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Abstract

This study highlights the value of museum collections in invasion biology. It focuses on introduced tilapias, *Oreochromis niloticus* and *Coptodon rendalli* in the Congo Basin and their monogenean (Platyhelminthes) gill parasite fauna. *Oreochromis niloticus* was introduced throughout the Congo Basin while *C. rendalli* was introduced into the Lower Congo, but is native to the Middle and Upper Congo. In order to study the impact of these stocking events on the native parasite community we investigate the co-introduction and host switching of their parasites. Post-introduction material is compared with pre-introduction samples from museum collections of 5 native tilapias in the Congo basin. Nine of the known parasites of *O. niloticus* were co-introduced, while one, *Cichlidogyrus rognoni*, is missing and possibly not established. In contrast, no parasite species were found co-introduced with *C. rendalli* into the Lower Congo. The parasite fauna of *Tilapia sparrmanii* shared no species with *O. niloticus*. *Oreochromis mweruensis* shared five species with *O. niloticus*, but these were also found on the pre-introduction samples, and are considered native to both hosts. We report three putative host switches: *Cichlidogyrus sclerosus* and *Cichlidogyrus tilapiae* to *Coptodon tholloni* in the Lower Congo Basin and *Gyrodactylus nyanzae* to *Coptodon rendalli* in the Upper Congo.

Keywords

Oreochromis niloticus – host switching – museum collections – fish stocking – parasite co-introduction

Introduction

The Congo River is the second largest river in the world in terms of discharge, and its basin is the largest in Africa, covering around 3,8 million km^2 (Dupré et al 1996; Markert et al. 2010). Its fish biodiversity is of global importance (Shumway et al. 2003) and it is the species richest basin of Africa, harbouring approximately 1000 species, not including Lake Tanganyika, and with an endemicity level of 65% (Froese and Pauly 2019). The Congo Basin is divided into three major sections: the Upper Congo, or Lualaba, the Middle Congo and the Lower Congo (Alter et al. 2015; Harrison et al. 2016) and further into 16 freshwater ecoregions (Thieme et al. 2005). However, many areas remain underexplored (Stiassny et al. 2008), and a comprehensive overview of the ichthyofauna has not yet beenpublished. Additionally, the fish fauna is of high economic importance because of both fishing and aquaculture, providing at least 30% of the animal protein intake for the local population (Béné and Heck 2005). A major part of that is the culture of tilapias (Canonico et al. 2005), cichlid fish belonging to *Oreochromis* Günther, 1889, *Sarotherodon* Rüppell, 1852, *Coptodon* Gervais, 1853 and *Tilapia* Smith, 1840. Global tilapia production quintupled to over 5 million tonnes yearly between 2000 and 2015 (FAO 2017) and is now practiced in 140 countries (Deines et al. 2016).

Tilapia culture is dominated by Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) and its hybrids, that together make up over 75% of the cultured tilapias in sub-Saharan African (FAO 2017). *Oreochromis niloticus* is native to the Nile River Basin, the coastal rivers of Israel, and various East African lakes including Lake Tanganyika, Lake Kivu, Lake Turkana, and the Omo, Suguta, Senegal, Gambia, Volta, Niger, Benue and Chad River systems (Trewavas, 1983). However, it can thrive in virtually any tropical freshwater and estuarine habitat, with the exception of torrential rivers (Shipton et al. 2008). The fish is very opportunistic and easily changes its feeding behaviour depending on which other fish species co-occur (Njiru et al. 2004, Zengeya et al. 2012). *Oreochromis niloticus* is a year-round spawner that exhibits parental brood care and that has a flexible growth rate and maturation size (Starling et al. 2002). All these factors contribute to the popularity of Nile tilapia as a culture species, but also make it a potentially dangerous invasive species (Zengeya et al. 2015). *Oreochromis niloticus* has escaped culture facilities numerous times and established feral populations in many tropical and sub-tropical countries (Cone et al. 1995, Jiménez-García et al. 2001, Mendoza-Franco et al. 2006,; Roche et al. 2010, Šimková et al. 2019). It is estimated that over half of all tropical countries have at least one feral tilapia population (Deines et al. 2016), with adverse effects such as competition for breeding grounds (Canonico et al. 2005), predation on eggs (Alcaraz et al. 2015), hybridization with native tilapias (Firmat et al. 2013, Deines et al. 2014) and stimulation of phytoplankton growth and eutrophication (Starling et al. 2002). Native species of *Oreochromis* can decline rapidly after introduction of *O. niloticus* (Shipton et al. 2008). In Lake Victoria, the

native *Oreochromis esculentus* Graham, 1928 was virtually wiped out by *O. niloticus* in 30 years (Goudswaard et al. 2002, Wise et al. 2007).

Introduction of tilapias for sub-Saharan aquaculture generally started in the late 1940s with a few species, but introductions are poorly documented (Thys van den Audenaerde 1966, Welcomme 1988). A precise introduction date for *O. niloticus* in the Congo Basin is missing from the literature, but the first record we have is from the Uele River in the North of the basin in 1964 (Thys van den Audenaerde 1964).

To combat weed growth and for aquaculture, another tilapia, *Coptodon rendalli* (Boulenger, 1897) is often introduced (Froese and Pauly 2019). It is native to the Middle Congo up to the Upper Lualaba and Bangwuelu area, the Cuanza, Catumbele, Malagarasi and Limpopo Rivers, Lake Tanganyika, Malawi and Zambezi and the coastal areas of the Zambezi delta, Okavango and Cunene (Froese and Pauly 2019). It was presumably introduced to the Lower Congo in the early 1950s. An exact introduction date is lacking from the literature, but in the collection of the RMCA (Royal Museum for Central Africa, Tervuren, Belgium) introduced specimens from 1957 are available, so introduction must have preceded 1957. Additionally, all reported introductions of *C. rendalli* into African countries are between 1951–1957 (Welcomme 1988).

Although several adverse effects of introduced tilapias have been studied, reports of co-introduction of parasites and their subsequent spillback and spillover effects are lacking for continental Africa (Deines et al. 2016). The parasites we focus on are monogenean flatworms belonging to *Cichlidogyrus* Paperna, 1960 (Dactylogyridae), *Scutogyrus* Pariselle and Euzet, 1995 (Dactylogyridae) and *Gyrodactylus* Von Nordmann, 1832 (Gyrodactylidae) . These parasites provide an ideal model to study the parasite fauna in historical host collections because, firstly, they have a direct life cycle (Kearn 2004). Parasites with direct life cycles are more likely to be co-introduced, since only one host has to be present in the introduced area (Lymbery et al. 2014, Goedknegt et al. 2016). Secondly, most monogenean species infecting cichlids are ectoparasites that attach to the gills with a specialized posterior attachment organ, the haptor (Pariselle and Euzet 2009). As such they are typically fixated together with their host when preserved, largely protected by the operculum of the fish against washing off and further manipulations of the host specimens. Thirdly, monogeneans are often highly host-specific, more so than other fish parasites (Whittington et al. 2000, Cribb et al. 2002). Parasite host switches, more specifically, spillback and spillover effects are therefore quite discernible by comparing parasite faunas. A spillback effect occurs when a native parasite host switches to an introduced host and a spillover effect occurs when an introduced parasite host switches to a native host (Goedknegt et al. 2016).

Reports of spillover and spillback of parasites from these genera exist from other continents and Madagascar, where tilapias are non-native (Cone et al. 1995, Jiménez-García et al. 2001, Mendoza-Franco et al. 2006, Roche et al. 2010, Šimková et al. 2019). In general, sub-Saharan Africa is underrepresented in the literature on invasion biology (Pyšek et al. 2008) and its helminthological diversity is also very much understudied (Poulin et al. 2019). In an effort to close the research gap, we have focused our studies on the largest basin that houses native tilapias, the Congo Basin. In order to study whether the parasite communities of native tilapias changed after introduction and if so, whether through invasive parasites, we follow up the characterizations of the present-day parasite communities of native cichlids the Congo Basin (Jorissen et al. 2018a, b), by complementing them with parasite communities from museum collections of the same cichlid species from before the introduction dates of *O. niloticus* and *C. rendalli.*

Material and Methods

Host and parasite sampling

Fish were collected during two recent field expeditions: one to the Luapula–Mweru subregion (sensu Thieme et al. 2005) in the Upper Congo Basin, with sampling localities in and near Lubumbashi, DRC and the other to the Lower Congo region with sampling localities west and south of Kinshasa (see Fig. 1a-e). Fish were caught in the field with gill nets or collected from aquaculture stations or were freshly bought at local fish markets. Live specimens were killed with an overdose of MS222 (tricaine mesylate). Four tilapia species, sympatric with introduced *O. niloticus* in either region or both, were most abundant in our catches and best suited for large-scale

study: *Coptodon rendalli*, *C. tholloni* (Sauvage, 1884), *Oreochromis mweruensis* Trewavas, 1983 and *Tilapia sparrmanii* Smith, 1840. Additional hosts were selected from the collection of the Royal Museum for Central Africa, Tervuren, Belgium (RMCA). These hosts are stored on ethanol, but were treated with formaldehyde before deposition, hampering molecular work. The hosts were selected to cover the broadest natural range of the respective cichlid species according to FishBase (Froese and Pauly 2019) with a focus on the regions sampled during recent fieldwork (Fig. 1a-e). A list of the selected fish specimens can be found in Addendum 1.

To distinguish *Oreochromis mweruensis* from *Oreochromis macrochir* (Boulenger, 1912) live specimens are needed to examine the colour pattern or nest shape (Schwanck, 1994). If those are not available, the sampling locality of museum specimens is used because both hosts are not sympatric (Trewavas 1983, Schwanck 1994). *Oreochromis mweruensis* is endemic to the Lower Luapula and Lake Mweru, which is where we concentrated the historical and recent samplings of the Luapula-Mweru subregion (see Table 3, Fig. 1b and Jorissen et al. 2018a). However, the available museum samples from these localities were limited and supplemented with specimens of *O. macrochir* from the Upper-Luapula, the Cuanza, Cunene and Kasai Basins. The parasite faunas of both hosts completely overlap, except for one specimen of *C.* sp.4 from the Luapula-Mweru subregion (see Table 2). Therefore, we consider *O. mweruensis* and *O. macrochir* together in Table 2.

The right gill arches were removed from the fish for parasitological screening. The left gill chamber was left intact for ichthyological research. Gills were exhaustively screened for ectoparasites using a Wild M5 stereomicroscope (Wild, Heerbrugg, Switzerland). Monogeneans were fixed on microscope slides with Hoyer's medium or glycerine-ammonium picrate and slides were permanently sealed with glyceel (Bates 1997). Identification of monogeneans to species level was carried out with a Leica DM2500 microscope (Leica, Wetzlar, Germany) with Nomarski interference contrast and based on the systematic revision of Pariselle and Euzet (2009) complemented with the more recent descriptions in Van Steenberge et al. (2015). Parasites were deposited in the invertebrate collection of the RMCA under accession numbers RMCA_VERMES_41866–43292. A list of these parasite specimens can be found in Addendum 2. We also included every parasite from Jorissen et al. 2018a,b from *C. rendalli*, *C. tholloni*, *O.mweruensis*/*macrochir* and *T. sparrmanii* in this study. Collection numbers for those specimens are listed in Jorissen et al. 2018a,b.

Calculation of infection parameters

Prevalence (P) and mean infection intensity (MI) were calculated following Bush et al. (1997). Prevalence was determined as the proportion of hosts infected with a particular parasite. We consider a prevalence below 0,1 as low. Mean infection intensity was calculated by taking the total number of parasite specimens of a particular species divided by the number of fish specimens infected by that parasite. To have an idea whether our effective sample size is large enough to detect a certain parasite species on a host, a corrected sample size, n, was calculated based on the formula in Paredes-Trujillo et al. (2016). This estimates the number of hosts needed to find at least one specimen of a certain parasite species. These authors assume a Poisson distribution for the probability of detecting a certain parasite on a particular fish species depending on the prevalence of that parasite. The equation is as follows: $n = \frac{-Ln(\frac{\alpha}{S})}{P}$, with α being the probability that a single fish is not infected in the sampling. While Paredes-Trujillo et al. (2016) set α to 0.05 as a standard value, we instead used the proportion of fish specimens of a species that was not infected by a single monogenean because it is more biologically relevant. S represents the sensitivity, the proportion of detected parasites by the researcher, and was taken to be 0.75 as a standard in Paredes-Trujillo et al. (2016) based on a study on the diagnosis of malaria (Ojurongbe et al. 2013). While we are unaware of a published sensitivity for detecting monogenean fish parasites, given that our samples were exhaustively screened, we can approximate it by taking the proportion of unidentified parasite specimens on the respective host species per region. These unidentified specimens may contain representatives of the parasite we are estimating the corrected sample size for and thus are important to take into account in our calculations. In some cases it was not possible to identify a monogenean to species level because of a less than optimal orientation on the slide, or because the specimen was a juvenile (no well-developed hard parts). Sample-based rarefaction curves, based on abundance data (absolute numbers of parasites), were constructed and extrapolated to estimate the parasite species diversity of the host species

between recent and historical samplings (see Fig. 2), using the iNEXT software and package in R (Chao et al. 2016). Images were finalized in GIMP V.2.10.10.

Results

A total of 393 fish were examined for this study. They harboured 1833 monogeneans, 1693 of which were identified to species level. The parasites belonged to 29 species (Table 2), with nine of them being new to science (Table 3). Fish were collected from 75 sampling sites, 22 of which were from recent expeditions and the other 53 from the collection of the RMCA. Samples were collected across 18 river systems within eight countries in Central and southern Africa (Fig. 1a-e, Table 4). The nine newly discovered parasite species are not described in this manuscript because species descriptions fall outside of the scope of this paper. While some species lack the material to describe and draw them fully (Table 4), others will be described in a future study. Nevertheless, we provide some additional morphological information on these undescribed worms so they are recognizable by other researchers. We also deposited these specimens in the collection of the RMCA so they are consultable. The new species were numbered and were not attributed binomials to avoid nomenclatural problems.

Seven species were found infecting *O. niloticus* in Lower Congo and eight in Luapula-Mweru. *Cichlidogyrus halli* Paperna and Thurston, 1969 and *Cichlidogyrus thurstonae* Ergens, 1981 dominate the fauna in both regions, complemented by *Cichlidogyrus tilapiae* Paperna, 1960 in Lower Congo and *Cichlidogyrus sclerosus* Paperna and Thurston, 1969 in Luapula-Mweru (Table 2). All other parasites had low prevalences *sensu* Valtonen et al. 1989 (≤ 0.1, see Table 2).

Tilapia sparrmanii does not share any gill monogenean species with *O. niloticus*, whilst *C. tholloni* shares parasite species with both *C. rendalli* and *O. niloticus* in the recent samples (Table 2). In the Lower Congo area, the parasite fauna of introduced *C. rendalli* does not overlap with the parasite fauna of the host's native range (Table 2). In the Lower Congo the parasite fauna consists of *Cichlidogyrus berradae* Pariselle and Euzet, 2003 and *Cichlidogyrus cubitus* Dossou, 1982. Both parasites also infect *C. tholloni* in the recent and historical samples (Table 2). Additionally, the introduced *C. rendalli* is also infected by *Cichlidogyrus flexicolpos* Pariselle and Euzet, 1995, but this parasite was not found on *C. tholloni*. Secondly, *C. tholloni* shares two parasite species with *O. niloticus*, but only in the recent samples (Table 2). In the Upper Congo, *Coptodon rendalli* shares *Gyrodactylus nyanzae* Paperna, 1973 with *O. niloticus*, but only in the recent samples. *Oreochromis mweruensis/macrochir* shares five parasite species with *O. niloticus* in both the recent and historical samples, namely *Cichlidogyrus cirratus* Paperna, 1964, *C. halli*, *C. sclerosus*, *C. tilapiae* and *G. nyanzae* (Table 2).

Rarefaction curves show our sampling effort to be near or on the asymptotic plateau except for the historical samples of *C. rendalli*, *T. sparrmanii* and *O. mweruensis/macrochir* (Fig. 2b). While higher sampling effort could reveal more, albeit rare parasite species, this would not change our conclusions with regard to parasite species co-introduced with Nile tilapia. We also observe that the estimated diversity is the highest for *O. mweruensis*, with the largest confidence interval and the lowest for *T. sparrmanii* (Fig. 2). Lastly, the corrected n showed that our sampling effort was sufficient to find almost all parasite species recorded in this study. As such, if the estimated effort was higher, it did not differ much from the actual sampling effort (Table 2).

Discussion

Based on recent field expeditions, we compared the monogenean gill parasite fauna of the native *C. rendalli*, *O. macrochir* and *T. sparrmanii* with the introduced *O. niloticus* in the Luapula-Mweru subregion and the native *C. tholloni* with the introduced *C. rendalli* and *O. niloticus* in the Lower Congo in order to detect parasite co-introductions and to determine species shared between native and introduced hosts (Fig. 3), a situation which may result from parasite spillback or spillover (Goedknegt et al. 2016). To verify this, recent parasite faunas were compared with pre-introduction faunas, using museum samples from capture dates before the introduction of the introduced hosts.

Co-introduced species

Nine species of Monogenea were found on introduced specimens of *O. niloticus* in the Congo Basin (Table 2) that also infect native Nile tilapia populations (Pariselle and Euzet 2009, Přikrylová et al. 2009, Zahradníčková et al. 2016). However, one species that infects *O. niloticus* in its native range is missing in our samples, namely *Cichlidogyrus rognoni* Pariselle, Bilong Bilong and Euzet, 2003. It is possible that it was not co-introduced into the Congo Basin or that it could not establish, following the enemy-release hypothesis (Prenter et al. 2004), which points to the reduced parasite load of introduced hosts. However, it is only known from the Senegal River (Pariselle et al. 2003) and it is more likely that the stocking of the Congo Basin did not originate in Senegal and the introduced hosts did not carry *C. rognoni* to begin with. There is variation in the parasite community composition of *O. niloticus* in its native range, as predicted by the geographic mosaic of coevolution theory (Gomulkiewicz et al. 2000). This is true for most parasite-host systems and can be influenced by host genetics, (e.g. in metazoan parasites of cyprinids Seifertová et al. 2008), geographic distance (e.g. for ectoparasites of birds Gómez-Diaz et al. 2008) or environmental distance (e.g. on mites of rodents Krasnov et al. 2005, Vinarski et al. 2007).

Host switching or natural host range?

Six parasite species have spilled over or spilled back as suggested by our data. Firstly, our data suggest a spillover of *C. sclerosus* from *O. niloticus* to *C. tholloni* in the Lower Congo Basin, Luidi River (locality 21, Table 4) and the Ogowe River (locality 13, Table 4). *Cichlidogyrus sclerosus* does not infect a representative of *Coptodon* in its native range, but is widely reported from *O. niloticus* (Pariselle and Euzet 2009, Lim et al. 2016).

Secondly, the presence of *C. tilapiae* on a single individual of *C. tholloni* in a culture setting from Ndimba Leta pond 1 in the Lower Congo Region, was suggested to be the result of a spillover from *O. niloticus* (Jorissen et al. 2018b). As these two fish species were farmed together in a closed system, this spillover is not surprising (see e.g. Justine 2009). In Madagascar, *C. tilapiae* was found to infect introduced *C. rendalli*, originating from *O. niloticus* or *O. mossambicus* (Šimková et al. 2019), proving that it is capable of successfully establishing on a representative of *Coptodon*. Representatives of *Cichlidogyrus* and *Gyrodactylus* are certainly capable of species jumps over even larger phylogenetic distances. For example, *G. cichlidarum* has been reported to spillover from introduced tilapia to poeciliid fish in Mexico (García-Vásquez et al. 2017) and *C. tilapiae* was found infecting the native endemic aplocheilid *Pachypanchax omalonotus* (Duméril, 1861) in the Anjingo River, Madagascar (Šimková et al. 2019).

Thirdly, three parasites of more closely related hosts also show host switching dynamics. In the Lower Congo area, the introduced *C. rendalli* are infected with the native parasites *C. berradae*, *C. cubitus* and *C. flexicolpos* (Jorissen et al. 2018b). The three specimens of *C. rendalli* infected with these parasites all originate from the Tondé River (locality 10, Table 4) from our recent field expedition to Lower Congo in 2015. Using pre-introduction host specimens of *C. tholloni*, we can confirm that *C. berradae* and *C. cubitus* are native to the Lower Congo and likely infected *C. rendalli* through a spillback from the native *C. tholloni*. *Cichlidogyrus flexicolpos* was not found on any other host in our study, but infects *Coptodon guineensis* also (Pariselle and Euzet 1995), which occurs in Lower Congo (Froese and Pauly 2019). The nine other specimens of *C. rendalli* from the Lubuzi and Mvuazi in the Lower Congo (localities 11 and 12, Table 4) were not infected and date from 1957 and 1958. In this case it may be that *C. rendalli* was introduced without parasites and was then free to be colonized, following the enemy-release hypothesis (Prenter et al. 2004).

Lastly, in the Luapula-Mweru subregion, *G. nyanzae* is shared between *O. niloticus* and *C. rendalli*, but only in the recent samples (Table 2). We suggest that this is a spillover from *O. niloticus* to *C. rendalli*, because we observed that *G. nyanzae* only infects *C. rendalli* in case *O. niloticus* occurs sympatrically, as also is the case in Lake Kariba and Chirundu, Zimbabwe, where samplings occurred in 2011 and 2012 and where *O. niloticus* was introduced (Zahradníčková et al. 2016). *Gyrodactylus nyanzae* has so far not been found on any other samples of *C. rendalli* (Table 2). However, the parasite is native to Luapula-Mweru because we did find it in historical samples of *O. mweruensis* there (Table 2).

In two of the three above mentioned spillover events (*C. tilapiae* and *C. sclerosus* on *Coptodon tholloni*), the parasites have a low prevalence and are considered rare. In the third event, *G. nyanzae* on *C. rendalli*, the prevalence is only slightly higher. Because of the low prevalences and because of the jump between quite distantly related hosts (Dunz and Schliewen 2013), we suspect that the parasite has a suboptimal fitness after the spillover. We propose these new host-parasite associations are a result of ecological fitting (Janzen 1985) via "sloppy fitness space" rather than stemming from a shared evolutionary history (see Agosta and Klemens 2008; Araujo et al. 2015). For two of the three spillback effects, *C. berradae* and *C. cubitus* on *Coptodon rendalli*, the prevalence is higher and the hosts are congeners. We therefore suspect that both *C. tholloni* and *C. rendalli* present a similar resource to the parasites. In this case the novel species association would result from a spillback effect through ecological fitting via resource tracking within existing fitness space (Agosta and Klemens 2008). For the third spillback effect, *C. flexicolpos* on *C. rendalli*, the source host is unknown, but might be *C. guineensis*.

Finally, five monogenean species are shared between *O. niloticus* and *O. mweruensis/macrochir*. However, these parasites were also present in the pre-introduction samples, so they are native to *O. mweruensis/macrochir* and to the Upper Congo (Table 2). The parasite fauna of *T. sparrmanii* shared no species with *O. niloticus* and was hence not taken into consideration for either spillback or spillover events from Nile tilapia.

Conclusions

Although we report three spillover and three spillback events, only one spillover from *O. niloticus* seems established in each region; *C. sclerosus* on *C. tholloni* in Lower Congo and the Ogowe River and *G. nyanzae* on *C. rendalli* in the Luapula-Mweru subregion. *Coptodon rendalli* even had its complete fauna replaced by local species in Lower Congo. In general, the parasitological impact of introduced tilapia on the native hosts seems limited, especially compared to some areas of Madagascar, where the complete native ectoparasitic monogenean fauna was found replaced by spilled over species (Šimková et al. 2019). Indigenous Malagasy monogeneans could only persist on native cichlids in an area that was more recently colonised by tilapias (Šimková et al. 2019). The authors of that study indicate that the timing and origin of introduction may shape introduced parasite communities. We agree with that statement, but our data adds that the introductions to the Congo Basin are sufficiently old and diverse, albeit poorly documented, to expect a larger effect. Therefore, we suspect that also other factors limit parasite spillover in the Congo Basin, such as host density. Secondly, an ecological niche modelling study on the theoretical distribution range of *O. niloticus* in mainland Africa indicates that it would not perform optimally in the Congo Basin (Zengeya et al. 2012) and that it might occur in lower densities than native tilapias. Lastly, immune response might also play a role. It was shown that lake specimens of *Gasterosteus aculeatus* Linnaeus 1758 (three-spined stickleback) exert lower and different immune response to infection by *Gyrodactylus* spp. than stream specimens (Brunner et al. 2017). In Lake Kariba, Zimbabwe, it was observed that parasite infection was facilitated on tilapias and monogeneans had a broader host range in general compared to the Luapula-Mweru subregion, where the same species occur (Douëllou 1993, Jorissen et al.2018a). However, more needs to be done to complete our, now fragmentary understanding of parasite spillover from tilapias.

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List of tables

Table 1 Earliest reported introduction of *O. niloticus* for all countries sampled in this study. We use the dates in this table as a cut-off between pre and post-introduction samples. Precise introduction dates for the DRC, Gabon and Mozambique are unknown. However, *O. niloticus* was exported from the DRC to Burundi in 1951 (Welcomme 1988), but it is possible that these specimens came from regions of the DRC where it is native (Lake Tanganyika and Kivu). Thys Van Den Audenaerde (1964) reported the first confirmed introduction of *O. niloticus* in the DRC in the Uele River, Northern Congo Basin. The first specimen of *O. niloticus* reported from Gabon is in 1990, but introduction might have been somewhat earlier

Country	Year	Reference
Angola	No reported introduction	/
Botswana	No reported introduction	/
DR Congo	<1964	Thys Van Den Audenaerde 1964
Gabon	<1990	FAO 2002
Mozambique	Early 1990s	Firmat et al. 2013
Tanzania	1954	Lowe-McConell 1982 Ogutu-Ohwayo 1990
Zambia	1982	Schwanck 1995
Zimbabwe	1982	Schwanck 1995

Table 2 Parasitological data of the collected monogenean gill parasites. Columns represent species, while rows represent sample area. Parasite infections resulting from a putative spillover or spillback are marked with an '*' and in bold. A '-' indicates that a species was not found. Recent, historical and introduced specimens are indicated by a R, H and I in superscript, respectively. 'N' represents the number of fish studied and '#infected' the number of infected host specimens. The parasitological data is represented as 'P/MI/n', with P being the prevalence, MI the mean intensity of infection and n the sampling effort needed to find a species of parasite at least once, the latter only represented in the total rows

O. niloticus	**N**	**#infected**	***C. cirratus***	***C. halli***	***C. mbirizei***	***C. sclerosus***	***C. thurstonae***	***C. tilapiae***	***G. ergensi***	***G. nyanzae***	***S. longicornis***
Lower Congo[RI]	73	53	0,02/1/95	0,3/1,9/5	0,02/1/95	0,06/1,5/24	0,42/3,2/4	0,42/1,6/4	-	-	0,07/1/19
Luapula-Mweru[RI]	57	45	0,08/1/22	0,62/3,6/3	-	0,5/4,6/4	0,51/2,7/3	0,18/2,3/9	0,04/11/43	0,08/1/22	0,08/1,3/22

O. mweruensis/macrochir	**N**	**#infected**	***C. cirratus***	***C. dossoui***	***C. halli***	***C. papernastrema***	***C. sclerosus***	***C.* sp.4**	***C. tiberianus***	***C. tilapiae***	***G. nyanzae***	***S. gravivaginus***
Luapula-Mweru[R]	32	22	0,22/4,3/6	0,29/1,3/4	0,16/3,4/8	0,04/2/36	0,07/2/18	-	0,04/1/36	0,04/1/36	0,1/13,7/12	0,25/2,3/5
Cuanza[H]	3	2	-	0,33/2	0,67/1,5	-	0,33/1	-	-	-	-	-
Cunene[H]	4	3	-	0,25/2	0,5/1	-	-	-	-	-	-	-
Kasai[H]	2	1	-	-	-	-	-	-	-	0,5/4	-	-
Loge[H]	1	0	-	-	-	-	-	-	-	-	-	-
Lualaba[H]	5	1	0,2/5	-	-	-	0,2/1	-	-	-	-	0,2/1
Luapula-Mweru[H]	24	12	0,08/1	0,29/3,14	0,2/2,6	0,13/2,67	-	0,04/1	-	-	0,04/1	0,13/4
Total historical	39	19	0,08/2,4/8	0,23/2,9/3	0,23/2/3	0,08/2,7/8	0,05/1/11	0,03/1/22	-	0,03/4/22	0,03/1/22	0,13/2,6/5

C. rendalli	**N**	**#infected**	***C. berradae***	***C.* sp.2**	***C. cubitus***	***C. dossoui***	***C. flexicolpos***	***C.* sp.3**	***C. papernastrema***	***C. quaestio***	***C.* sp.5**	***C. tiberianus***	***G. chitandiri***	***G. nyanzae***
Luapula-Mweru[R]	19	17	-	-	-	0,69/5,4/4	-	-	0,11/2,5/20	0,48/5,9/5	-	0,32/2,5/7	-	**0,11/3/20***
Lower Congo[RI]	12	3	**0,24/2,7/1***	-	**0,24/9,4/1***	-	**0,08/5/3***	-	-	-	-	-	-	-
Cuanza[H]	1	1	-	-	-	1/6	-	-	1/3	1/3	-	-	-	-

Kasai[H]	2	2	-	0,5/2	-	-	-	0,5/1	0,5/1	-		-	-	-
Lualaba[H]	15	9	-	0,34/3,8	-	0,42/2,8	-	-	0,09/1	-	-	0,25/3	0,09/2	-
Malagarasi[H]	2	0	-	-	-	-	-	-	-	-	-	-	-	-
Luapula-Mweru[H]	21	12	-	0,05/6	-	0,43/6	-		0,15/2,4	-	0,05/1	0,39/2,4	-	-
Okavango[H]	1	1	-	-	-	1/1	-	-	-	-	-	-	-	-
Zambezi[H]	2	2	-	-	-	-	-	-	-	-	-	0,5/3	-	-
Total historical	45	28	-	0,16/3,9/6	-	0,39/4,7/3	-	0,02/1/41	0,16/1,9/6	0,02/3/41	0,02/1/41	0,3/3,6/4	0,02/2/41	-

C. tholloni	**N**	**#infected**	***C. berradae***	***C.* sp.1**	***C.* sp.2**	***C.* sp.6**	***C. cubitus***	***C. reversati***	***C. sclerosus***	***C. tilapiae***	***G. chitandiri***
Lower Congo[R]	16	11	-	0,13/2	0,32/6,4	0,13/1,5	0,32/1,8	0,07/8	**0,07/1***	**0,07/4***	0,07/21
Ogowe[R]	4	2	0,25/1	-	-	-	-	-	**0,25/3***	-	-
Total recent	20	13	0,05/1/21	0,1/2/11	0,25/6,4/5	0,1/1,5/11	0,25/1,8/5	0,05/8/21	**0,1/2/11***	**0,05/4/21***	0,05/21/21
Lower Congo[H]	20	14	-	0,05/2	0,5/4,4	-	0,15/1,7	0,1/3,5	-	-	-
Pool Malebo[H]	3	1	-	0,34/1	0,34/1	-	-	-	-	-	-
Ogowe[H]	2	1	-	-	0,5/2	-	-	-	-	-	-
Total historical	25	16	-	0,08/1,5/13	0,48/3,9/3	-	0,12/1,7/9	0,08/3,5/13	-	-	-

T. sparrmanii	**N**	**#infected**	***C.* sp.7**	***C. dossoui***	***C. papernastrema***	***C.* sp.8**	***C. tiberianu***	***C.* sp.9**	***G. chitandiri***	***G.* sp**

							s			
Luapula-Mweru[R]	20	13	-	0,4/3/3	0,6/5,5/2	-	-	-	-	-
Changana[H]	1	0	-	-	-	-	-	-	-	-
Kasai[H]	12	9	-	0,5/1,9	0,75/1	0,17/2,5	0,25/1	-	0,05/8	-
Lualaba[H]	5	5	-	0,4/3	1/23	-	-	0,2/3	-	-
Luapula-Mweru[H]	19	10	0,06/1	0,16/3,7	0,43/9,3	-	0,06/2	-	-	0,1/1
Okavango[H]	5	5	-	0,4/4,5	0,6/4,7	-	-	-	-	-
Tanganyika[H]	3	3	-	0,67/2	0,34/1	-	0,67/1	-	-	-
Zambezi[H]	6	4	-	0,4/2	0,5/6,4	-	0,2/7	-	-	-
Total historical	51	36	0,1/1/59	0,33/2,7/3	0,57/8/2	0,04/2/29	0,2/2/9	0,1/3/59	0,02/8/57	0,1/1/59

Table 3 List of sampling localities, coordinates, river system, country and sampling date. Numbers correspond with Figure 1. Locality 75 lacks coordinates and is not shown on Figure 1. Locality 47 lacks a precise date, but it was possible to deduct that is was from before 1936. Recent localities include sample sites from the recent field expeditions and samples of introduced species from the museum collection. Locality 11 and 12 pre-date the introduction of *O. niloticus* in the DRC (see Table 1), but introduced *C. rendalli* was sampled there, so these are post-introduction localities. Sampling localities with a '*' indicate that no parasites were found there

Recent	Number	Coordinates	River system	Country	Date
Monzi	1	05°37'00,6''S 13°14'01,5''E	Lower Congo	DRC	30-6-2015
Pond Near Kila Kindinga and Lukasi River	2	5°29'7"S 14°53'3,8"E	Lower Congo	DRC	26-6-2015
Ndimba-Leta, Mbanza-Ngungu	3	05°15'10"S 14°51'24,3"E	Lower Congo	DRC	29-6-2015
Inkisi near Kisantu	4	05°08'02,6"S 15°03'51,5"E	Lower Congo	DRC	10-2005, 2015
Lake Kipopo	5	11°34'S 27°21"E	Luapula-Mweru	DRC	27-8-2014
Zoo Lubumbashi	6	11°39'S 27°28'E	Luapula-Mweru	DRC	3-9-2014
Bumaki Farm	7	11°34"S 27°30'E	Luapula-Mweru	DRC	4-9-2014
Futuka Farm	8	11°29'S 27°39'E	Luapula-Mweru	DRC	29-8-2014
Luapula River off Kashobwe	9	09°40'S 28°37'E	Luapula-Mweru	DRC	7-9-2014
Tondé	10	05°57'35,9''S 12°21'58,4''E	Lower Congo	DRC	29-6-2015
Lubuzi River	11	05°00'S 12°56'E	Lower Congo	DRC	1958
Mvuazi	12	05°19'S 15°07'E	Lower Congo	DRC	3-1957, 2015
Ogowe River near Mpere	13	1°38'S 9°37'E	Ogowe	Gabon	1-9-1993
Congo River at Nganda Flash station	14	06°02'01,8"S 12° 31'48,2"E	Lower Congo	DRC	25-8-2014
Muila Nzenze, mangroves near Kibamba	15	06°00'06,8"S 12°40'27"E	Lower Congo	DRC	24-8-2014
Inkisi River	16	04°47'22,4"S 14°54'35,5"E	Lower Congo	DRC	12-2006
Muala River near Muala Kinsende	17	05°16'11,4"S 14°57'42,1"E	Lower Congo	DRC	2-2007
Moke Inkisi	18	05°16'30"S 14°58'02"E	Lower Congo	DRC	2-2007*
Ngeba Inkisi	19	05°11'01,5"S 15°12'23,1"E	Lower Congo	DRC	1-2007
Lukusu River	20	05°13'36,9"S 15°13'02"E	Lower Congo	DRC	8-2006*
Luidi River near Kinua-Nsudi	21	05°28'22,8"S 15°13'11,1"E	Lower Congo	DRC	2-2007
Wungu River near Kiyenga	22	05°31'47,9"S 15°16'56"E	Lower Congo	DRC	9-2006
Historical					
Cunene River near Ruakana	23	17°25'S 14°14'E	Cunene	Angola	25-9-1953
Cunene River near Capelongo	24	14°54'S 15°08'E	Cunene	Angola	25-9-1959
Lagunes of the Cutato river	25	14°23'S 16°28'E	Cuanza	Angola	25-9-1959

Loge River near Uige	26	07°45'S 15°00'E	Loge	Angola	8-1973*
Luembe River near Limboma	27	07°51'S 21°27'E	Kasai	Angola	11-1972
Bambesa	28	03°22'S 25°44'E	Lualaba	DRC	8-1954*
Dikuluwe River near Shimama	29	09°38'S 27°08'E	Lualaba	DRC	10-9-1951
Kimilolo	30	11°43'S 27°26'E	Luapula-Mweru	DRC	7-1963*
Lukonzolwa	31	08°47'S 28°39'E	Luapula-Mweru	DRC	11-1911, 2-1931
Lake Mweru	32	9°17'18,9"S 28°43'42,4"E	Luapula-Mweru	Zambia	10-1911
Pembe Lagoon near Katotoma	33	9°55'24,2"S 28°43'47,5"E	Luapula-Mweru	Zambia	8-1993*
Kasenga near Luapula River	34	10°20'S 28°38'E	Luapula-Mweru	DRC	18-7-1911
Kabunda	35	12°26'S 29°22'E	Luapula-Mweru	DRC	31-5-1937
Cuchi river	36	14°39'27,8"S 16°54'13,3"E	Okavango	Angola	10-1959
Lake Calundo	37	11°48'S 20°52'E	Kasai	Angola	1-1955
Sankuru River near Inkongo	38	04°53'S 23°16'E	Kasai	DRC	10-1937
Congo river near Yaekela	39	00°48'S 24°16'E	Lualaba	DRC	2-1955
Kisangani	40	00°30'N25°12'E	Lualaba	DRC	1-1920*
Lualaba River near Kindu	41	02°57'S 25°56'E	Lualaba	DRC	3-1950
Luvua River near Kiambi	42	07°20'S 28°01'E	Luapula-Mweru	DRC	5-1931
Lake Upemba near Bukama	43	09°10'S 25°50'E	Lualaba	DRC	6-1937
Nyonga, Lake Upemba	44	08°38'S 26°18'E	Lualaba	DRC	5-1925
Lubumbashi	45	11°39'S 27°28'E	Luapula-Mweru	DRC	1-1920
Delta Malagarazi right arm	46	05°13'S 29°48'E	Malagarasi	Tanzania	8-1993*
Crossing of the Passa river into the Ogowe near Franceville	47	01°36'S 13°31'E	Ogowe	Gabon	<1936
Banana	48	06°00'S 12°24'E	Lower Congo	DRC	1936, 6-1948
Congo river near Boma	49	05°50'S 13°03'E	Lower Congo	DRC	8-1937
Congo river near Inga	50	05°29'S 13°34'E	Lower Congo	DRC	10-1957
Ndimba Leta Ponds, Mbanza-Ngungu	51	05°15'S 14°51'E	Lower Congo	DRC	7-1957
Pool Malebo	52	04°18'S 15°18'E	Pool Malebo	DRC	1964, 1969*
Kinkole, Pool Malebo	53	04°14'S 15°22'E	Pool Malebo	DRC	1957
Molondo island, Pool Malebo	54	04°17,08'S 15°27,52'E	Pool Malebo	DRC	2007*
Kinkole, Pool Malebo	55	04°20'S 15°30'E	Pool Malebo	DRC	7-1957
Porto Changanine	56	24°06,72'S 33°47,16'E	Changa	Mozambique	7-2004*

Okavango River near Thohatsebe	57	19°55'S 23°30'E	Okavango	Botswana	5-1985
rail bridge at Kafué River	58	15°47'16"S 28°10'36"E	Zambezi	Zimbabwe	7-1965
Lunzua River at the Abercorn-Kasama road	59	09°04'S 31°25'E	Tanganyika	Zambia	7-1967
Cuebe River	60	14°36'S 17°48'E	Okavango	Angola	10-10-1959
Luombwa River near Kikenya	61	12°21'S 29°38'E	Luapula-Mweru	DRC	11-1930, 12-1932
Kilwa, Lake Mweru	62	09°18'S 28°25'E	Luapula-Mweru	DRC	2-1931*
Lake Mweru	63	08°37'S 28°29'E	Luapula-Mweru	DRC	1-10-1911
Pweto, Lake Mweru	64	08°28'S 28°56'E	Luapula-Mweru	DRC	12-1911
Kandulu	65	12°46'S 28°38'E	Luapula-Mweru	DRC	5-1937
Mipoposhi near Luongwo River	66	9°38'29,6"S 29°26'40,6"E	Luapula-Mweru	Zambia	10-1997
Kikondja near Lake Kisale	67	08°10'S 26°25'E	Lualaba	DRC	8-1937
Bukama	68	09°10'S 25°50'E	Lualaba	DRC	6-1937
Tshikapa	69	06°25'S 20°47'E	Kasai	DRC	9-1946*
Dilolo	70	10°42'S 22°20'E	Kasai	DRC	2-1948
Lubi River	71	07°20'S 23°00'E	Kasai	DRC	1905
mare Tchifuca	72	10°00'S 19°30'E	Kasai	Angola	6-1954
Lucoge river	73	07°33'S 20°27'E	Kasai	Angola	4-1964*
Luachimo river near Mwaoka	74	07°39'S 20°51'E	Kasai	Angola	6-1964
Ogowe River near Lécéni	75	/	Ogowe	Gabon	1957

Table 4 Morphological information on undescribed species. Abbreviations: AP= accessory piece, DB= dorsal bar, He= heel, Pe= penis, VB= ventral bar, Vg= vagina, UncI-VII= hooklets pair I-VII

Species	n	Host	Locality	Collection number (RMCA_VERMES_)	Morphological info
Cichlidogyrus sp.1	6	*C. tholloni*	15, 19, 49, 50, 53	38305, 42513, 42554, 43266, 43291	Resembles *C. aegypticus* Ergens 1981 and is distinguished from it by the morphology of the Vg, which is sinuous and has a constant diameter instead of having a swollen portion and bend as in *C. aegypticus*. Mentioned in Jorissen et al. 2018b as *C.* sp.1
C. sp.2	106	*C. rendalli*, *C. tholloni*	15, 16, 17, 19, 21, 28, 38, 39, 42, 44, 49, 50, 53, 75	38305-07, 42266-67, 42273, 42282, 42288-89, 42337-38, 42403, 42417-25, 42427-29, 42438, 42443-44, 42454, 42467, 42470-72, 42481-82, 42484-90, 42504-07, 42511-12, 42514, 42516-18, 42520, 42529-33, 42535-39, 42543-49, 43257-60, 43263-65, 43267-90, 43293	Resembles *C. arthracantus* Paperna 1960, but with larger He and furcula-like distal end of AP, Vg coiled. Mentioned in Jorissen et al. 2018b as *C.* sp.2
C. sp.3	1	*C. rendalli*	38	42339	Haptor and He correspond with *C. arthracanthus*, but Vg present, spirally coiled over 2 loops, AP not observed, Pe shorter than *C. arthracanthus*.
C. sp.4	1	*O. macrochir*	35	42561	Material unfit for description, MCO 2 simple tubes, Pe 180° turn, AP straight, tubes of equal thickness, UncI short, UncIII-VII long, DB & VB very small, Vg not observed.
C. sp.5	1	*C. rendalli*	31	42387	Resembles *C. maeander* Geraerts & Muterezi Bukinga, 2020(Geraerts et al. 2020)
C. sp.6	4	*C. tholloni*	19, 22	42491, 42495-96, 42515	Resembles *C. ornatus* Pariselle & Euzet 1996.
C. sp.7	1	*T. sparrmanii*	64	41866	Resembles *C. franswittei* Pariselle & Vanhove 2015, but He with coarse surface instead of being smooth such as in *C. franswittei*, also AP and Pe are simple tubes of equal length and do not meet distally. Single specimen, but of sufficient quality for description.

C. sp.8	5	*T. sparrmanii*	72	41942-43, 41945-46, 42167	Representative of *Cichlidogyrus* with two basal bulbs in sequence at the proximal end of the MCO. Unknown within the genus. Material of insufficient quality for a detailed description.
C. sp.9	3	*T. sparrmanii*	67	42177, 42183, 42185	Resembles *C. dossoui*, but is distinguished from it by having a much larger and broader Vg, which appears to be made up of two hollow tubes nested in each other. Additional material is needed to fully describe the MCO.

List of Figures

Fig1a-e Maps of sample localities of a) *Oreochromis niloticus* b) *O. mweruensis/macrochir* c) *Coptodon rendalli* d) *C. tholloni* and e) *Tilapia sparrmanii*. Top left, field image of fish species. Bottom left, sampling area within Africa. ▲: Recently sampled localities; ●: localities from pre-introduction collections, corresponding with the numbers in Table 3. River systems and large freshwater bodies in blue. Ecoregions in black. Scale bottom left. Maps were reconstructed with MapInfo V9.0 using GIS data from the sampling sites with overlays of the major African river systems, water bodies and freshwater ecoregions (Thieme et al. 2005)

Fig2a-b Sample-based rarefaction curves and extrapolation split by host species. X-axis represents the number of parasite specimens collected. Y-axis represents the estimated gill parasite diversity. a) Recent samples and b) pre-introduction samples. Confidence interval of 95% is highlighted. Sample cut-off at 600 for the recent samples and at 300 for the historical samples

Fig3 Visual representation of parasite host switches. Red ● with an 'I': introduced fish species. Green ● with an 'N': native fish species. Direction of arrows indicate the direction of the host switch. The arrows towards *Coptodon rendalli* are spill backs and the arrows from *Oreochromis niloticus* are spill overs. Species of monogeneans colour-coded. From left to right; purple: *Cichlidogyrus cubitus*; brown: *Cichlidogyrus berradae*; light blue: *Cichlidogyrus flexicolpos*; green: *Cichlidogyrus tilapiae*; orange: *Cichlidogyrus sclerosus* and dark blue: *Gyrodactylus nyanzae*

Fig1a

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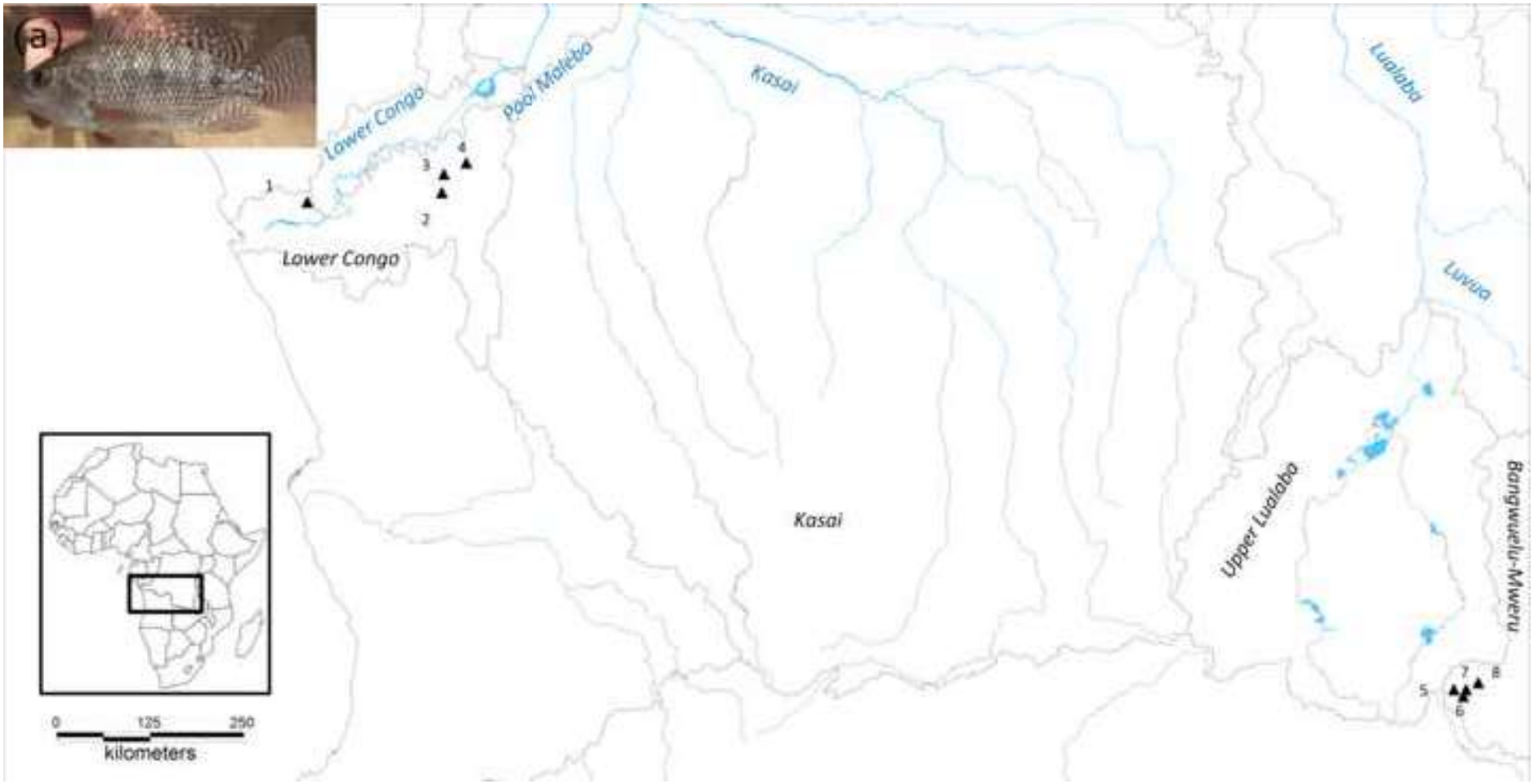

Fig1b

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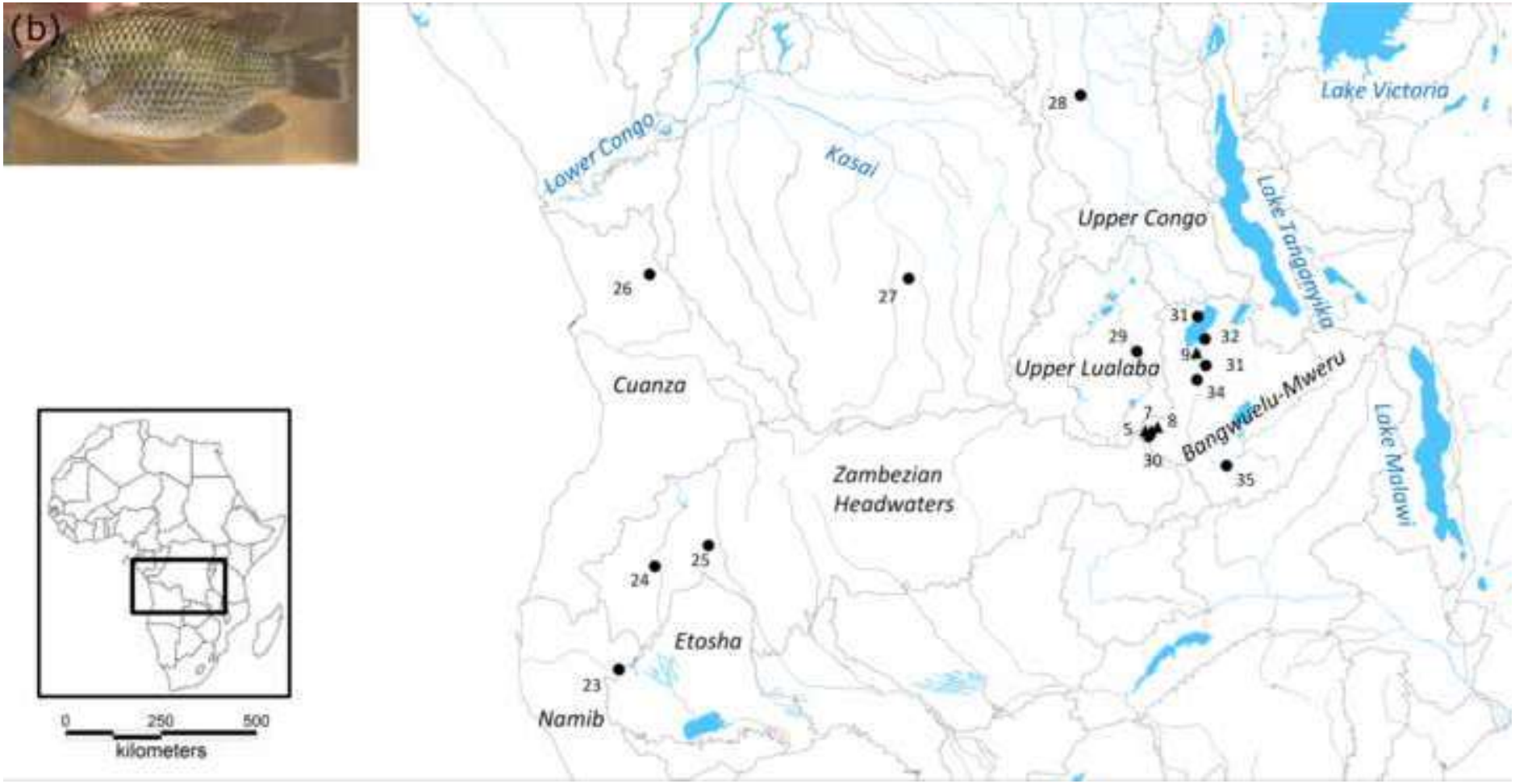

Fig1c

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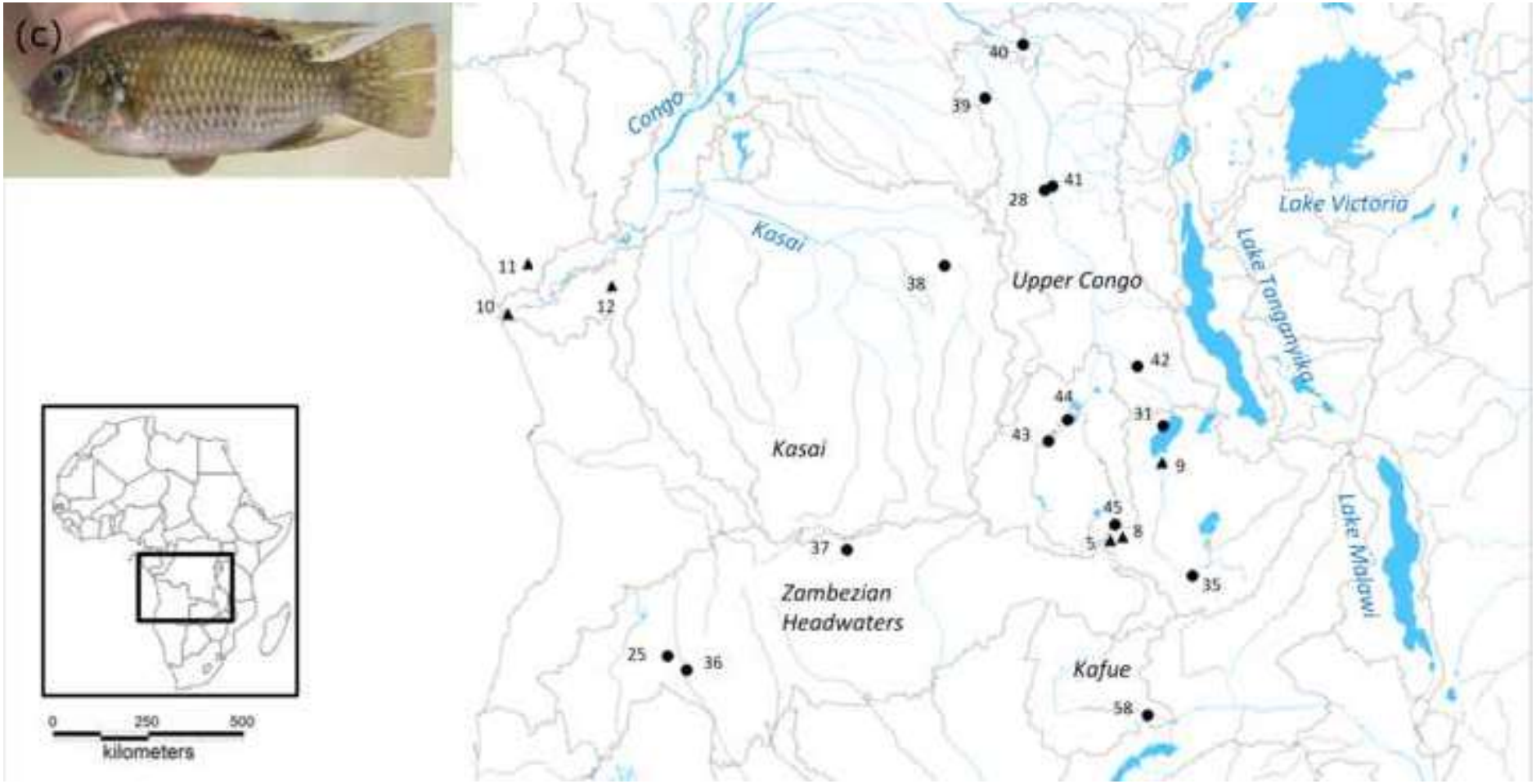

Fig1d

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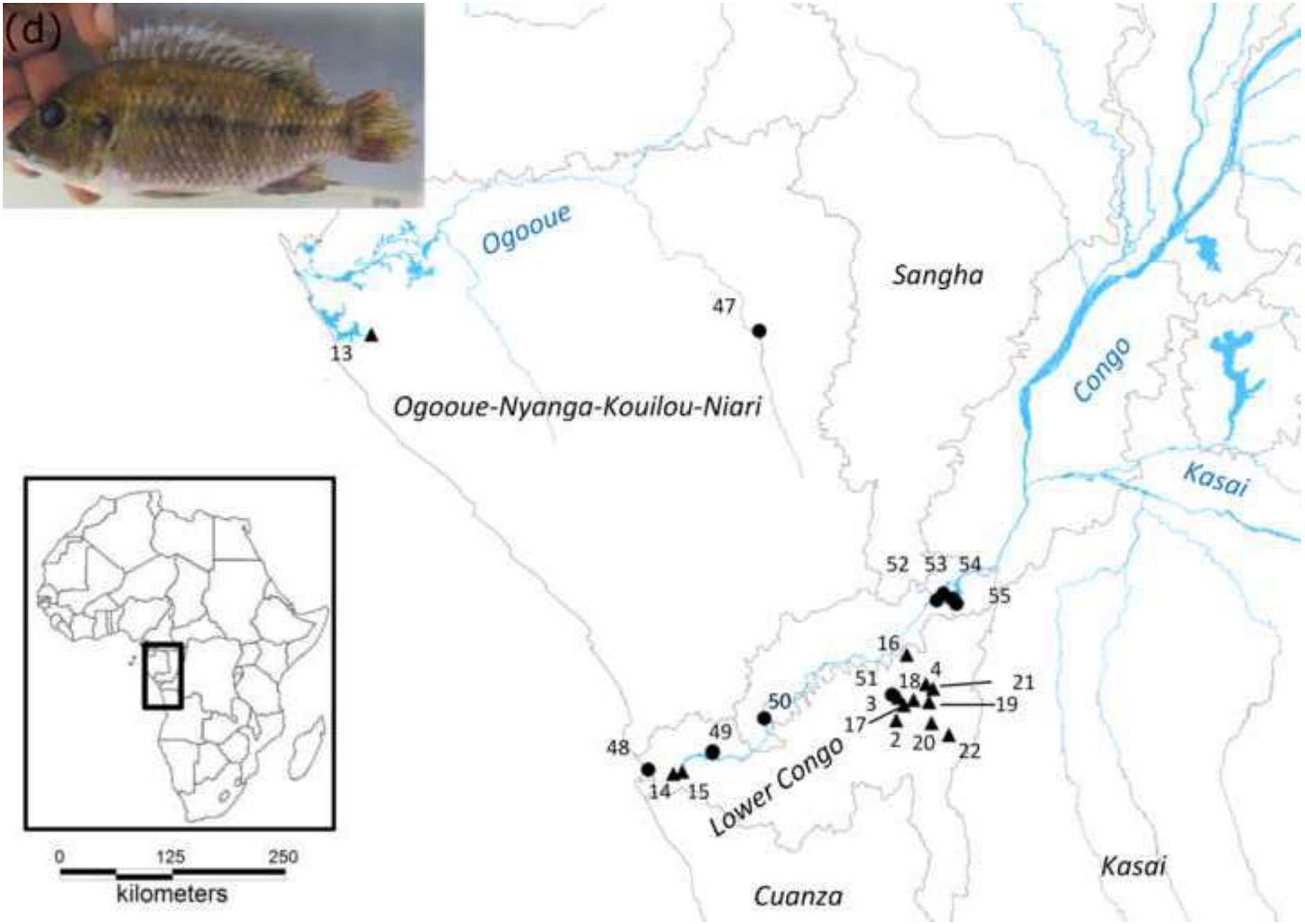

Fig1e

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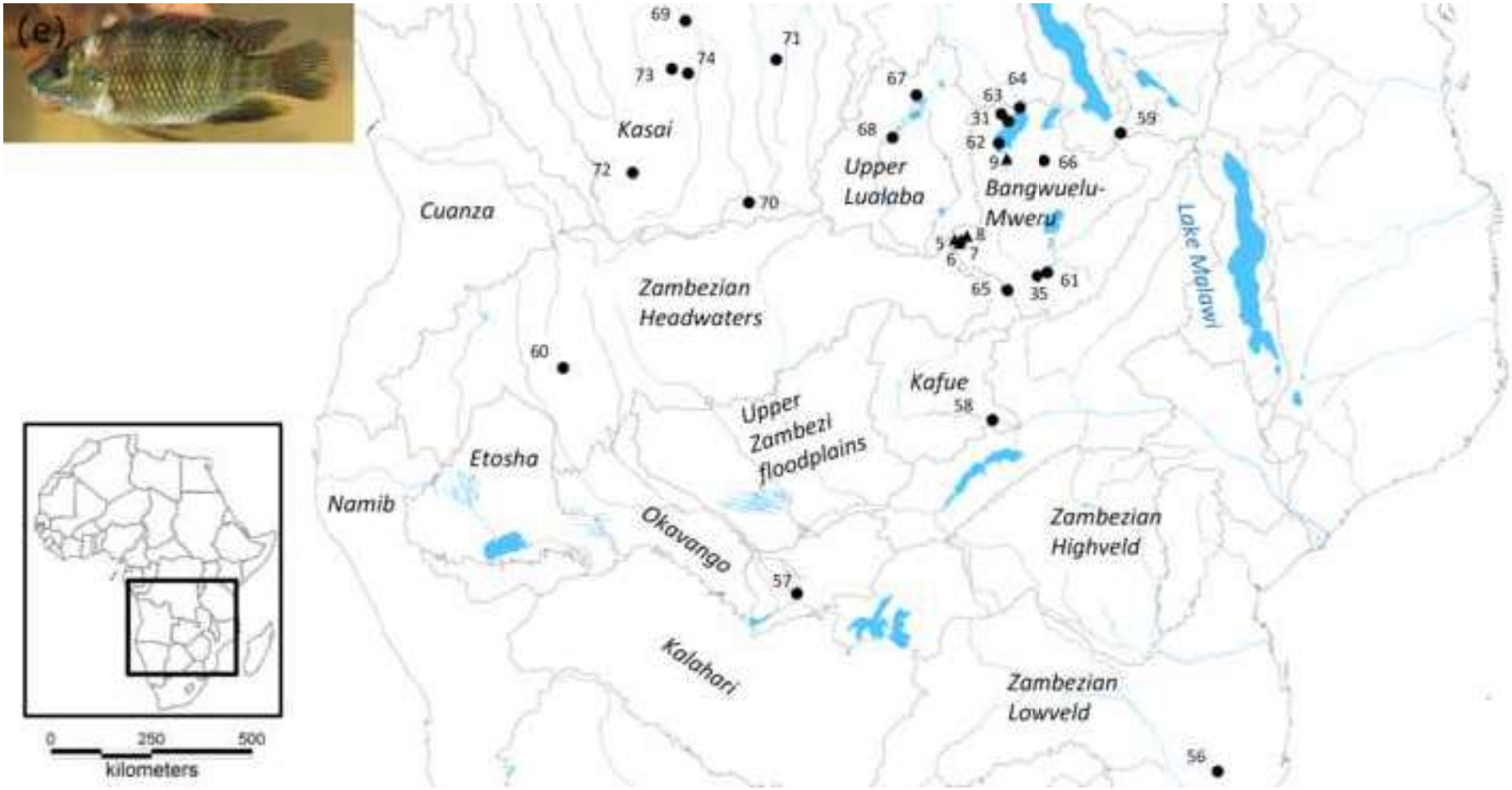

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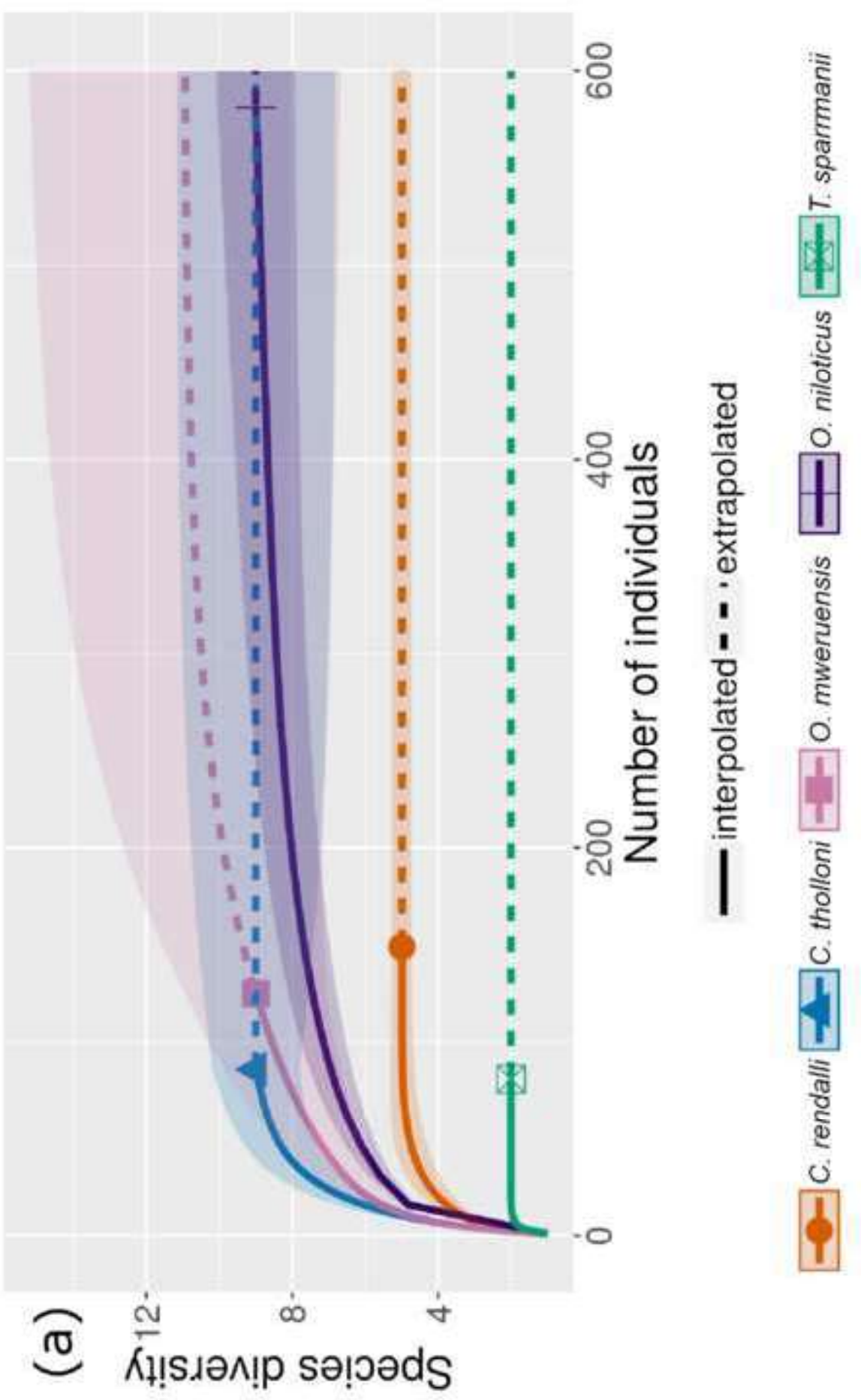

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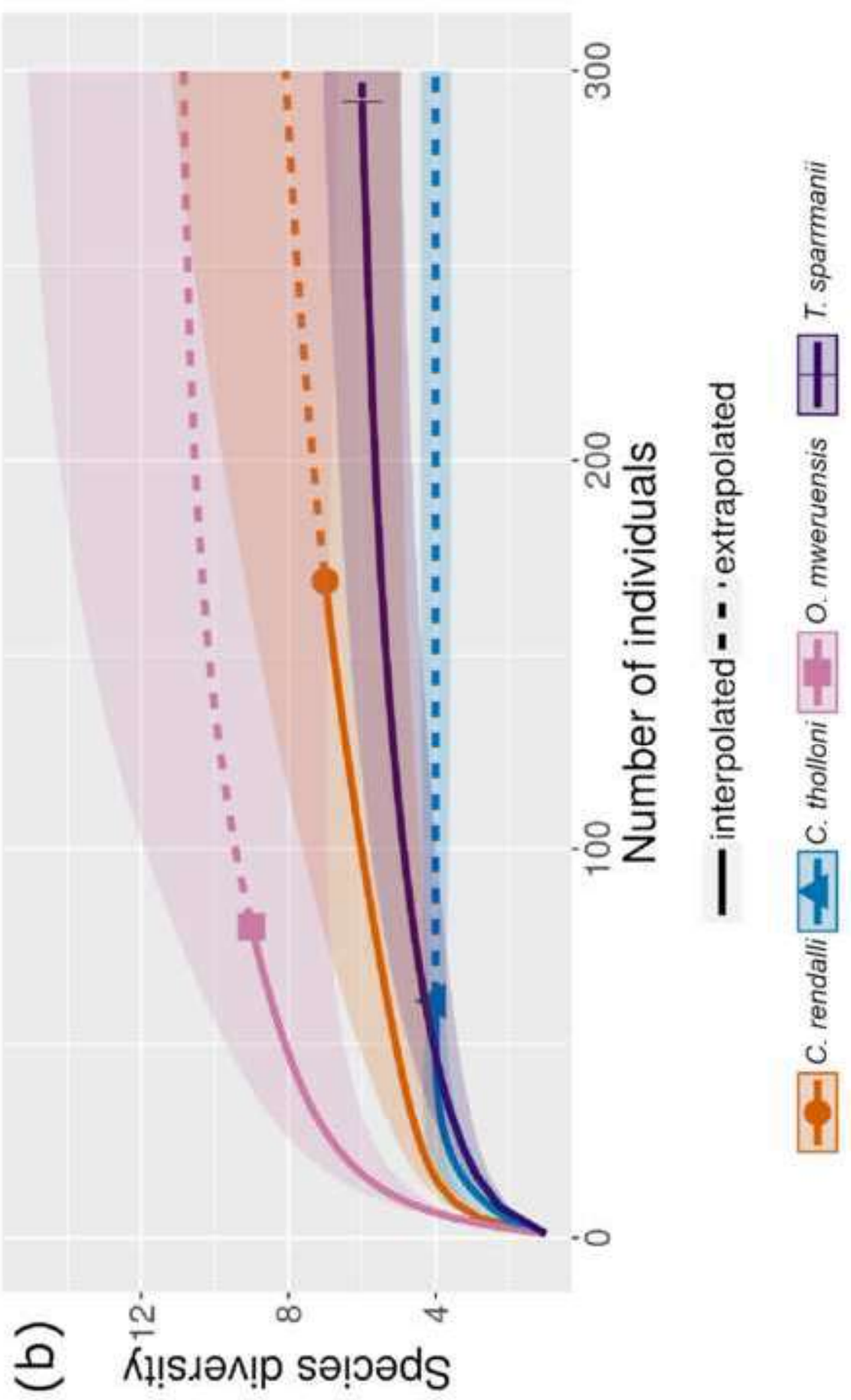

Fig3

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