	PV216842

Table 6. Pairwise genetic distances (*p*-distances) between the partial cox1 gene sequences obtained from cercariae shed by *Bittium reticulatum* and reference *Curtuteria arguinae* sequences.

Sequence	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1) B3_trem_COI_TremCOI_S2	-	-	-	-	-	-	-	-	-
(2) B2_trem_COI_TremCOI_S2	0.135	-	-	-	-	-	-	-	-
(3) B4_trem_COI_TremCOI_S2	0.129	0	-	-	-	-	-	-	-
(4) *C. arguinae* (PP988234)	0.120	0.004	0.004	-	-	-	-	-	-
(5) *C. arguinae* (MT002920)	0.125	0	0	0.004	-	-	-	-	-
(6) *C. arguinae* (PP988236)	0.125	0	0	0.004	0	-	-	-	-
(7) *C. arguinae* (PP988237)	0.125	0	0	0.004	0	0	-	-	-
(8) *C. arguinae* (PP988238)	0.125	0	0	0.004	0	0	0	-	-
(9) *C. arguinae* (PP988239)	0.125	0	0	004	0	0	0	0	-

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

Table 7. Overview of the known life cycles and morphometrics of cercariae of trematode species of the genus *Curtuteria*

	C. arguinae	*C. australis*	*C. numenii*	*C. grummti*	*C. haematopodis*
First intermediate host	*Bittium reticulatum* (Cerithiidae) [1]	*Cominella glandiformis* (Cominellidae) [6]	-	-	-
Second intermediate host	*Cerastoderma edule* (Cardiidae) [2,3] *Ruditapes phillipinarum* (Veneridae) [4,5] *R. decussatus* [4] *Polititapes aureus* (Veneridae) [4]	*Austrovenus stutchburyi* (Veneridae) [6] *Macomona liliana* (Tellinidae) [6,7] *Semele australis* (Semelidae) [6]	-	-	-
Definitive host	*Haematopus ostralegus* [1]	*H. finschi* [6] *H. unicolor* [8] *Larus* spp. [8,9]	*Numenius phaeopus* [10]	*Somateria mollissima* [11]	*H. ostralegus* [12]
Number of collar spines	33 [1,2]	31 [6]	29 [10]	29 [11]	33 [12]
Cercarial body length (mm)	0.3 [1]	0.69 [6]	-	-	-
Cercarial body width (mm)	0.12 [1]	0.26 [6]	-	-	-
Cercarial tail length (mm)	0.35 [1]	0.49 [6]	-	-	-
Cercarial oral	0.05 [1]	0.05 [6]	-	-	-

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

sucker diameter (mm)					
Cercarial ventral sucker diameter (mm)	0.075 [1]	0.15 [6]	-	-	-
Material fixation	Ethanol-fixed, under coverslip pressure [1]	Alive, under coverslip pressure [6]	-	-	-

References: [1] Present study, [2] Desclaux *et al.*, 2006; [3] de Montaudouin *et al*., 2021; [4] Dang *et al*., 2009; [5] Alfeddy *et al.*, 2024; [6] Allison *et al*., 1979; [7] Leung and Poulin, 2008; [8] Bennett *et al.*, 2023; [9] McFarland *et al*., 2003; [10] Reimer, 1963; [11] Odening, 1963; [12] Smogorzhevskaya and Iskova, 1966.

Figure 1. Figure panel of the different species of marine gastropods sampled on Banc d'Arguin (Arcachon Bay, France). A: *Tritia reticulata*; B: *Tritia neritea*; C: *Bittium reticulatum;* D: *Peringia ulvae*; E: *Steromphala umbilicalis*. Scale bars represent 1 cm.

Figure 2. Microphotographs of rediae of *Curtuteria arguinae* infecting *Bittium reticulatum* tissue. Left: *B. reticulatum* tissue sample (digestive glands and gonads) with apparent rediae (white arrows). Right: Closer view of a redia.

Figure 3. Figure panel of cercariae of *Curtuteria arguinae*. A. Drawing with the 33-spine circumoral collar. os: oral socker; cs: collar spine; ph: pharynx, in: intestines; ev: excretory vesicles; vs: ventral sucker; t: tail; B. Photograph under a stereomicroscope; C. SEM microphotograph.

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press

Figure 4. Figure panel of different larval stages of *Curtuteria arguinae* (cercariae and metacercariae), with a focus on the circumoral collar spines, present or absent, under stereo- and light microscopy. Arrows indicate the presence of spines. Scale bars represent 50 µm. A) Cercaria without spines. B) Cercaria with spines. C) Metacercaria with spines encysted in the digestive glands of a cockle observed under a stereomicroscope. D) Metacercaria with collar spines under light microscopy after extraction from the host tissue.

Figure 5. Scheme of the putative life cycle of *Curtuteria arguinae*. FIH, first intermediate host: *Bittium reticulatum* (needle snail); SIH, second intermediate host: *Cerastoderma edule* (edible cockle); DH, definitive host: *Haematopus ostralegus* (European oystercatcher). The adult worm, the miracidium and the eggs are not represented as their morphologies remain unknown.

https://doi.org/10.1017/S0031182025100267 Published online by Cambridge University Press