Gill monogeneans of '*Gnathochromis*' and *Limnochromis* (Teleostei, Cichlidae) in Burundi: do the parasites mirror host ecology and phylogenetic history?

Nikol Kmentová, Milan Gelnar, Stephan Koblmüller, Maarten P.M. Vanhove

Monogenea is one of the most species-rich groups of parasitic flatworms. Research on this taxon in Africa has been recently intensified. For example, already more than 100 nominal species belong to *Cichlidogyrus*, a genus mostly occurring on cichlids. Twenty-two of these were described from Lake Tanganyika which is a biodiversity hotspot in which many vertebrate and invertebrate taxa underwent unique evolutionary radiations. Parasites were also used as a potential tool to uncover host species relationships. This study presents the first investigation of the monogenean fauna occurring on the gills of endemic *Gnathochromis* species along the Burundese coastlines. We test whether their monogenean fauna reflects the different phylogenetic position and ecological niche of *'G.' pfefferi* and *G. permaxillaris.* Worms collected from specimens of *Limnochromis auritus*, a cichlid belonging to the same tribe as *G. permaxillaris*, were used for comparison. Morphological as well as genetic characterisation was used for parasite identification. In total, all 73 *Cichlidogyrus* individuals collected from *'G.' pfefferi* were identified as *C. irenae*. This is the only representative of *Cichlidogyrus* previously described from *'G.' pfefferi*, its type host. *Gnathochromis permaxillaris* is infected by a species of *Cichlidogyrus* morphologically very similar to *C. gillardinae*. The monogenean species collected from *L. auritus* is considered as new for science, but sufficient specimens lack for a formal description. Our results confirm previous suggestions about *'G.' pfefferi* as a good disperser infected by a single monogenean species. Although *G. permaxillaris* and *L. auritus* are placed in the same tribe, there is closer morphological similarity between species of *Cichlidogyrus* occurring on *G. permaxillaris* and *C. irenae* from *'G.' pfefferi*, compared to the monogeneans of *L. auritus*. This pattern therefore can be caused by various processes in the parasite-host system's evolutionary history, such as host-switching or duplication events. Additional samples, allowing phylogenetic analysis, from the species of *Cichlidogyrus* occurring on *G. permaxillaris* and *L. auritus* are needed to reveal their history.

Gill monogeneans of '*Gnathochromis*' and *Limnochromis* (Teleostei, Cichlidae) in Burundi: do the parasites mirror host ecology and phylogenetic history?

Nikol Kmentová[1], Milan Gelnar[1], Stephan Koblmüller[2], Maarten P. M. Vanhove[1,3,4,5]

[1] Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

[2] Institute of Zoology, University of Graz, Universitätsplatz 2, A-8010 Graz, Austria

[3] Biology Department, Royal Museum for Central Africa, Leuvensesteenweg 13, B-3080 Tervuren, Belgium

[4] Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, University of Leuven,Ch. Deberiotstraat 32, B-3000 Leuven, Belgium

[5] *Present address:* Capacities for Biodiversity and Sustainable Development, Operational Directorate Natural Environment, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium

Corresponding author:

Nikol Kmentová

Kamenice 5, Brno-Bohunice, 625 00, Czech Republic

Email address: kmentovan@mail.muni.cz

Abstract

Monogenea is one of the most species-rich groups of parasitic flatworms. Research on this taxon in Africa has been recently intensified. For example, already more than 100 nominal species belong to *Cichlidogyrus*, a genus mostly occurring on cichlids. Twenty-two of these were described from Lake Tanganyika which is a biodiversity hotspot in which many vertebrate and invertebrate taxa underwent unique evolutionary radiations. Parasites were also used as a potential tool to uncover host species relationships. This study presents the first investigation of the monogenean fauna occurring on the gills of endemic *Gnathochromi*s species along the Burundese coastlines. We test whether their monogenean fauna reflects the different phylogenetic position and ecological niche of *'G.' pfefferi* and *G. permaxillaris*. Worms collected from specimens of *Limnochromis auritus*, a cichlid belonging to the same tribe as *G. permaxillaris*, were used for comparison. Morphological as well as genetic characterisation was used for parasite identification. In total, all 73 *Cichlidogyrus* individuals collected from '*G.' pfefferi* were identified as *C. irenae*. This is the only representative of *Cichlidogyrus* previously described from *'G.' pfefferi*, its type host. *Gnathochromis permaxillaris* is infected by a species of *Cichlidogyrus* morphologically very similar to *C. gillardinae*. The monogenean species collected from *L. auritus* is considered as new for science, but sufficient specimens lack for a formal description. Our results confirm previous suggestions about *'G.' pfefferi* as a good disperser infected by a single monogenean species. Although *G. permaxillaris* and *L. auritus* are placed in the same tribe, there is closer morphological similarity between ~~species of~~ *Cichlidogyrus* ~~occurring~~ on *G. permaxillaris* and *C. irenae* from *'G.' pfefferi*, compared to the monogeneans of *L. auritus*. This pattern therefore can be caused by various processes in the parasite-host system's evolutionary history, such as host-switching or duplication events.

Additional samples, allowing phylogenetic analysis, from the species of *Cichlidogyrus* occurring on *G. permaxillaris* and *L. auritus* are needed to reveal their history.

Introduction

Cichlids are a unique target group for biologists because of their remarkable evolutionary history characterized by diverse speciation and adaptive radiation processes (Salzburger et al., 2005; Turner, 2007; Muschick, Indermaur & Salzburger, 2012). Studies about cichlid adaptation mechanisms provided important information, generally applicable in evolutionary biology (Kocher, 2004; Koblmüller, Sefc & Sturmbauer, 2008). Cichlids range from Central and South America, across Africa, Iran, the Middle East and Madagascar to India and Sri Lanka, but most species are concentrated in the Neotropics and in Africa (Chakrabarty, 2004). A place famous for its extraordinary cichlid diversity is Lake Tanganyika (Koblmüller, Sefc & Sturmbauer, 2008). It is considered a prime study area for evolutionary research because it shows the greatest diversity in speciation mechanisms of the African Great Lakes (Salzburger et al., 2002; Salzburger, 2009). In Lake Tanganyika, there are more than 200 described cichlid species belonging to 53 genera (Snoeks, 2000; Takahashi, 2003; Koblmüller, Sefc & Sturmbauer, 2008), usually classified into 16 tribes (Takahashi, 2003, 2014).

Although cichlids have been subjects of interest for many decades, there are still gaps in the understanding of their phylogenetic history and taxonomy (Koblmüller, Sefc & Sturmbauer, 2008). According to recent molecular findings, the two species of *Gnathochromis*, *G. permaxillaris* (L. R. David 1936) and '*G.*' *pfefferi* (G. A. Boulenger 1898) belong to different cichlid tribes (Limnochromini, Tropheini, respectively) and their classification therefore needs revision (Salzburger et al., 2002; Duftner, Koblmüller & Sturmbauer, 2005; Koblmüller et al.,

2010; Muschick, Indermaur & Salzburger, 2012; Kirchberger et al., 2014). A possible source for a better understanding of cichlid taxonomy and phylogeny, and a particularly diverse group of organisms in Lake Tanganyika, are monogenean parasites (Mendlová et al., 2012; Vanhove et al., 2015; Van Steenberge et al., 2015). Monogenea E. J. M. van Beneden 1858 is a group of parasitic flatworms mainly occurring on fish gills, skin and fins (Pugachev et al., 2009). These often tiny animals have a direct life cycle. Relatively strong host specificity was reported on cichlid hosts (Pariselle & Euzet, 2009; Gillardin et al., 2012; Muterezi Bukinga et al., 2012; Řehulková, Mendlová & Šimková, 2013). These characteristics make them an ideal model for investigating co-evolutionary processes in host-parasite systems (Pouyaud et al., 2006). While there is no record characterizing the monogenean fauna on any of the tribe members of Limochromini, most of the species of Tropheini were already investigated. Earlier studies suggested '*G.*' *pfefferi, Limnotilapia dardennii* (G. A. Boulenger 1899) and '*Ctenochromis*' *horei* (A. Günther 1894) are infected by a single dactylogyridean monogenean species each: *Cichlidogyrus irenae, C. steenbergei* and *C. gistelincki* C. Gillardin, M. P. M. Vanhove, A. Pariselle et al. 2012, respectively (Gillardin et al., 2012). The closely related *Astatotilapia burtoni* (A. Günther 1894) is infected by *C. gillardinae* F. Muterezi Bukinga, M. P. M. Vanhove, M. Van Steenberge et al. 2012 ~~(Gillardin et al., 2012;~~ Muterezi Bukinga et al., 2012). These observations are hitherto only based on reports from several localities along the Congolese and Zambian coasts of the lake (Gillardin et al., 2012; Muterezi Bukinga et al., 2012; Vanhove et al., 2015). Thorough sampling covering as many host localities as possible is however needed to conclude about the full extent of a species' parasite fauna (Price & Clancy, 1983; Brooks et al., 2006; Caro, Combes & Euzet, ~~2009~~).

As mentioned above, '*Gnathochromis*' is a polyphyletic genus and no comparison of the parasite fauna of its two species has been performed to date. Do the parasites reflect the phylogenetic and ecological situation of their hosts? We investigate the monogenean fauna of both *Gnathochromis* species to answer the following questions:

(1) Does the Burundese population of '*G.*' *pfefferi* confirm that this host is only infected by a single species of *Cichlidogyrus*?

(2) Since *Gnathochromis* is considered polyphyletic, is the dissimilarity between its two representatives reflected in their parasite fauna?

Material & Methods

Sampling

Fish specimens were obtained from commercial fishermen along the Burundese coastline. We examined two '*G.*' *pfefferi* individuals from Mvugo (4°15' S, 29°34' E) and four from Mukuruka (4'14' S, 29°33' E), as well as seven hosts belonging to *G. permaxillaris* and six *Limnochromis auritus* (G. A. Boulenger 1901) individuals from Bujumbura (3°23′S 29°22′E) (Fig. 1). The latter species was included to allow a comparison between the monogeneans of *G. permaxillaris* and another member of Limnochromini, a tribe from which no monogeneans have been described previously. Fish were sacrificed by severing the spinal cord and dissected immediately. Gills were removed according to the standard protocol of Ergens & Lom (1970) and immediately preserved in pure ethanol in plastic tubes until further inspection in the lab. Some fresh gills were also inspected *in situ* for monogenean parasites using dissecting needles and a stereomicroscope. Slides prepared *in situ* were stained in GAP (glycerine ammonium picrate) (Malmberg, 1957) or

in Hoyer's solution (Humason, 1979). Monogeneans were isolated in the lab using a dissecting needle and an Olympus SZX7 stereomicroscope. They were mounted on a slide under a cover slip. Parasite individuals utilized for genetic characterisation were identified using an Olympus BX51 microscope with incorporated phase contrast at a magnification of 100x (oil immersion, 10x ocular) with Micro Image software and photographed for *post hoc* confirmation of species identity. They were stored in 1.2 ml Eppendorf tubes with 99,8 % ethanol for subsequent DNA isolation. The research was approved by the Ethics Committee of Masaryk University. The approval number which allows us to work with vertebrate animals is CZ01308.

Morphometrics

The morphometrics part was based on 26 different metrics measured according to Řehulková, Mendlová & Šimková (2013) and Gillardin et al. (2012). Measurements and photos were taken using the same configuration as above. In some cases an extra magnification of 2x had to be used. Voucher specimens were deposited in the invertebrate collection of the Royal Museum for Central Africa under accession numbers 37792-802.

DNA extraction and genetic characterisation

Ethanol evaporation took place in a vacuum centrifuge. DNA was extracted using the Qiagen Blood and Tissue Isolation Kit according to the manufacturer's instructions with some modifications (samples in ATL buffer (180µl) with protein kinase (20µl) were kept in 1.5 ml Eppendorf tubes overnight at room temperature). The DNA extract was then concentrated to a volume of 80µl in 1.5 ml Eppendorf tubes using a vacuum centrifuge and stored at a temperature of -20°C until polymerase chain reaction amplification. Part of the 18S nuclear ribosomal DNA gene, together with the first Internal Transcribed Spacer (ITS-1) region was amplified for 5 individuals using the S1 (5'-ATTCCGATAACGAACGAGACT-3') (Matějusová et al., 2001)

and IR8 (5'-GCAGCTGCGTTCTTCATCGA-3') (Šimková et al., 2003) primers. Each amplification reaction contained 1.5 unit of *Taq* Polymerase, 1X buffer containing 0.1 mg/ml BSA, 1.5 mM $MgCl_2$, 200 mM dNTPs, 0.5 mM of each primer and 30 ng of genomic DNA in a total reaction volume of 30 µl under the following conditions: 2 min at 94°C, 39 cycles of 1 min at 94°C, 1 min at 53°C and 1 min and 30 sec at 72°C, and finally 10 min at 72°C. The obtained nucleic acid sequences were aligned using MUSCLE (Edgar, 2004) under default distance measures and sequence weighting schemes, implemented in MEGA 6.06 (Tamura et al., 2013), together with GenBank sequences of *Cichlidogyrus* retrieved from hosts belonging to '*G.*' *pfefferi* (KT037169-73). Sequences and their alignment were visually inspected and corrected using the same software. Uncorrected pairwise distances were calculated in MEGA. The newly obtained haplotype sequence was deposited in GenBank under accession number KT692939.

Results

All 73 adult monogeneans collected from '*G.*' *pfefferi* specimens were identified as *C. irenae* on the basis of Gillardin et al. (2012). The prevalence was 83.3%, infection intensity 18.2 and abundance 15.1 (calculated using adult monogeneans only). Our set of measurements matches with the original description of *C. irenae* (Gillardin et al., 2012) (Table 1). Differences in heel length are caused by different metrics (measuring up to the base of the heel *versus* to the base of the copulatory tube).

Only one specimen of *G. permaxillaris* showed a monogenean infection. It was infected by a single representative of a species of *Cichlidogyrus* similar in morphology ~~as well as~~

159 ~~morphometrics (Table 2)~~ to an already described species from *A. burtoni* ~~called~~ *C. gillardinae*.
160 Unfortunately, we cannot confidently confirm conspecificity based on only one specimen and
161 therefore we refer to it as *C.* cf. *gillardinae*. Its pairs of anchors are asymmetrical: the dorsal
162 anchor has a much longer guard than shaft while in the ventral anchor, guard and shaft are equal
163 in size. The auricles and ventral bar branches are relatively short. Its male copulatory organ is
164 characterized by a short heel, a simple copulatory tube with constant diameter and an accessory
165 piece with easily overlooked distal bulb. No sclerotized vagina was observed. Despite these
166 similarities with *C. gillardinae*, we can see some differences compared to the original
167 description. *Cichlidogyrus* cf. *gillardinae* from *G. permaxillaris* has a more ~~slender heel and a~~
168 ~~shorter ventral anchor root~~.
169
170 Two monogenean specimens of an undescribed species of *Cichlidogyrus* were collected from
171 one individual of *L. auritus*. One of the most noticeable structures within this parasite's haptor
172 are the ~~relatively~~ long auricles of the dorsal transverse bar. There is no visible difference between
173 the length of guard and shaft in any of the anchors. The copulatory tube is thin with a constant
174 diameter; a heel was not recognized. The accessory piece is robust and thick with a fork-shaped
175 ending. No sclerotized vagina was observed. Micrographs of the collected monogenean species
176 are presented in Fig. 2.
177
178 The rDNA dataset included four successfully amplified sequences of parasites collected from
179 '*G.*' *pfefferi*. Only one haplotype (1060 base pairs) was recognised. The maximum overlap with
180 sequences of more southern specimens of *C. irenae* obtained from GenBank was 571 base pairs,
181 situated within ITS-1. The uncorrected pairwise genetic distance reached a maximum of 0.8%,

182 which is below the species-level cut-off of 1%, suggested for this region for the best-studied
183 monogenean, *Gyrodactylus* (Zietara & Lumme, 2002). This result confirms the identification,
184 based on morphology and morphometrics, of a single monogenean species infecting '*G.*' *pfefferi*,
185 namely *C. irenae*.
186
187 **Discussion**

188 The monogenean fauna of the cichlid '*G.*' *pfefferi* in Burundi was characterised morphologically
189 and genetically. We confirmed the occurrence of *C. irenae*, representing the first record of this
190 species in Burundi. According to previous results, the species richness of *Cichlidogyrus* on
191 Tanganyika cichlids is influenced by the dispersal ability or isolation of the host species
192 (Pariselle et al., 2015a; Grégoir et al., 2015). Our results therefore support previous suggestions
193 about '*G.*' *pfefferi* as a cichlid with good dispersal ability hosting only a single representative of
194 *Cichlidogyrus*, now recorded from several localities in the northern as well as the southern part
195 of the Lake (Vanhove et al., 2015) (see Fig. 3).
196
197 Monogenean parasites belonging to *Cichlidogyrus* were also used as an additional way to look at
198 species interrelationships within *Gnathochromis*. The parasite from *G. permaxillaris* was
199 identified as *C.* cf. *gillardinae*. Since *C. gillardinae* was originally described from the
200 haplochromine *A. burtoni*, a fish also occurring in aquatic systems along Lake Tanganyika's
201 shores, it is most likely a generalist parasite infecting representatives of two unrelated cichlid
202 genera with different habitat preferences (Konings, 1998; Muterezi Bukinga et al., 2012).
203 Although the limnochromine *G. permaxillaris* is hence infected by a monogenean species
204 different from *C. irenae* described from '*G.*' *pfefferi*, its parasite seems more similar to its

congeners infecting tropheine hosts like '*G.*' *pfefferi* (Gillardin et al., 2012; Pariselle et al., 2015a). *Cichlidogyrus* can be divided into ~~main~~ lineages based on the configuration of ~~the~~ haptoral hard parts, in particular the relative length of the pairs of hooks (also termed uncinuli) (Pariselle & Euzet, 2003; Vignon, Pariselle & Vanhove, 2011). Indeed, both parasites' haptor shares important characteristics: asymmetry between anchors, small (*sensu* Pariselle & Euzet, 2009) hooks. *Cichlidogyrus* cf. *gillardinae* differs substantially from the *Cichlidogyrus* species collected from the closely related host *L. auritus*, also a limnochromine cichlid. In the latter flatworm, the extremely long dorsal bar auricles represent an evident similarity with *C. vandekerkhovei* M. P. M. Vanhove, F. A. M. Volckaert and A. Pariselle 2011 and *C. makasai* M. P. M. Vanhove, F. A. M. Volckaert and A. Pariselle 2011 (Vanhove, Volckaert & Pariselle, 2011) collected from species of *Ophthalmotilapia* J. Pellegrin 1904, belonging to the endemic Ectodini. This feature was hitherto never found in other monogenean congeners. The gill monogenean retrieved from *Limnochromis* hence seems to belong to an endemic Tanganyika lineage. There is still discussion about the evolution of the haptoral sclerotized structures of these monogeneans. Morand et al. (2002) assume that haptoral structures do not reflect a phylogenetic pattern as a result of adaptation to microhabitat within the host~~. Moreover, Messu Mandeng et al.(in press) reveal an adaptive component presented in attachment organ morphology of *Cichlidogyrus*~~. However, other studies suggest the existence of a phylogenetic signal in sclerite morphology and shape within dactylogyridean monogeneans (Šimková et al., 2002, 2006) and specifically within *Cichlidogyrus* (Vignon, Pariselle & Vanhove, 2011). Possible explanations for the affinities of monogenean species on *Gnathochromis* are host history as well as habitat characteristics. While '*G.*' *pfefferi* is a typical rock dwelling littoral cichlid occurring at depths between 1 and 15 metres with maternal mouthbrooding care, *G. permaxillaris* is a biparental

mouthbrooder occurring over muddy bottoms rarely seen in water shallower than 30 metres (Maréchal & Poll, 1991; Konings, 1998). *Limnochromis auritus* is placed together with *G. permaxillaris* in Limnochromini (Muschick, Indermaur & Salzburger, 2012), prefers a similar habitat with muddy bottoms at depths ranging from 5 to 125 metres and also exhibits biparental mouthbrooding care (Maréchal & Poll, 1991; Konings, 1998). According to Mendlová & Šimková (2014) the host specifity of *Cichlidogyrus* parasitizing African cichlid fishes is significantly influenced by fish phylogeny and by the form of parental care. No *Cichlidogyrus* species was hitherto observed to infect cichlid species with different parental care systems (i.e. substrate brooders as well as mouthbrooders) (Pouyaud et al., 2006). Given that the haplochromine *A. burtoni* is a maternal mouthbrooder mainly occurring in wetlands adjacent to the lake, it is unclear how it came to share a species with *G. permaxillaris* from which it differs ecologically, phylogenetically and in reproductive behaviour. Due to the lack of genetic data, we cannot perform (co-)phylogenetic analyses. According to Mendlová et al. (2012) duplication and host-switching events have played the most important role in the evolutionary history of African cichlid dactylogyrideans. Vanhove et al. (2015), however, also observed a role for co-speciation in species of *Cichlidogyrus* infecting Lake Tanganyika tropheine cichlids. Although representatives of *Cichlidogyrus* occuring on littoral cichlid assemblages including Tropheini display strong host specificity (Gillardin et al., 2012; Muterezi Bukinga et al., 2012; Vanhove et al., 2015), a lower specificity was observed within members of Bathybatini in the deepwater habitat (Pariselle et al., 2015b). Hence, some lineages of *Cichlidogyrus* in Lake Tanganyika were already shown to have a wide host range. Given the low prevalence and infection intensities observed in this study, and the deepwater habitat of the limnochromine hosts, it is a challenge to

retrieve additional material for species identification and molecular analyses. These are needed to uncover the whole co-phylogenetic history of *Gnathochromis* and its monogenean fauna.

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests

The authors declare there are no competing interests.

Author Contributions

Nikol Kmentová prepared samples, obtained and analysed the data and wrote the paper. Milan Gelnar provided experience in monogenean taxonomy and revised the manuscript. Stephan Koblmüller identified the hosts, provided experience in cichlid biology and revised the manuscript. Maarten P. M. Vanhove designed and led the study, analysed the data and wrote the paper.

Funding

This study was realized with financial support from the Czech Science Foundation (GBP505/12/G112 – ECIP).

ACKNOWLEDGEMENTS

We would like to thank Maarten Van Steenberge, Tine Huyse, Antoine Pariselle and the parasitology group at Masaryk University, Brno for their hospitality and cooperation. Eva Řehulková, Šárka Mašová, Iva Přikrylová, Radim Blažek, Veronika Nezhybová, Gaspard Banyankimbona and the Schreyen-Brichard family and the technical staff of Fishes of Burundi are thanked for their help in collecting samples, and Wolfgang Gessl for providing fish pictures.

REFERENCES

Brooks DR., León-Règagnon V., McLennan DA., Zelmer D. 2006. Ecological fitting as a determinant of the community structure of platyhelminth parasites of anurans. *Ecology* 87:76–85.

Caro A., Combes C., Euzet L. 2009. What makes a fish a suitable host for Monogenea in the Mediterranean? *Journal of Helminthology* 71:203.

Duftner N., Koblmüller S., Sturmbauer C. 2005. Evolutionary relationships of the Limnochromini, a tribe of benthic deepwater cichlid fish endemic to Lake Tanganyika, East Africa. *Journal of Molecular Evolution* 60:277–289.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research* 32:1792–7.

Ergens R., Lom J. 1970. *Causative agents of fish diseases*. Prague: Academia.

Gillardin C., Vanhove MPM., Pariselle A., Huyse T., Volckaert FAM. 2012. Ancyrocephalidae (Monogenea) of Lake Tanganyika: II: description of the first *Cichlidogyrus* spp. parasites from Tropheini fish hosts (Teleostei, Cichlidae). *Parasitology Research* 110:305–313.

Grégoir AF., Hablützel PI., Vanhove MPM., Pariselle A., Bamps J., Volckaert FAM., Raeymaekers JAM. 2015. A link between host dispersal and parasite diversity in two sympatric cichlids of Lake Tanganyika. *Freshwater Biology* 60:323–335.

Humason GL. 1979. *Animal Tissue Techniques:4th.* San Francisco: Freeman and Company.

Chakrabarty P. 2004. Cichlid biogeography: Comment and review. *Fish and Fisheries* 5:97–119.

Kirchberger PC., Sefc KM., Sturmbauer C., Koblmüller S. 2014. Outgroup effects on root

294 position and tree topology in the AFLP phylogeny of a rapidly radiating lineage of cichlid
295 fish. *Molecular Phylogenetics and Evolution* 70:57–62.

296 Koblmüller S., Egger B., Sturmbauer C., Sefc KM. 2010. Rapid radiation, ancient incomplete
297 lineage sorting and ancient hybridization in the endemic Lake Tanganyika cichlid tribe
298 Tropheini. *Molecular Phylogenetics and Evolution* 55:318–34.

299 Koblmüller S., Sefc KM., Sturmbauer C. 2008. The Lake Tanganyika cichlid species
300 assemblage: Recent advances in molecular phylogenetics. *Hydrobiologia* 615:5–20.

301 Kocher TD. 2004. Adaptive evolution and explosive speciation: the cichlid fish model. *Nature*
302 *reviews. Genetics* 5:288–298.

303 Konings A. 1998. *Guide to Tanganyika Cichlids*. El Paso: Cichlid Press.

304 Malmberg G. 1957. On the occurrence of *Gyrodactylus* on Swedish fishes. *Skrifter utgivna av*
305 *Södra Sveriges Fiskeriföreningen*:19–76.

306 Maréchal C., Poll M. 1991. *Bathybates, Hemibates*. In: Daget J, Gosse J-P, Teugels GG, Thys
307 van den Audenaerde DFE eds. *Checklist of the Freshwater Fishes of Africa (CLOFFA)*.
308 Tervuren: Royal Museum for Central Africa, 21–24,186.

309 Matějusová I., Gelnar M., McBeath AJA., Collins CM., Cunningham CO. 2001. Molecular
310 markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei).
311 *International Journal for Parasitology* 31:738–745.

312 Mendlová M., Desdevises Y., Civáňová K., Pariselle A., Šimková A. 2012. Monogeneans of
313 west African cichlid fish: Evolution and cophylogenetic interactions. *PLoS ONE* 7:e37268.

314 Mendlová M., Šimková A. 2014. Evolution of host specificity in monogeneans parasitizing

African cichlid fish. *Parasites & Vectors* 7:69.

Messu Mandeng FD., Bilong Bilong CF., Pariselle A., Vanhove MPM., Bitja Nyom AR., Agnèse J-F. A phylogeny of ~~Cichlidogyrus~~ species (Monogenea, Dactylogyridea) clarifies a host switch between fish families and reveals an adaptive component to attachment organ morphology of this parasite genus. *Parasites & Vectors*~~:(in press)~~.

Morand S., Šimková A., Matějusová I., Plaisance L., Verneau O., Desdevises Y. 2002. Investigating patterns may reveal processes: evolutionary ecology of ectoparasitic monogeneans. *International Journal for Parasitology* 32:111–119.

Muschick M., Indermaur A., Salzburger W. 2012. Convergent ~~E~~volution within an ~~A~~daptive ~~R~~adiation of ~~C~~ichlid ~~F~~ishes. *Current Biology* 22:2362–2368.

Muterezi Bukinga F., Vanhove MPM., Van Steenberge M., Pariselle A. 2012. Ancyrocephalidae (Monogenea) of Lake Tanganyika: III: *Cichlidogyrus* infecting the world's biggest cichlid and the non-endemic tribes Haplochromini, Oreochromini and Tylochromini (Teleostei, Cichlidae). *Parasitology Research* 111:2049–2061.

Pariselle A., Van Steenberge M., Snoeks J., Volckaert FAM., Huyse T., Vanhove MPM. 2015a. Ancyrocephalidae (Monogenea) of Lake Tanganyika: Does the *Cichlidogyrus* parasite fauna of *Interochromis loocki* (Teleostei, Cichlidae) reflect its host's phylogenetic affinities? *Contributions to Zoology* 84:25–38.

Pariselle A., Muterezi Bukinga F., Van Steenberge M., Vanhove MPM. 2015b. Ancyrocephalidae (Monogenea) of Lake Tanganyika: IV: *Cichlidogyrus* parasitizing species of Bathybatini (Teleostei, Cichlidae): reduced host-specificity in the deepwater realm? *Hydrobiologia* 748:99–119.

337 Pariselle A., Euzet L. 2003. Four new species of *Cichlidogyrus* (Monogenea: Ancyrocephalidae),
338 gill parasites of *Tilapia cabrae* (Teleostei: Cichlidae), with discussion on relative length of
339 haptoral sclerites. *Folia Parasitologica* 50:195–201.

340 Pariselle A., Euzet L. 2009. Systematic revision of dactylogyridean parasites (Monogenea) from
341 cichlid fishes in Africa, the Levant and Madagascar. *Zoosystema* 31:849–898.

342 Pouyaud L., Desmarais E., Deveney M., Pariselle A. 2006. Phylogenetic relationships among
343 monogenean gill parasites (Dactylogyridea, Ancyrocephalidae) infesting tilapiine hosts
344 (Cichlidae): Systematic and evolutionary implications. *Molecular Phylogenetics and*
345 *Evolution* 38:241–249.

346 Price PW., Clancy KM. 1983. Patterns in Number of Helminth Parasite Species in Freshwater
347 Fishes. *The Journal of Parasitology* 69:449–454.

348 Pugachev ON., Gerasev PI., Gussev A V., Ergens R., Khotenowsky I. 2009. *Guide to*
349 *Monogenoidea of freshwater fish of Palaeartic and Amur regions*. Milan: Ledizione-
350 LediPublishing.

351 Řehulková E., Mendlová M., Šimková A. 2013. Two new species of *Cichlidogyrus* (Monogenea:
352 Dactylogyridae) parasitizing the gills of African cichlid fishes (Perciformes) from Senegal:
353 Morphometric and molecular characterization. *Parasitology Research* 112:1399–1410.

354 Salzburger W., Meyer A., Baric S., Verheyen E., Sturmbauer C. 2002. Phylogeny of the Lake
355 Tanganyika cichlid species flock and its relationship to the Central and East African
356 haplochromine cichlid fish faunas. *Systematic Biology* 51:113–135.

357 Salzburger W., Mack T., Verheyen E., Meyer A. 2005. Out of Tanganyika: genesis, explosive
358 speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC*

359 *Evolutionary Biology* 5:17.

360 Salzburger W. 2009. The interaction of sexually and naturally selected traits in the adaptive
361 radiations of cichlid fishes. *Molecular Ecology* 18:169–185.

362 Snoeks J. 2000. *Ancient Lakes: Biodiversity, Ecology and Evolution*. Elsevier.

363 Šimková A., Ondráčková M., Gelnar M., Morand S. 2002. Morphology and coexistence of
364 congeneric ectoparasite species: reinforcement of reproductive isolation? *Biological Journal*
365 *of the Linnean Society* 76:125–135.

366 Šimková A., Plaisance L., Matějusová I., Morand S., Verneau O. 2003. Phylogenetic
367 relationships of the Dactylogyridae Bychowsky, 1933 (Monogenea: Dactylogyridea): The
368 need for the systematic revision of the Ancyrocephalinae Bychowsky, 1937. *Systematic*
369 *Parasitology* 54:1–11.

370 Šimková A., Verneau O., Gelnar M., Morand S. 2006. Specificity and specialization of
371 congeneric monogeneans parasitizing cyprinid fish. *Evolution* 60:1023.

372 Takahashi T. 2003. Systematics of Tanganyikan cichlid fishes (Teleostei: Perciformes).
373 *Ichthyological Research* 50:367–382.

374 Takahashi T. 2014. Greenwoodochromini Takahashi from Lake Tanganyika is a junior synonym
375 of Limnochromini Poll (Perciformes: Cichlidae). *Journal of Fish Biology* 84:929–36.

376 Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. 2013. MEGA6: Molecular
377 Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30:2725–
378 2729.

379 Turner GF. 2007. Adaptive radiation of cichlid fish. *Current Biology* 17:827–831.

Vanhove MPM., Pariselle A., Van Steenberge M., Raeymaekers JAM., Hablützel PI., Gillardin C., Hellemans B., Breman FC., Koblmüller S., Sturmbauer C., Snoeks J., Volckaert FAM., Huyse T. 2015. Hidden biodiversity in an ancient lake: phylogenetic congruence between Lake Tanganyika tropheine cichlids and their monogenean flatworm parasites. *Scientific Reports* 5:13669.

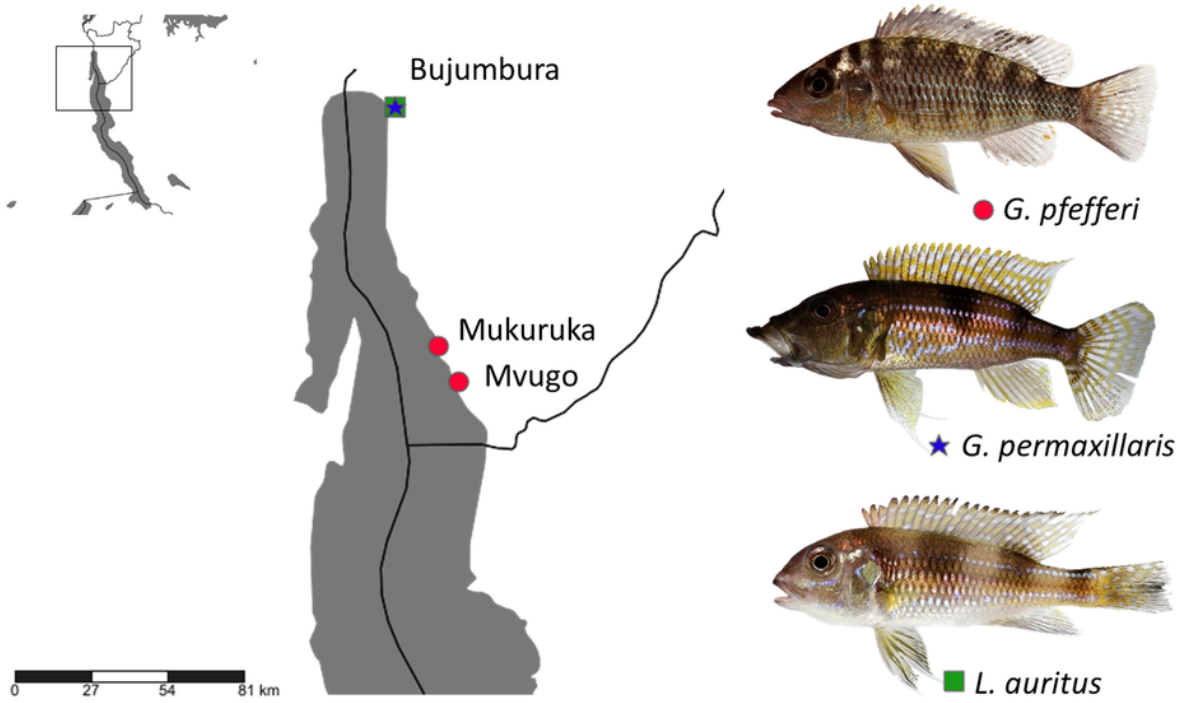
Vanhove MPM., Volckaert FAM., Pariselle A. 2011. Ancyrocephalidae (Monogenea) of Lake Tanganyika: I: Four new species of *Cichlidogyrus* from *Ophthalmotilapia ventralis* (Teleostei: Cichlidae), the first record of this parasite family in the basin. *Zoologia (Curitiba, Impresso)* 28:253–263.

Vignon M., Pariselle A., Vanhove MPM. 2011. Modularity in attachment organs of African *Cichlidogyrus* (Platyhelminthes: Monogenea: Ancyrocephalidae) reflects phylogeny rather than host specificity or geographic distribution. *Biological Journal of the Linnean Society* 102:694–706.

Zietara MS., Lumme J. 2002. Speciation by host switch and adaptive radiation in a fish parasite genus *Gyrodactylus* (Monogenea, Gyrodactylidae). *Evolution; International Journal of Organic Evolution* 56:2445–58.

1

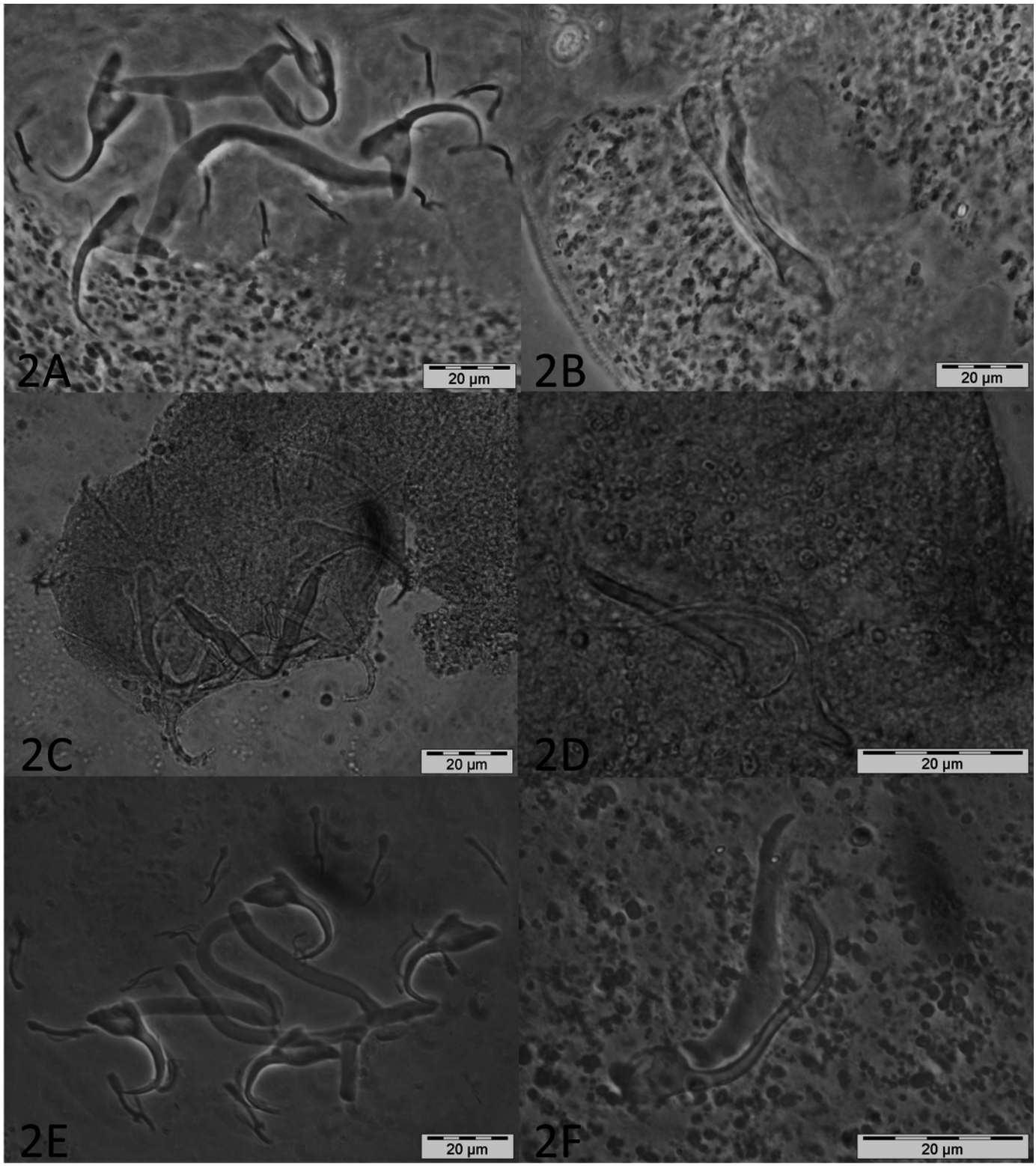
Sampling localities in Lake Tanganyika with indication of host species (photos by Wolfgang Gessl).



2

Micrograph of haptoral and male genital sclerotized structures from monogenean species belonging to *Cichlidogyrus*.

Host species: A) *'G.' pfefferi* (opisthaptor, Hoyer's medium, phasecontrast); B) *'G.' pfefferi* (MCO, Hoyer's medium, phasecontrast); C) *G. permaxillaris* (opisthaptor, GAP); D) *G. permaxillaris* (MCO, GAP); E) *L. auritus* (opisthaptor, Hoyer's medium, phasecontrast); F) *L. auritus* (MCO, Hoyer's medium, phasecontrast).


2A
20 µm
2B
20 µm
2C
20 µm
2D
20 µm
2E
20 µm
2F
20 µm

3

Geographical position of records of *C. irenae*, monogeneans infecting *'G.' pfefferi*.

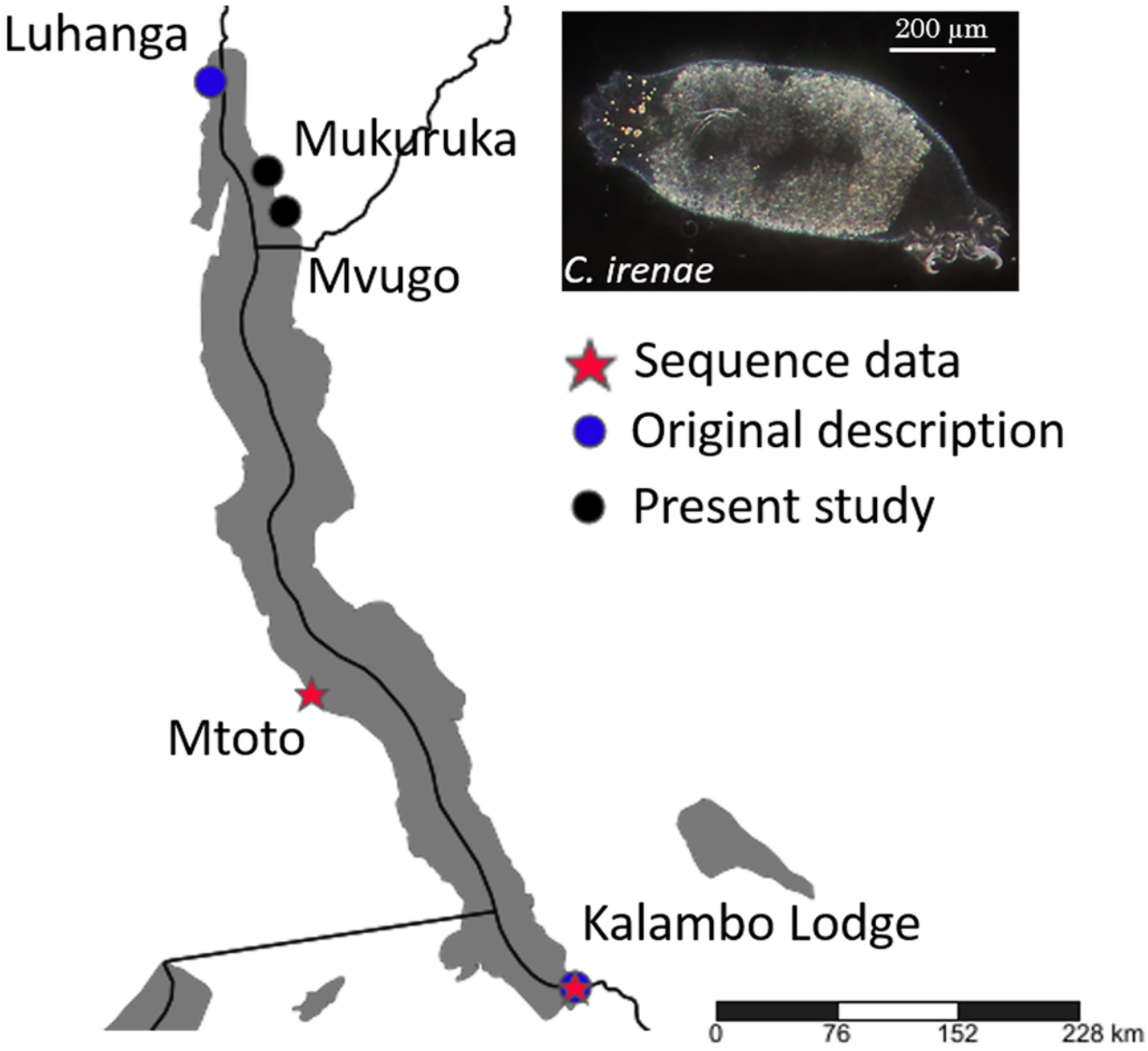


Table 1(on next page)

Table 1

Comparison of measurements (in µm) on Burundese C. irenae with the original description.

1 Comparison of measurements (in µm) on Burundese *C. irenae* with the original description.

	***C. irenae* from Burundi (n=30[a])**	***C. irenae*** (Gillardin et al., 2012)
Ventral anchor		
Total length	30,3±2,3[b] (n=28)[c]; (26,9-36,4)[d]	31,4±1,6 (n=14); (29,3-34,6)
Length to notch	25,7±0,9 (n=25); (22,6-29,8)	28,5±1,4 (n=14); (26,1-30,2)
Inner root length	8,7±1,7 (n=24); (5,6-10,8)	8,1±1,3 (n=14); (5,9-10,1)
Outer root length	5,5±0,7 (n=18); (4,9-6,8)	5,4±1,2 (n=14); (3,2-7,8)
Point length	8,5±1,1 (n=25); (6,9-10,4)	10,0±1,5 (n=14); (7,9-12,8)
Dorsal anchor		
Total length	30,5±2,6 (n=22); (27-37,5)	35,0±2,8 (n=15); (30,0-38,5)
Length to notch	21,8±1,1 (n=16); (19,8-23,9)	25,8±1,6 (n=15); (22,4-28,8)
Inner root length	10,6±1,3 (n=16); (7,9-13,4)	12,3±1,5 (n=15); (9,6-14,7)
Outer root length	5,3±0,9 (n=16); (4,1-7,2)	4,6±0,7 (n=15); (3,6-5,9)
Point length	7,1±1 (n=12); (5,7-8,7)	9,1±1,0 (n=15); (6,9-11,1)
Ventral bar		
Branch length	38,4±4,4 (n=22); (32-49,5)	31,6±4,6 (n=15); (24,8-39,5)
Branch maximum width	6±0,9 (n=28); (3,6-8,1)	4,8±0,9 (n=15); (3,2-6,5)
Dorsal bar		
Maximum straight width	40,1±4,1 (n=14); (35-48,6)	32,7±7,0 (n=15); (17,9-45,8)
Thickness at middle length	7,5±1,2 (n=28); (5,7-10,3)	6,1±1,1 (n=15); (4,2-8,2)
Distance between auricles	15,2±1,9 (n=28); (12,1-18,4)	11,5±1,8 (n=15); (8,3-15,2)
Auricle length	15,3±2,3 (n=15); (12,2-19,9)	14,2±2,4 (n=15); (9,6-19,0)
Hooks		
Pair I	12,3±0,6 (n=26); (11,5-13,2)	11,6±0,4 (n=15); (10,8-12,1)
Pair II	18,5±2,1 (n=28); (14,8-22,8)	-
Pair III	20,6±1,2 (n=25); (18,4-22,2)	-
Pair IV	21,1±1,5 (n=25); (19,4-25)	-
Pair V	10,1±0,9 (n=10); (9,4-12,2)	11,4±0,9 (n=15); (9,2-12,6)
Pair VI	21,4±2,4 (n=10); (16,1-22,8)	-
Pair VII	20,6±3,3 (n=18); (17,5-25,7)	-
Average size of pairs II, III, IV, VI, VII	20,2 2,5 (n=105); (13,3-27,3)	16,3±2,1 (n=15); (11,9-19,3)
Medium size		
Copulatory tube curved length	69,9±5,3 (n=30); (59,3-81,4)	69,5±5,7 (n=20); (48,0-73,3)
Accessory piece curved length	68,8±8,2 (n=30); (54-91)	59,5±5,8 (n=20); (37,8-64,8)
Heel straight length	11,1±3,9 (n=30); (6-22,6)	4,1±0,2 (n=20); (3,6-4,4)

2

3

Table 2(on next page)

Table 2

Comparison of measurements (in µm) on Burundese C. cf gillardinae with the original description.

1 Comparison of measurements (in µm) on Burundese *C. cf gillardinae* with the original
2 description.

	***C. cf. gillardinae* from Burundi (n=30[a])**	***C. gillardinae*** (Muterezi Bukinga et al., 2012)
Ventral anchor		
Total length	30,3±2,3[b] (n=28)[c]; (26,9-36,4)[d]	32 (27–37)
Length to notch	25,7±0,9 (n=25); (22,6-29,8)	28 (23–32)
Inner root length	8,7±1,7 (n=24); (5,6-10,8)	10 (8–13)
Outer root length	5,5±0,7 (n=18); (4,9-6,8)	6 (4–9)
Point length	8,5±1,1 (n=25); (6,9-10,4)	8 (6–11)
Dorsal anchor		
Total length	30,5±2,6 (n=22); (27-37,5)	33 (29–38)
Length to notch	21,8±1,1 (n=16); (19,8-23,9)	23 (19–29)
Inner root length	10,6±1,3 (n=16); (7,9-13,4)	12 (9–16)
Outer root length	5,3±0,9 (n=16); (4,1-7,2)	5 (4–7)
Point length	7,1±1 (n=12); (5,7-8,7)	7 (5–8)
Ventral bar		
Branch length	38,4±4,4 (n=22); (32-49,5)	31 (27–35)
Branch maximum width	6±0,9 (n=28); (3,6-8,1)	5 (3–6)
Dorsal bar		
Maximum straight width	40,1±4,1 (n=14); (35-48,6)	33 (27–39)
Thickness at middle length	7,5±1,2 (n=28); (5,7-10,3)	6 (4–8)
Distance between auricles	15,2±1,9 (n=28); (12,1-18,4)	12 (9–15)
Auricle length	15,3±2,3 (n=15); (12,2-19,9)	11 (8–14)
Hooks		
Pair I	12,3±0,6 (n=26); (11,5-13,2)	11 (9–13)
Pair II	18,5±2,1 (n=28); (14,8-22,8)	22 (19–24)
Pair III	20,6±1,2 (n=25); (18,4-22,2)	15 (13–17)
Pair IV	21,1±1,5 (n=25); (19,4-25)	17 (15–21)
Pair V	10,1±0,9 (n=10); (9,4-12,2)	10 (8–12)
Pair VI	21,4±2,4 (n=10); (16,1-22,8)	21 (18–26)
Pair VII	20,6±3,3 (n=18); (17,5-25,7)	14 (11–17)
Copulatory tube curved length	69,9±5,3 (n=30); (59,3-81,4)	47 (42–55)
Accessory piece curved length	68,8±8,2 (n=30); (54-91)	35 (29–42)
Heel straight length	11,1±3,9 (n=30); (6-22	5 (4–7)

3