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Working towards a conservation plan for fish parasites: Cyprinid parasites from the south African cape fold freshwater ecoregion as a case study

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ABSTRACT

The preservation of the world's biodiversity for future generations has been a global objective for many years, with the establishment of the International Union for Conservation of Nature (IUCN) Red List of Threatened Species in 1964. However, the conservation of parasites is a more recent development and, due to the difficulty in obtaining data and studying some of the parasitic species, comes its own series of challenges. Using parasites of cyprinid hosts (one critically endangered, one endangered and three near threatened) collected from South Africa's Cape Fold freshwater ecoregion (CF) as a case study, this paper discusses the challenges and possible solutions for implementing a fish parasite conservation project. Novel data on the fish parasites (1819 metazoan parasite individuals, representing the Acanthocephala, Cestoda, Copepoda, Digenea, Monogenea and Nematoda) of the CF are provided from the five endemic hosts, *Cheilobarbus serra* (Peters, 1864), *Labeobarbus seeberi* (Gilchrist et Thompson, 1913), *Pseudobarbus phlegethon* (Barnard, 1938), *Sedercypris calidus* (Barnard, 1938), and *Sedercypris erubescens* (Skelton, 1974). Conservation statuses for selected parasite taxa are also proposed based on the conservation statuses of the fish hosts, according to the Conservation Assessment Methodology for Animal Parasites (CAMAP).

1. Introduction

The importance of parasite conservation has been extensively debated and the motivation for the conservation of a group of organisms, which has traditionally been the focus of eradication and control, is now well established (Lymbery and Smit 2023). To further the conservation of parasites, a working group comprising parasitologists and ecologists put forward a comprehensive strategy aimed at safeguarding non-pathogenic parasites that pose no risk to humans or domesticated animals (Carlson et al., 2020). As species conservation efforts and related funding are usually based on the species' conservation status, Kwak et al. (2020) adapted the International Union for Conservation of Nature (IUCN) assessment criteria to develop the Conservation Assessment Methodology for Animal Parasites (CAMAP). The CAMAP consists of seven criteria, with the first five focussing on the ecological data of the parasite and criteria 6–7 on the conservation status of the definitive and intermediate (if present) hosts. Kwak et al. (2020) acknowledge that the lack of ecological data on many poorly studied parasite species can make it difficult to assess the conservation status of these organisms using criteria 1–5, therefore, using host status, especially for host-specific parasites, might be more practical.

This is specifically true for South Africa's Cape Fold freshwater ecoregion (CF) (as defined by Abell et al., 2008), known for its high levels of biotic diversity and endemism, where limited information exists on the diversity of fish parasites. Furthermore, there is no information on the ecology of the few fish parasite species that have been documented from this region. The CF is also of high importance from a freshwater conservation point, since, of the 27 indigenous fish species present, 16 (59%) are endemic and 15 species (56%) are threatened, including two critically endangered species (Chakona et al., 2022). Thus, there is a very high probability that the CF is also host to a large number of threatened fish parasite species. Dobson et al. (2008) suggested that it is highly probable that a considerable number of parasitic helminths will be lost before being identified and classified. Due to the lack of research on the fish parasites from the CF, as well as the high level of endemism of freshwater fish and a large number of threatened

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hosts, the risk of undiscovered and described CF freshwater fish parasite species going extinct, is very real. To date, there are records for 21 parasite species from ten host species (four endemic and six invasive hosts) in the CF. However, two parasite species are endemic to the CF (reported from the four endemic hosts) and the remaining 19 have the following introduction statuses in South Africa: three parasites are co-invasive, seven are co-introduced with invasive and translocated hosts, one parasite species have an uncertain invasion status, the remaining eight parasite species (one reported from an CF endemic) have yet to be assessed and their origin and host range needs further investigation (see Fryer 1977; Van As and Basson 1984; Moravec et al., 2016; Smit et al., 2017; Truter et al., 2023). Thus, it is clear that knowledge on diversity of the parasitic communities of native and endemic fishes in the CF is greatly understudied and warrant extensive investigation to determine not only diversity, but also distribution and host specificity. To address this information gap, a project was initiated in 2022 that aimed to update and generate species data (barcode and catalogue the biodiversity) on the freshwater fish parasites of South Africa, to assist in the assessment of the risks of species extinctions, and make informed conservation decisions to mitigate impacts on South Africa's biodiversity. During the first year of the project, we came across a series of challenges that needed to be overcome in order to successfully work towards the conservation of fish parasites. Using the data collected on five cyprinid species (one critically endangered, one endangered and three near threatened) collected in the CF, the aims of this paper were to provide novel data on the fish parasites of the CF, use the Kwak et al. (2020) CAMAP to propose conservation statuses for selected taxa found, and to discuss challenges (and provide possible solutions) for implementing a fish parasite conservation project.

2. Materials and methods

2.1. Hosts

The International Union for Conservation of Nature (IUCN) Red List of Threatened Species is a widely recognised and commonly used tool for monitoring the status of endangered and threatened species worldwide. This valuable tool helps to inform decision-making related to conservation and biodiversity management. The five host species from this study have been recognised in three of the different IUCN Red List categories. The three Near Threatened (NT) species studied are the Clanwilliam sawfin *Cheilobarbus serra* (Peters, 1864), the Clanwilliam yellowfish *Labeobarbus seeberi* (Gilchrist et Thompson, 1913), and the Clanwilliam redfin *Sedercypris calidus* (Barnard, 1938). These fish are vulnerable to endangerment in the near future and are noted as having declining population sizes or distributions. All three of these fish are endemic to the Olifants-Doring River System (ODRS), Western Cape Province, South Africa (Skelton 2001). According to the latest IUCN records (Impson et al. 2017a, 2017b; Van der Walt et al., 2017b), there are 13 subpopulations (11 riverine and two dam) of the sawfin, 15 populations of the Clanwilliam yellowfish, and an ongoing decline occurring within several subpopulations of the Clanwilliam redfin. The latter species is further of interest as it is a small range-restricted species known from 19 threat-defined locations in the ODRS. The Endangered (EN) species, at risk of imminent extinction, is the fiery redfin *Pseudobarbus phlegethon* (Barnard, 1938). This is also a range-restricted species, known from five tributaries along the ODRS (Van der Walt et al., 2017a). These five subpopulations are small and severely fragmented attributing to their deteriorating numbers. The last species studied is Critically Endangered (CR) and has an extremely high risk of going extinct in its natural habitat. The Twee River redfin *Sedercypris erubescens* (Skelton, 1974) is endemic only to the Twee River catchment. This species has been diminishing in numbers since 1987, with the latest numbers between 4100 and 6838 (from two subpopulations that are small and isolated, see Jordaan et al., 2017a). One subpopulation is from the interconnected rivers in the Twee River System, while the second is from an introduced subpopulation to the Tuinskloof Dam (Bills 2011; Jordaan et al., 2017b). Invasive fish and habitat degradation have played a role in the dwindling population numbers of all the threatened ODRS fishes (Impson et al., 2007).

2.2. Sampling localities and parasite collection

Individuals of the above five cyprinid fish species were sampled alongside the annual monitoring surveys of CapeNature from various rivers and streams in the ODRS within the Cederberg Wilderness Area, Western Cape Province, South Africa, during December 2022 (Fig. 1). Fish were collected using fyke nets, seine nets and electrofishing. Ethical approval for this study was obtained from the North-West University AnimCare Research Ethics Committee (NWU-00781-22-A5), and the permit for fish collection was obtained from CapeNature (CN44-87-23462; CN16-87-23461). All collected fishes were transported in aerated containers with water from the sampled locality to a field station following the NWU-approved protocol (SOP NWU-00267-17-A5) for the temporary holding of fish. At the field station, each fish was killed by percussive stunning and cervical transection (SOP NWU-00267-17-A5). The body surface, fins, gills, and all internal organs were screened for the presence of parasites using a Nikon SMZ445 Zoom Stereo Microscope (Nikon, Tokyo, Japan). Parasites were preserved as per the standard approach for each parasitic group following Gelnar et al. (2018). All collected parasites were morphologically studied and microphotographs were captured using a compound microscope (Nikon Eclipse N*i*, Nikon, Tokyo, Japan) equipped with a computerised digital camera system for image analysis with differential interference contrast, and NIS-Elements BR 4.60© software. Full parasite species descriptions including both morphological and molecular analyses of the parasite taxa found will be published separately.

2.3. Data analyses

Ecological descriptors for the different taxa (prevalence with 95% confidence interval and mean intensity) were calculated on the online tool Quantitative Parasitology on the Web (QPweb, http://www.zoologia.hu/qp/qp.html) (Reiczigel et al., 2019). All other descriptors were calculated following Bush et al. (1997). Plotting of a species accumulation curve to determine completeness of parasity diversity discovery based on sampling effort was done using the iNEXT Online (iNEXT Online, shinyapps.io) tool to estimate potential diversity for the three hosts that had a species richness of 2 or more (Chao et al., 2016).

3. Results

Sixty-seven fish were collected and a total of 1819 metazoan parasite individuals, representing the Acanthocephala, Cestoda, Copepoda, Digenea, Monogenea and Nematoda were collected from various organs (Table 1). In addition, Myxozoa plasmodia were found on the gills of one of the host species (spores per plasmodium not counted). The three NT species were hosts to the most species-rich parasitic communities. *Cheilobarbus serra* (Fig. 2 A) harboured the most species (six), which included the monogeneans *Paradiplozoon* sp. (Fig. 2 B – D), *Gyrodactylus* sp. (Fig. 2 E – F) and pre-metamorphic copepod stages of a Lernaeidae species (Fig. 2 G), all from the gills. Adult and sub-adult stages of the nematode *Rhabdochona* sp. 1 (Fig. 2 H – I) and a cestode of the Caryophyllidea (Fig. 2 J) were recovered from the intestine. The parasitic community of *L. seeberi* (Fig. 3 A) were the second most species-rich, with myxozoan plasmodia, *Myxobolus* sp. (Fig. 3 B) and monogenean, *Dactylogyrus* sp. (Fig. 3 C – E), collected from the gills. A second nematode species, *Rhabdochona* sp. 2 (Fig. 3 F – H) was recovered from the intestine, and diplostomid metacercariae (Fig. 3 I) were excysted from black cysts on the skin. *Sedercypris calidus* (Fig. 4 A) were also infected with the same *Paradiplozoon* sp. (Fig. 4 B) as *C. serra*, while cystacanths of *Acanthogyrus* sp. (Fig. 4 C) and third-stage larvae of *Contracaecum* sp.

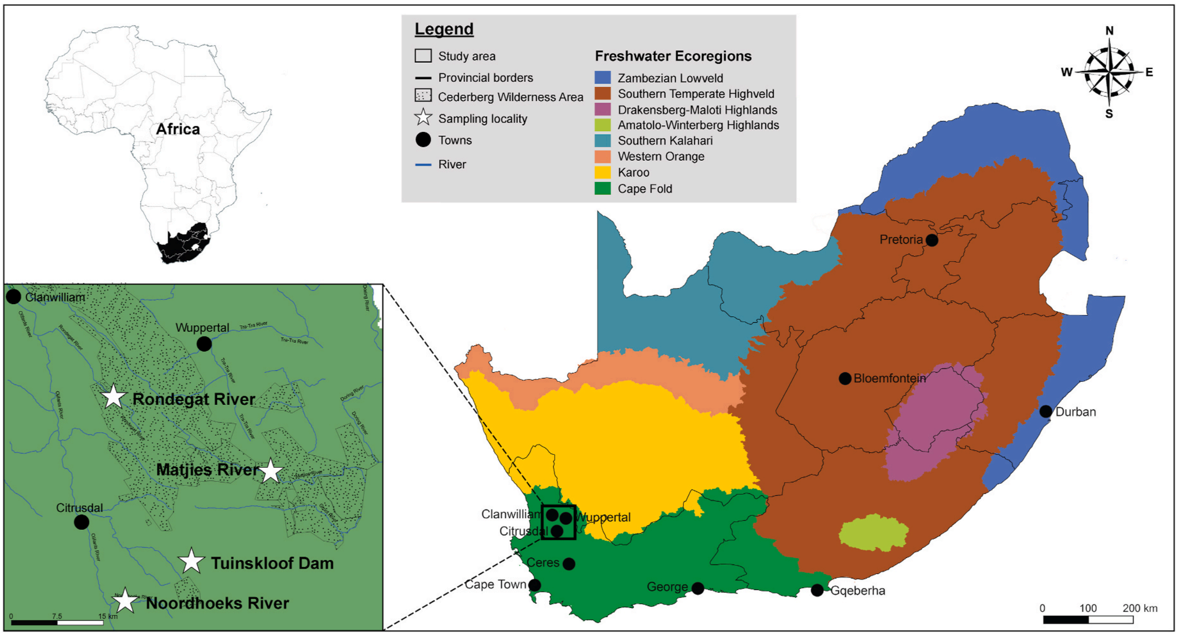

Fig. 1. Map illustrating localities where the five cyprinid hosts were collected in the Cape Fold ecoregion in the Western Cape, South Africa.

Table 1
List of threatened fish species and their associated parasitofauna collected from rivers within the Olifants-Doorn River system in the Cape Fold ecoregion, Western Cape, South Africa. Abbreviations: CR – Critically Endangered, EN – Endangered, NT – Near Threatened. N/n – number of host individuals infected with any parasite/ number of host individuals screened. Values for parasitic infection are indicated as: Prevalence (%) [95% confidence interval], mean intensity (range), unless indicated otherwise.

	Cheilobarbus serra (Peters, 1864)	*Labeobarbus seeberi* (Gilchrist et Thompson, 1913)	*Pseudobarbus phlegethon* (Barnard, 1938)	*Sedercypris calidus* (Barnard, 1938)	*Sedercypris erubescens* (Skelton, 1974)
IUCN status	NT	NT	EN	NT	CR
N/n	15/15	10/10	1/12	15/15	1/15
Total length in mm [mean ± SD (range)]	232.3 ± 50.4 (140–350)	222.8 ± 24.3 (190–270)	42.8 ± 26.1 (52–65)	92.8 ± 15.1 (75–125)	99.1 ± 28.4 (95–120)
Parasite richness	6	4	1	3	1
Myxozoa					
Myxobolus sp.[b]	–	40 [0.122, 0.738], 9 (1–16)[d]	–	–	–
Acanthocephala					
Acanthogyrus sp.[c]	–	–	8 [0.002, 0.385], 1 (1)	93 [0.681, 0.998],17.1 (1–42)	–
Monogenea					
Dactylogyrus sp.[a]	–	100 [0.692, 1], 34 (1–111)	–	–	–
Gyrodactylus sp.[a]	33 [0.118, 0.616], 1.4 (1–2)	–	–	–	–
Paradiplozoon sp.[a]	66 [0.384, 0.882], 3.0 (1–6)	–	–	60 [0.323, 0.837], 3.0 (1–8)	–
Cestoda					
Caryophyllidea gen. sp.[b]	86 [0.595, 0.983], 19.9 (1–80)	–	–	–	–
Digenea					
Diplostomidae gen. sp.[c]	40 [0.163,0.677], 2.0 (1–5)	40 [0.122, 0.738], 6.3 (1–2)	–	–	–
Nematoda					
Contracaecum sp.[c]	–	–	–	26.7 [0.078, 0.551], 1.0 (1)	6.7 [0.002, 0.319],1 (1)
Rhabdochona sp. 1[b]	80 [0.519, 0.957], 12.5 (1–54)	–	–	–	–
Rhabdochona sp. 2[b]	–	70 [0.348, 0.933], 100 (2–199)	–	–	–
Copepoda					
Lernaeidae gen. sp.[a,c]	6 [0.002, 0.319], 2 (2)	–	–	–	–

[a] Monoxenic parasite species that utilise fish as host for both immature and adult parasite life stage forms.
[b] Polyxenic parasite species that utilise the vertebrate fish host as a definitive host.
[c] Indicate intermediate life stages of parasite (i.e., metacercariae, acanthocysts, larvae) present.
[d] Intensity range for number of plasmodia per fish individual fish host, spores in each plasmodium not counted.

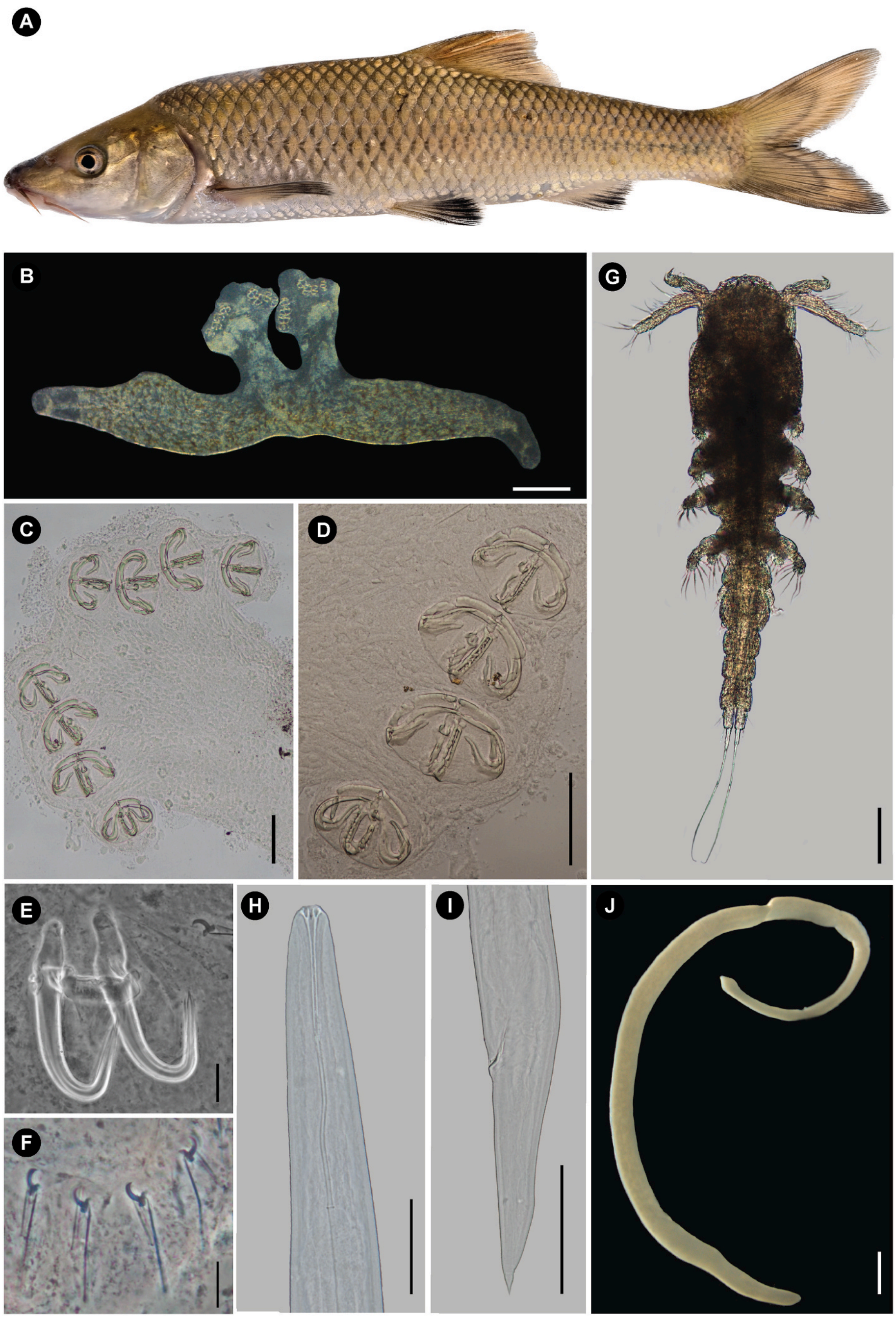

Fig. 2. *Cheilobarbus serra* (Peters, 1864) (max. length: 350 mm) (**A**); adult *Paradiplozoon* sp. (**B**) and sclerites in attachment clamps (**C**, **D**) found on the gills; hamuli of *Gyrodactylus* sp. (**E**) and marginal hooks (**F**), and a pre-metamorphic stage of the copepod belonging to the Lernaeidae (**G**), both from the gills. Anterior (**H**) and posterior (**I**) ends of *Rhabdochona* sp. 1 (lateral view) from the intestine; (**J**) whole specimen of the Caryophyllidea. Scale bars: 10 μm (E, F); 100 μm (C, D, G, H, I); 500 μm (B); 1000 μm (J).

(Fig. 4 D – E) were found in the body cavity. The endangered *P. phlegethon* (Fig. 5 A) and critically endangered *S. erubescens* (Fig. 6 A) were only infected with a single specimen of *Acanthogyrus* sp. (Fig. 5 B) and larval *Contracaecum* sp. (Fig. 6 B – C), in the body cavity, respectively. The parasite species richness of three host species (*C. serra*, *L. seeberi* and *S. calidus*) and associated sampling effort during the present study indicates that the true parasitic diversity of these three hosts (at infra- and component community level) has not been fully sampled within its endemic distribution range within the ODRS (Fig. 7).

4. Discussion

4.1. Parasite diversity

This is the first comprehensive parasitological survey conducted on any of the endemic and threatened species in the CF. Previous surveys consisted of sporadic encounters of parasitic taxa collected during exploratory surveys by fish taxonomists and ecologists (Barnard 1955; Fryer 1977; Moravec et al., 2016). To date, only three parasite species have been reported from four endemic fish species within the CF. Two of these hosts are *S. calidus* and *S. erubescens* collected during the present study and are hosts of the branchiuran, *Chonopeltis minutus* Fryer (1977) (see Van As and Van As 1999). However, *C. minutus* was not found during this survey (see discussion below). Our discovery of 11 parasitic taxa that did not morphologically conform to that of any known species, indicates that there is most definitely undiscovered parasitic diversity from these endemic CF fishes. The species accumulation curve (Fig. 7) further supports this notion and warrants further investigation into the parasitic communities of the selected hosts from other localities or populations within their narrow distribution range.

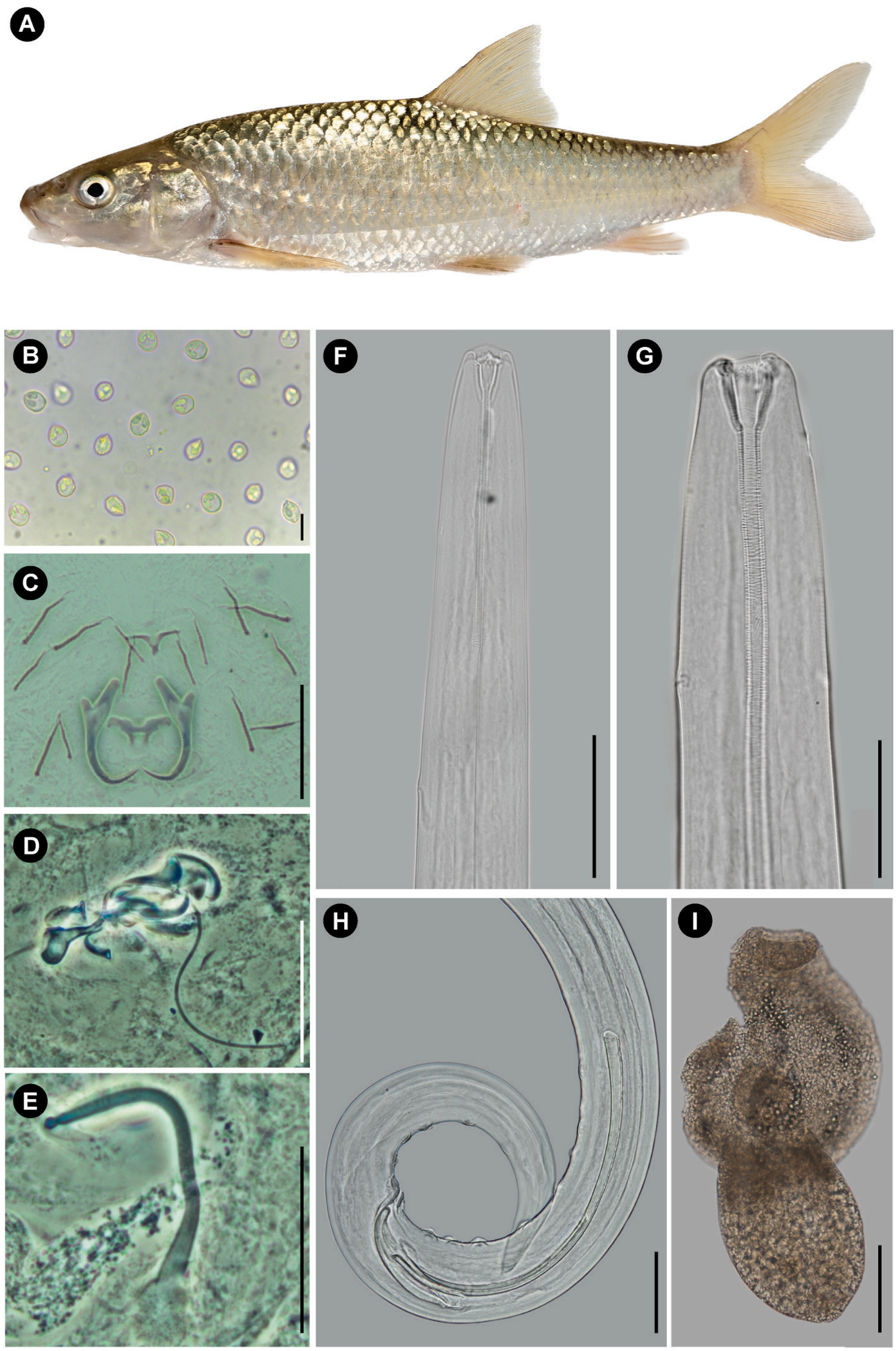

Fig. 3. *Labeobarbus seeberi* (Gilchrist et Thompson, 1913) (max. length: 270 mm) (**A**); *Myxobolus* sp. (**B**) and *Dactylogyrus* sp. from the gills of *L. seeberi*, hamuli and marginal hooks (**C**), male copulatory complex (**D**) and vagina (**E**). Lateral view of *Rhabdochona* sp. 2 from the intestine, anterior end of female (**F**) and male (**G**), posterior end of male (**H**); metacercariae of Diplostomidae (**I**) from black cysts on skin. Scale bars: 10 μm (B); 50 μm (D, E); 100 μm (C, F, G, H, I).

4.2. Parasite conservation status

As mentioned previously, the most suitable criteria to assess parasite conservation within the framework of the CAMAP would be to infer criteria 6–7 until additional data is available for a full re-assessment. Similar to the case study demonstrating the application of the CAMAP on a host-specific tick of the critically endangered pygmy possum (Kwak et al., 2018) in Australia, no comprehensive parasitic surveys have been undertaken on the abundance and frequency of these 11 parasitic species across the populations of the five selected cyprinids in the ODRS. Due to this lack of comprehensive temporal abundance and frequency of infection data for the cyprinid hosts studied, the following monoxenic parasitic species, *Dactylogyrus* sp., *Gyrodactylus* sp., *Paradiplozoon* sp., and the pre-metamorphic lernaeid copepod most probably constitute Near Threatened species, based on criterion 6, since they all utilise their respective hosts as both immature and adult forms. Similarly, the polyxenic species *Myxobolus* sp., the two *Rhabdochona* spp. and the caryophyllidean cestode that utilise the vertebrate fish host as definitive hosts are proposed as Near Threatened. The following three species, the larval *Acanthogyrus* sp., *Contracaecum* sp. and Diplostomidae metacercariae, are assessed under criterion 7, since these parasites utilise their cyprinid hosts as intermediate hosts. These three are regarded as Data Deficient as there is currently no information available on the hosts of their other life stages. It should be noted that placement of species in

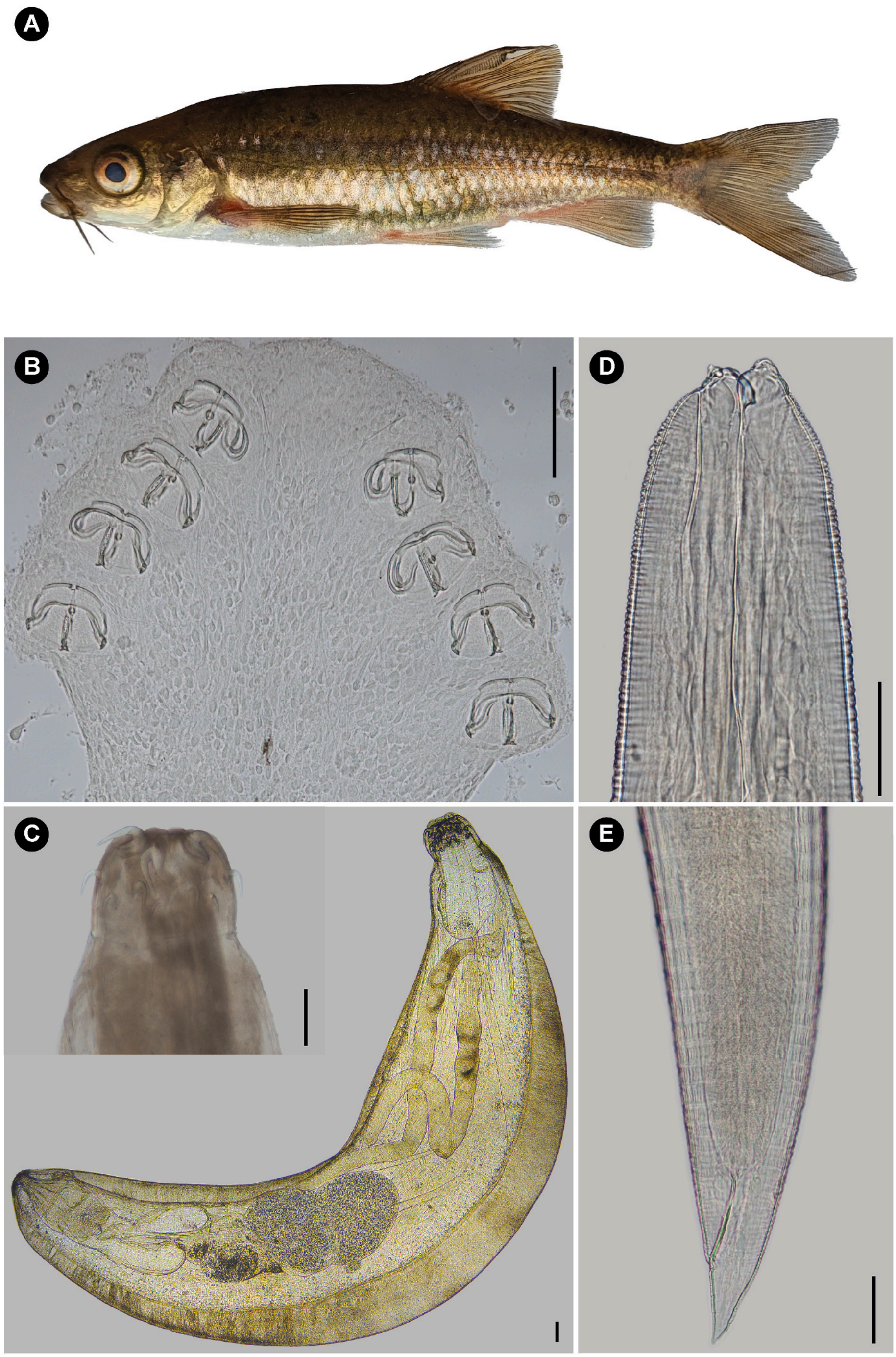

Fig. 4. *Pseudobarbus calidus* (Barnard, 1938) (max. length: 125 mm) (**A**). Sclerites of *Paradiplozoon* sp. from the gills (**B**). Acanthocephala from the body cavity, whole specimen (**C**) and hooks on proboscis (top left insert). Larval *Contracaecum* sp. from the body cavity, anterior (**D**) and posterior (**E**) ends, lateral view. Scale bars: 100 μm (B, C, D, E).

the Data Deficient category is suitable in cases where there is a deficiency in data on diversity, distribution and host ranges and is provided for within the category guidelines of the IUCN. Placement of specimens within this category supports continued and extensive investigation to inform on geographic ranges, and in the case of parasites their specificity and utilisation of intermediate and definitive hosts which, alongside complete morphological and molecular identification will warrant re-assessment within the framework of the IUCN and CAMAP (see Kwak et al., 2020; Lymbery and Smit 2023).

The only known fish parasite from the ODRS, *C. minutus* was originally described from *S. calidus* and *S. erubescens*. These fish were collected in 1974 from the Twee and Tra Tra rivers and the branchiurans were located in the gill chambers of their hosts (although some of the small larvae were also located inside the mouth of the host). *Chonopeltis minutus* was not found on any of the cyprinids (including *Cheilobarbus capensis* (Smith, 1841); *C. serra*; *Enteromius anoplus* (Weber, 1897); and *P. phlegethon*) nor on the Clanwilliam catfish *Austroglanis gilli* (Barnard, 1943) examined by the same collector (see Fryer 1977). Fryer (1977) reported a prevalence of 12% from the Tra Tra River (44/362) and 96% from the Twee River (48/50), with an intensity of one to eight parasites per infected fish (with the exception of a single fish host that had 25 parasites infesting it), clearly showing that it was, especially in the Twee

Fig. 5. *Pseudobarbus phlegethon* (Barnard, 1938) (max. length: 65 mm) (**A**); *Acanthogyrus* sp. found from the body cavity (**B**). Scale bar: 500 μm.

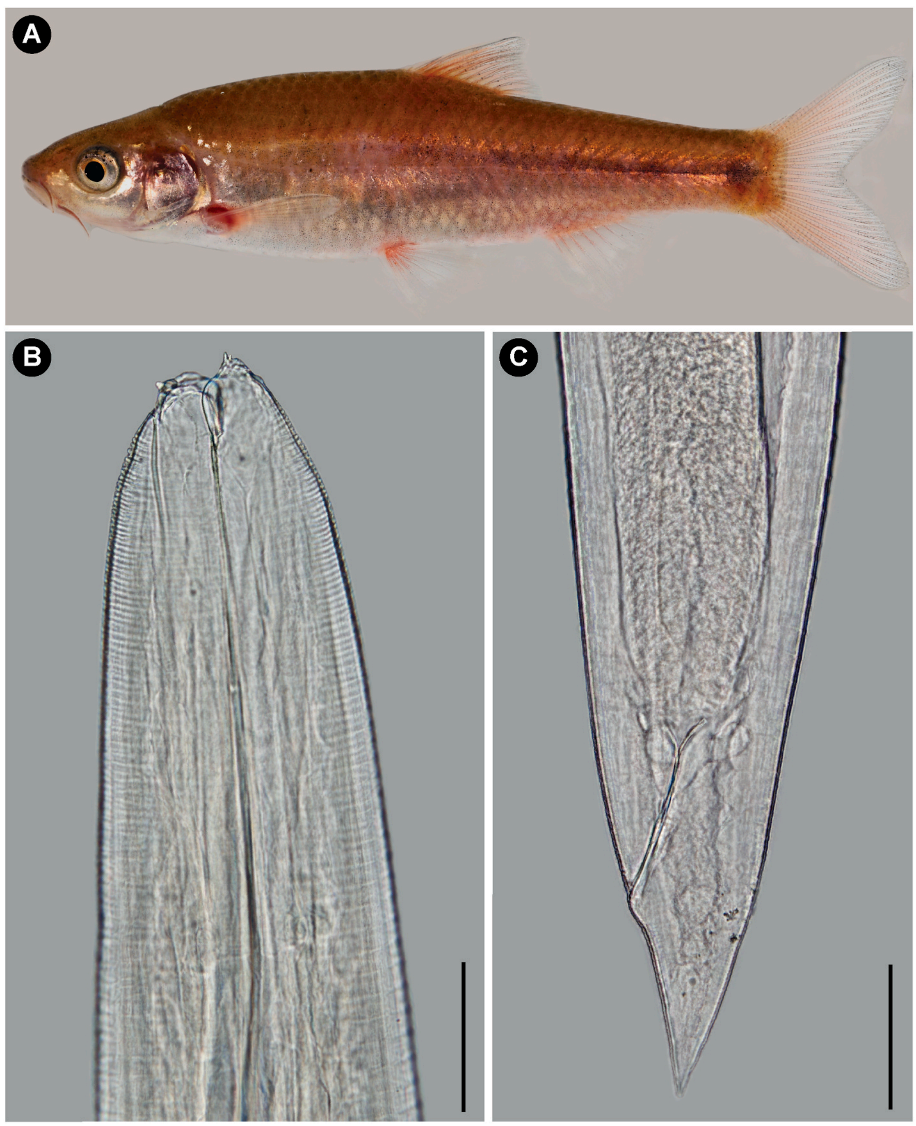

Fig. 6. *Sedercypris erubescens* (Skelton, 1974) (max. length: 120 mm) (**A**). Larval *Contracaecum* sp. from the body cavity, anterior (**B**) and posterior (**C**) ends, lateral view. Scale bars: 100 μm (B, C).

River, a common and abundant species. In the same publication, Fryer (1977) also described *Chonopeltis australissimus* Fryer, 1977 from museum material from the adjacent Great Berg River, originally collected in 1937 from *Pseudobarbus burgi* (Boulenger, 1911), another fish species currently listed as Endangered. In 1999, Van As and Van As (1999) redescribed *C. minutus* based on the 1974 type material and synonymised *C. australissimus* with *C. minutus*. The authors proposed that the distribution of the specimens into the two water systems could have occurred prior to the isolation of the two rivers, or even across the watershed thereafter as the headwaters of the rivers are in close proximity and fish translocation could have occurred during heavy rain (Van As and Van As, 1999). Unfortunately, additional specimens of *C. minutus* have not been collected and recorded again since 1974, including the present study. Based on the originally reported high prevalence in the Twee River, this is concerning and led us to believe that this parasitic species may no longer be extant. As all three hosts are threatened species

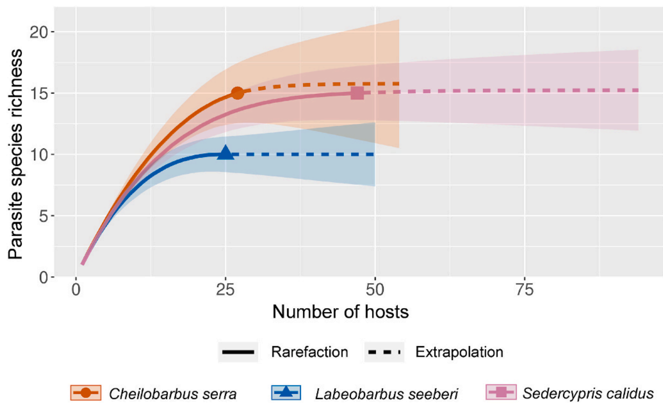

Fig. 7. Rarefaction/extrapolation curve estimating the diversity of parasites as a function of sampling effort for three of the five hosts collected in the Olifants-Doorn River System, Western Cape Province, South Africa. Shaded area represents the 95% confidence interval obtained using the bootstrap method based on 100 repetitions. Created using iNEXT Online (Chao et al., 2016).

(one NT, one EN and one CR), and *C. minutus* has not been collected in 50 years, it can only be assumed that this endemic and rare branchiuran parasite is either already extinct or in danger of extinction and should be classified as Critically Endangered.

4.3. Challenges

4.3.1. Lethal collection of species at risk of extinction

The three main points of concern when doing research on the parasites of threatened host species are the lethal collection of the host, the number of individuals to be dissected, and the methods used for humane killing of the fishes. The lethal collection of species at risk of extinction has been the subject of lively debate ranging from the justification of lethal sampling (Heupel and Simpfendorfer, 2010) as well as proposing non-lethal approaches (Simmons and Palace 2022). In fact, the IUCN already produced a policy statement in 1989 on research involving species at risk, and in the same year provided guidelines for the implementation of the policy, specifically in reference to scientific collection of threatened species (IUCN 1989). From our experience, we can with confidence acknowledge that these criteria are considered of high importance during the ethical and permit application process in the South African context. The entities and guidelines implemented at institutional and provincial level to which researchers must answer before any animal (despite its conservation status) is utilised for research purposes comprises a rigorous review process. When threatened species are required for the study, it may be necessary to require assistance from local liaison or environmental intelligence officers who will need to determine if the fish populations in question are stable and viable for sampling. This could potentially be a time-consuming and resource intensive process to determine population sizes and their viability, which may discourage research on these species and their parasites. From this study, it was also clear that it is imperative to liaise with local conservation agencies or permitting offices in a timely manner. Furthermore, maintaining good relationships with these agencies, fostering a culture of care, using an integrative approach to understand the purpose and value of the intended research and its outcomes, as well as valuing advice, time, and assessment from both a scientific and management perspective, are all of utmost importance. In South Africa, similar to many other developing countries, we are currently facing a deficit in knowledge on biodiversity, with a great shortfall in past knowledge to guide the policy on the management and protection of parasitic species along with their application in the conservation of ecological units (parasites and hosts) for the future. Currently, the application of non-lethal tools, such as eDNA, to assess parasitic communities is still in the developing and refinement stages, and these are not yet accepted as default tools for detection (Thomas et al., 2022). In addition, comparative libraries for the datasets obtained using non-lethal tools first need to be populated prior to its consideration as the primary method of assessment, and in South Africa, there is much to be done before such tools can be utilised effectively.

When considering sample size, the aim is to limit utilisation to the minimum number of individuals needed to provide statistically sound data sets. In practice, there is not necessarily a “golden number” for a given species, and at times we can only utilise the maximum approved (permitted) sample size, usually between 10 and 20 individuals. Although unknown, the approximate number of specimens required to determine taxa richness can be calculated using predictive models, or through implementing species accumulation curves to visualise how much of a community has been recorded or how the effort reflects the proportion of data that was obtained from a sampled community (Dove and Cribb 2006). To exemplify this point, Erasmus et al. (2022) showed that there is still much diversity to be discovered for the super klipfish *Clinus superciliosus* (Linnaeus, 1758) after estimating the diversity of parasites as a function of sampling effort in this host with a smaller sample size ($n = 16$ to 20). Morris et al. (2019), using parasitic data from a much larger sample size of the elephant fish *Callorhinchus capensis* Duméril, 1865, were able to predict host population structure and characterise the parasitic community and potential interactions between parasitic species and potential hosts within that ecosystem. At times it would require lethal sampling to provide baseline and taxonomic data to complement non-lethal tools, yet it is possible to perform these without diminishing species that are vulnerable. Thus, it is imperative that continued inventory is taken of our ecosystems and their components, in this case, parasitic species.

The manner of killing the host fish is also a point for consideration. Following international guidelines most ethics committees consider a two-step procedure of killing a fish to be the most humane (usually a form of percussive stunning followed by a cervical transection). Although this may work for larger fish, smaller fish may prove to be more complicated and may require a suitable chemical agent (such as clove oil or MS-222). Although a natural agent such as clove oil has been shown to be a good and environmentally friendly anaesthesia agent for fishes (Keene et al., 1998; López-Cánovas et al., 2020), to our knowledge no studies have been conducted on whether these chemicals have an impact on the ability to successfully retrieve and study ectoparasites for taxonomic purposes and warrants further research.

4.3.2. Isolated and refuge populations

It is clear from ongoing conservation efforts of threatened species that very little is known about their co-existing symbionts, and this is portrayed in the absence of parasites on conservation priority lists to date. Conservation strategies and management plans primarily focus on maintaining the genetic integrity of a species considered for augmentation, and strategies rather focus on determining the lowest viable number of individuals for translocation or introduction into *in situ* or *ex situ* environments. This is done to safeguard the genetic integrity of the species of concern by ensuring there is low potential for i) genetic bottlenecking, ii) inbreeding depression, and iii) the introduction of pathogens and parasitic species that may compromise host health or falter the success of conservation strategies (Bills and Impson 2013; Northover et al., 2018). It is therefore clear that until recently, the translocation of hosts for conservation has been concerned with the prevention of parasitic introduction and spread, rather than the conservation of natural symbionts (mutualists, commensals, or parasites). Anti-parasitic treatment has thus been the primary cause for harming the conservation attempts of natural parasites.

In the present assessment, the parasitic community from five cyprinids in the ODRS was studied. However, this is also the first investigation on the parasitic community of a translocated population of the critically endangered *S. erubescens*, 17 years after the introduction of 48 individuals into the Tuinskloof Dam, from the upper reaches of the

Suurvlei River within the Twee River system. At that point, a single parasitic species, the branchiuran *C. minutus*, were known to parasitise the gill chambers of *S. erubescens* within the Twee River system (Fryer 1977; Jordaan et al., 2017b). However, the parasitic community of the seed population nor that of the translocated individuals to the Tuinskloof Dam were assessed prior to translocation. During the present study we only found a single larval nematode (*Contracaecum* sp.) in the 15 fish screened and thus it can be postulated that i) there were initially no parasites present in the founder population, or ii) if there were, the small founder population introduced into the Tuinskloof Dam had a naturally low parasite diversity and abundance that could not be sustained along with the adjustment to the lentic system to which it was translocated (Colwell et al., 2012; Northover et al., 2018).

An additional point of consideration for *in situ* or *ex situ* conservation interventions is the probability or potential for co-extinction of a parasitic species, particularly those that are highly host-specific or isolated to geographic regions (Strona 2015). Co-extinction refers to the disappearance of a parasitic species due to its dependence on a host that has become extinct, or where the host population has declined below a critical level to sustain its associated parasite community (Dunn et al., 2009; Colwell et al., 2012). Thus, when the parasite and host as an ecological unit is translocated for conservation purposes, host numbers should be considered alongside the ecological need for its parasites to persist in the novel environment, as well as maintain genetic integrity once host populations have been restored. Similarly, the specificity of the parasitic species associated with the host, as well as potential alternative hosts in the recipient environment should be taken into account if the parasite can utilise a range of hosts, without causing harm, to ensure its future persistence.

5. Conclusion

To our knowledge, this is the first dedicated effort to survey threatened and near threatened fish hosts in order to resolve the conservation status of their parasites. In doing so, a number of challenges had to be overcome, including receiving permits and ethical clearance for the research as well as dealing with isolated and refuge populations. One of the main findings from this research is the difference in parasite diversity between the near threatened and the threatened hosts, with the former hosting between three and six times the number of species than the latter. This indicates that some of the parasites of the two endangered species are most probably already extinct and further highlights the importance of protecting the entire threatened ecological community (the host and its parasites) of the ODRS and other similar systems with a large number of endemic and threatened species. One of the biggest challenges in fish parasite conservation research is obtaining permits and ethical approval for the lethal collection of threatened hosts species. Therefore, we as parasitologists, should take responsibility and consider the current and future value of the knowledge to be gained through the lethal collection of a host and how it can contribute to the conservation of the threatened host and their parasites.

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