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GERAERTS, Mare; Huyse, Tine; VANHOVE, Maarten & ARTOIS, Tom (2022) A new species of Cichlidogyrus Paperna, 1960 (Platyhelminthes: Monogenea: Dactylogyridae) infecting tilapias in Lake Kariba (Zimbabwe), with a discussion on its phylogenetic position. In: SYSTEMATICS AND BIODIVERSITY, 20 (1) , p. 1 -18 (Art N° 2143594).

DOI: 10.1080/14772000.2022.2143594
Handle: http://hdl.handle.net/1942/39339

Research Article

A new species of *Cichlidogyrus* Paperna, 1960 (Platyhelminthes: Monogenea: Dactylogyridae) infecting tilapias in Lake Kariba (Zimbabwe), with a discussion on its phylogenetic position

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Running title: A new species of *Cichlidogyrus* infecting tilapias

Monogeneans dominate the external parasite fauna of bony fish. During recent years, examination of more populations and species of *Cichlidogyrus* Paperna, 1960 has led to the (re)description of several species. *Cichlidogyrus halli* (Price & Kirk, 1967) Price, 1968, for example, has been redescribed several times in the past and has been proposed to encompass many (pseudo)cryptic species. In Lake Kariba (Zimbabwe), specimens of a species of *Cichlidogyrus* were found that morphologically resemble *C. halli*. These specimens were found on the gills of native *Oreochromis* cf. *mortimeri* and *Coptodon rendalli* (Boulenger, 1897), and introduced Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758). A detailed study of the morphology of these specimens, including morphometrics, and a thorough comparison with specimens of *C. halli* is presented. Part of the *COI* gene and 18S-ITS1 fragment were sequenced and analysed to provide insight into the phylogenetic placement of these specimens within the *Cichlidogyrus-Scutogyrus* monophylum. We found that *C. halli* and the new specimens sp. nov. are sister clades within the same monophyletic clade, and that clear morphological and morphometric differences are present in the dorsal bar of the haptor (auricles almost twice as long as in *C. halli*) and the male copulatory organ (wider penis stylet, longer accessory piece with a more elongated and less pronounced terminal triangular cap, narrower heel, as compared to *C. halli*). Based on these results, the new specimens are described as a new species: *C. chloeae* sp. nov. The role of introduced Nile tilapia as a potential reservoir for native parasites raises concern for potential spillbacks and stresses the need for further monitoring of monogeneans on native and introduced tilapias.
http://zoobank.org/urn:lsid:zoobank.org:DAF7DD13-6A31-4271-970B-A03DEEDD3938

Key words: *Cichlidogyrus*, Monogenea, Dactylogyridae, Lake Kariba, Zimbabwe, tilapia, *Oreochromis*

Introduction

Monogenea is a taxon of parasitic platyhelminthes that dominates the external parasite fauna of bony fish (Cribb et al., 2002; Paladini et al., 2017; Pugachev et al., 2010). It is a group of small, hermaphrodite flatworms (ranging from ca. 100 μm to 4 cm) with a direct life cycle and with most species being host specific (Paladini et al., 2017; Řehulková et al., 2018; Rahmouni et al., 2022). Species identification is traditionally based on the morphology of the sclerotised parts of the posterior attachment organ, called (opist)haptor and the male copulatory organ (MCO) and vagina (e.g. Pariselle & Euzet 2009). Among African monogeneans, *Cichlidogyrus* Paperna, 1960 is the most speciose genus (Pariselle & Euzet, 2009; Řehulková et al., 2018) with 128 described species from a total of 117 African cichlid species (Cruz-Laufer et al., 2021a).

In recent years, the examination of more populations of *Cichlidogyrus* spp. has led to the (re)description of several species (Fannes et al., 2017; Gobbin et al., 2021; Igeh et al., 2017; Jorissen et al., 2018b; Pariselle et al., 2003). Additionally, several species of Monogenea, including species of *Cichlidogyrus*, have been reported to display intraspecific morphological variability correlated with host species and geographic distribution (Kmentová et al., 2018; Rahmouni et al., 2021). *Cichlidogyrus halli* (Price & Kirk, 1967) Price, 1968, for example, is a morphologically variable species having been redescribed several times in the past (El-Naggar & Khidr, 1985; Ergens, 1981) and incorporates several synonymised (sub)species such as *Cichlidogyrus tubicirrus magnus* Paperna & Thurston, 1969, treated by Dossou (1982) as *Cichlidogyrus magnus*. Moreover, several morphotypes (e.g. Jorissen et al., 2018a) and subspecies (e.g. Paperna, 1979) within this species have been proposed, though the conspecific status of these subspecies has been questioned (Douëllou, 1993; Jorissen et al., 2018a; Jorissen et al., 2022; Pouyaud et al., 2006). *Cichlidogyrus halli* is widespread in Africa mainly on oreochromine cichlids (Pariselle & Euzet, 2009). It has also been frequently co-introduced

outside of continental Africa as a result of anthropogenic translocations of tilapias, sometimes leading to transmissions towards native host species (Shinn et al., in press).

In the 1990s, Douëllou (1993) examined specimens of *C. halli* infecting cichlids in Lake Kariba (Zimbabwe) and found that their morphology deviates from the one in the original species description in having longer auricles. However, she refrained from describing them as a separate taxon (Douëllou, 1993). During a field expedition in 2019, specimens of '*C. halli*', morphologically similar to the specimens reported by Douëllou (1993) were found infecting several tilapia species present in Lake Kariba.[1]

Lake Kariba is a man-made lake created in 1958 by damming the fast flowing middle Zambezi River (Reeve, 1960). Only three tilapia species are indigenous in the middle Zambezi Basin: *Oreochromis mortimeri* (Trewavas, 1966), *Coptodon rendalli* (Boulenger, 1897), and *Tilapia sparrmanii* Smith, 1840 (Marshall, 1988; Skelton, 1993). Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), has been introduced for aquaculture purposes and has become the most dominant tilapia species in the lake (Froese & Pauly, 2021; Maulu & Musuka, 2018). In the present study, the re-evaluation of additional specimens of '*C. halli*', and the morphological and genetical comparison of these specimens with *C. halli*, have led to the description of a new species *Cichlidogyrus chloeae* sp. nov.

Material and methods

Collection, sample preparation and conservation

During a field expedition at Lake Kariba in October–November 2019, tilapias were purchased from local fishermen, who caught the fish in the lake by drift netting. These specimens belong

[1]The term 'tilapia' will be used in the present study to refer to a paraphyletic group of cichlids consisting of several haplotilapiine tribes, including commercially important genera, such as *Oreochromis* Günther 1889, *Tilapia* Smith 1840, *Coptodon* Gervais 1853, and *Sarotherodon* Rüppell 1852 (Dunz & Schliewen, 2013; Trewavas, 1982).

to three different species: *Oreochromis niloticus*, *O.* cf. *mortimeri* and *Coptodon rendalli*. Details about the sampling locations are found in Fig. 1 and Table 1. identification of the specimens resembling *O. mortimeri* (Trewavas, 1966) is uncertain as they show the enlarged jaws typical of *O. mossambicus* which could point towards hybridisation. Additionally, several specimens of *O. niloticus* were bought at two local fish farms, Lake Harvest and Nicholson Bream Farm, located nearby the lake (Fig. 1; Table 1). These fish were caught by seine netting. In cases in which fish were still alive, they were killed by severing the spinal cord. Fish were morphologically identified in the field. From each specimen, a fin clip was taken and stored in 99% (v/v) ethanol for later genetic identification. Fish gills from both gill chambers were dissected and stored in 99% (v/v) ethanol. In the laboratory the gills were exhaustively screened for monogeneans using a Nikon C-DS stereomicroscope and an entomological needle. Some monogeneans were mounted for morphological examination on a glass slide, fixed with lactophenol, and covered with a coverslip. Coverslips were sealed with kolophonium-lanoline wax. The remaining flatworms were stored in 99% (v/v) ethanol for genetic identification.

Fin clips were deposited in the ichthyology collection at the Royal Museum for Central Africa (RMCA) in Tervuren (Belgium) under the collection number RMCA 2022.007.P. Mounted parasite specimens were deposited in the invertebrate collection of the RMCA; the collection of the research group Zoology: Biodiversity and Toxicology at Hasselt University, Diepenbeek, Belgium (HU); and the Finnish Museum of Natural History, Helsinki, Finland (MZH) (see 'HOLOTYPE' and 'PARATYPE' in the Results section for details on repositories and accession numbers).

Microscopy and illustrations

Whole-mounted specimens were examined under a Leica DM2500 microscope using differential interference contrast (DIC). Species were identified to genus level following the

identification keys in Pariselle & Euzet (2009) and Řehulková et al. (2018) and to species level with the identification key in Pariselle & Euzet (2009). Throughout this paper, we follow the terminology, the method of measuring the different parts of the sclerites, and the numbering of the uncinuli as in Geraerts et al. (2020), which follows the numbering proposed by Euzet & Prost (1981). Species descriptions are focused on details of the sclerotised parts i.e. haptor (specifically dorsal and ventral bars, dorsal and ventral anchors, uncinuli), male copulatory organ (MCO) and vagina (if sclerotised). Additional measurements were taken for the ventral and dorsal bar to enable a morphological comparison with previous studies (Fig. 2). Measurements and photographs were taken with the Leica Application Suite X (LASX) software. Drawings were made freehand using a drawing tube at a magnification of 1000× (objective ×100 immersion, ocular ×10) and edited in Adobe Illustrator version 25.2.3. Drawings of the different sclerotised parts were based on multiple specimens in case not all structures were clearly visible in a single individual.

The diagnosis-based version of the phylogenetic species concept was adopted for the identification of the new species (Davis & Nixon, 1992). The phylogenetic species concept defines species as reproductively isolated groups of natural populations that originate through a speciation event and end with the next speciation or vanish through extinction (Wägele, 2005). The diagnosis-based version defines a group of specimens as a new species when they consistently differ from another group of specimens in at least one attribute (Davis & Nixon, 1992).

To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN) (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:DAF7DD13-6A31-4271-970B-A03DEEDD3938. The Life Science Identifier (LSID) for the new species is reported in the taxonomic summary.

Morphometric evaluation of interspecific variation

Because the new species closely resembles *C. halli*, the morphometric variation between specimens of the new species and *C. halli*, collected from *O. niloticus* from Lake Kariba and surrounding fish farms in the present study, was assessed by performing a Principal Component Analysis (PCA) in R version 4.1.0 (R Core Team, 2021). Plots were visualised with the R package *ggplot2* version 3.3.5 (Wickham, 2016). A first PCA was performed including the measurements on both the haptor and MCO. Because the haptor and MCO presumably evolve at a different evolutionary rate (Pouyaud et al., 2006), two additional PCAs were carried out, one including the measurements on the haptor, the other including measurements on the MCO.

DNA extraction, amplification, sequencing and alignment

In the genetic analyses, we focused on fragments of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene and the small subunit ribosomal DNA (*18S*) and internal transcribed spacer (ITS1) (later referred to as 18S-ITS1). For both gene fragments, specimens of *C. chloeae* sp. nov. infecting *O.* cf. *mortimeri* and *O. niloticus* were selected, as well as specimens of *C. halli* infecting *O. niloticus* and *Coptodon rendalli* (Table S1). Micrographs were taken from the sclerotised parts (MCO and haptor) with a Leica DM2500 microscope and the Leica Application Suite X (LASX) software and deposited as photo vouchers on MorphoBank under the link http://morphobank.org/permalink/?P4221. For DNA extraction, a modified salting-out protocol was followed (provided to us by C. Laumer). Specimens were digested by incubating them in a solution of TNES buffer (400 mM NaCl, 20 mM EDTA, 50 mM Tris pH 8, 0.5% SDS) and 20 mg/mL proteinase *K* at 55°C for one hour. DNA was precipitated by adding 5 M NaCl, 96% (v/v) ethanol, and yeast tRNA as a carrier, and subsequent stored at -20°C for at least one hour. The resulting pellet was purified by two rounds of centrifugation, removing the

supernatant and washing the pellet with 70% (v/v) chilled ethanol. The extracted DNA was eluted in 30 µl of 0.1x TE buffer with 0.02% Tween-20 and stored at -20 °C. Amplification was done by a Polymerase Chain Reaction (PCR) with a T100 thermal cycler (Bio-Rad) and BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Part of the *COI* gene was amplified and sequenced using the primer pair ASmit1 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and ASmit2 (5'-TAAAGAAAGAACATAATGAAAATG-3') (Littlewood et al., 1997). The PCR was performed in a reaction mix of 2.5 µL of 10x PCR buffer (Invitrogen), 1 µL of 50 mM $MgCl_2$ (Invitrogen), 0.5 µL of 10 mM dNTP mix (Biolegio), 2 µL of each primer (10 µM) (Biolegio), 0.2 µL of 5 U/µL Platinum™ *Taq* Polymerase (Invitrogen), 1 µL template DNA, and 15.8 µL Ultrapure™ DNAse/RNase-free distilled water (Invitrogen) to reach a total volume of 25 µL per reaction. Amplification was carried out under the following conditions: initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 94°C for 1 min, annealing for 1 min at 50°C, and elongation at 72°C for 1 min, and a final elongation at 72°C for 7 min. A nested PCR was performed when the yield of the amplicon was low with a first amplification round using the primer pair ASmit1 and Schisto_3 (5'-TCTTTRGATCATAAGCG-3') (Lockyer et al., 2003), following the same reaction mix concentrations and PCR conditions as described above, except for the annealing step, which was performed at 44°C. The second amplification round was performed by using the primer pair ASmit 1 and ASmit as described above, using the amplicon of the first round as template DNA.

The 18S-ITS1 fragment was amplified and sequenced using the primer pair S1 (5-ATTCCGATAACGAACGAGACT-3) (Matějusová et al., 2001) and IR8 (5-GCAGCTGCGTTCTTCATCGA-3) (Šimková et al., 2003). The reaction mix contained 3 µL of 10x PCR buffer, 0.9 µL of 50 mM $MgCl_2$, 0.6 µL of 10 mM dNTP mix, 1.5 µL of each primer (10 µM) (Biolegio), 0.2 µL of 5 U/µl Platinum™ *Taq* Polymerase, 5 µl template DNA,

and 17.3 µL Ultrapure™ DNAse/RNase-free distilled water to reach a total volume of 30 µL per reaction. The PCR was performed under the following conditions: initial denaturation at 94°C for 2 min, 40 cycles of denaturation at 94°C for 1 min, annealing for 1 min at 53°C, and elongation at 72°C for 1.5 min, and a final elongation at 72°C for 10 min. Amplicons were sequenced by Macrogen with bi-directional Sanger sequencing using a 3730xl DNA Analyzer, and the chromatograms of the resulting sequences were checked in Geneious Prime version 2021.2.2 for ends with low base call quality ends, which were manually trimmed. MUSCLE version 3.8.425 (Edgar, 2004) was used under default conditions to align forward and reverse reads, and the consensus sequence was extracted. Newly generated sequences were deposited in NCBI GenBank under accession numbers ON819294, ON819296, ON819306-7, ON819324-7 (18S-ITS1 fragment for *C. chloeae* sp. nov.), ON827384, ON827403-4 (COI fragment for *C. chloeae* sp. nov.), ON819300, ON819320, ON819341 (18S-ITS-1 fragment for *C. halli*), ON827382, ON827389, ON827400 (COI fragment for *C. halli*).

Sequence analyses

To investigate the phylogenetic position of *C. chloeae* sp. nov. within the *Cichlidogyrus-Scutogyrus* monophylum (in which *Scutogyrus* renders *Cichlidogyrus* paraphyletic), samples were supplemented with sequences of *C. halli* and other species of *Cichlidogyrus* and *Scutogyrus* from Jorissen et al. (2022), Cruz-Laufer et al. (2021b), and GenBank (**Table S1**).

For both gene fragments, sequences were aligned with MUSCLE under default conditions and overhanging ends were manually trimmed in Geneious Prime. For each gene fragment, the optimal molecular evolution model (GTR+I+G for both gene fragments) was selected based on the corrected Akaike Information Criterion (AICc) using jModelTest2 on the Cipres Science Gateway version 3.3 (Miller et al., 2010). A Bayesian phylogenetic tree was constructed in BEAST version 1.10.4 (Suchard et al., 2018) using a Markov Chain Monte Carlo

(MCMC) approach with the best fitting substitution model, a constant size coalescent tree prior (default), and a strict molecular clock model (default). All other operators and prior distributions were left at default settings. Five independent runs were performed from a random starting tree with one cold and one heated chain (deltaTemperature = 0.1) for 10000000 generations with a sampling frequency of 1000. The resulting log files were combined in Tracer version 1.7.2 (Rambaut et al., 2018) with a 50% burn-in to check for convergence in the trace plots. Tree files were combined with LogCombiner (implemented in BEAST) with a 50% burn-in. A Maximum Clade Credibility tree was inferred with default settings in TreeAnnotator (also implemented in BEAST). In addition, a Maximum Likelihood (ML) search was performed in MEGAX version 10.2.6 (Stecher et al., 2020) with 1000 bootstrap replicates using an extensive Subtree-Pruning-Regrafting (SPR level 5) method. Phylogenetic trees were visualised in FigTree version 1.4.4 (Rambaut, 2018). *Cichlidogyrus pouyaudi* Pariselle & Euzet, 1994 was used as an outgroup to root the phylogenetic trees based on the 18S-ITS1 fragment because of the basal position of this species in the phylogenetic tree of the *Cichlidogyrus-Scutogyrus* monophylum (Mendlová et al., 2012; Messu Mandeng et al., 2015). The *COI* fragment of *C. pouyaudi* is not available on GenBank. Therefore, the phylogenetic tree based on this gene fragment was rooted with *Cichlidogyrus falcifer* Dossou & Birgi, 1984, the *COI* fragment of which is available, because this species falls in a different clade than specimens of *C. halli* and *C. chloeae* sp. nov. in the phylogenetic tree based on the 18S-ITS1 fragment (see Results).

The intraspecific genetic distances between specimens of *C. chloeae* sp. nov. and the interspecific genetic distances between specimens of *C. halli* and *C. chloeae* sp. nov. were calculated in MEGAX using the Kimura-2-parameter (K2P) distance model (Kimura, 1980) based on both the *COI* and 18S-ITS1 fragment, supplementing our dataset with GenBank sequences where available (Table S1).

Results

A total of 27 fish specimens of *O.* cf. *mortimeri* and 29 specimens of *Coptodon rendalli* were caught in Lake Kariba. Additionally, 58 specimens of *O. niloticus* were collected: 27 from aquaculture facilities (Lake Harvest and Nicholson Bream Farm) and 31 from Lake Kariba. On *O.* cf. *mortimeri*, a total of 63 specimens of *C. chloeae* sp. nov. was found, while no specimen of *C. halli* was detected. On *Coptodon rendalli*, three specimens of *C. chloeae* sp. nov. and one specimen of *C. halli* were found. On *O. niloticus,* a total of 40 specimens of *C. chloeae* sp. nov. was found on feral fish from the lake, while none were found on farmed fish. A total of 16 specimens of *C. halli* were found on feral *O. niloticus*, and 203 specimens on farmed fish. An overview of these results is given in **Table 2**. Apart from specimens of *C. halli* and *C. chloeae* sp. nov., also specimens belonging to other species of Monogenea were found on the gills of these hosts (for detailed information see Geraerts et al. (2022b)).

The species description of *C. chloeae*sp. nov. is presented below, together with a morphological comparison with *C. halli*. For the measurements on the hard parts, 10 specimens of *C. chloeae* sp. nov. from *O.* cf. *mortimeri*, 18 specimens from *O. niloticus*, and three specimens from *Coptodon rendalli* were used. Additionally, measurements were taken from 21 specimens of *C. halli* from *O. niloticus* (20 specimens from farmed hosts and one from a feral host) that were available for morphological analyses. Measurements on both species can be found in **Table 3**.

Taxonomy

Family Dactylogyridae Bychowski, 1933

Genus *Cichlidogyrus* Paperna, 1960

Cichlidogyrus chloeae Geraerts sp. nov.

HOLOTYPE: KN.28605.

PARATYPES: KN.28606–KN.28613, RMCA_VERMES_43649–RMCA_VERMES_43658, and UH nos. 826–837.

TYPE LOCALITY: Lake Kariba, Zimbabwe.

HABITAT: Gills

TYPE HOST: *Oreochromis niloticus* (Linnaeus 1758) (Perciformes: Cichlidae).

OTHER HOSTS: *Oreochromis* cf. *mortimeri* and *Coptodon rendalli* (Boulenger, 1897) (Perciformes: Cichlidae).

PREVALENCE AND INTENSITY: see **Table 4**.

ZOOBANK REGISTRATION: The Life Science Identifier (LSID) for *Cichlidogyrus chloeae* sp. nov. is urn:lsid:zoobank.org:act:EACD0A65-5035-4A6A-882F-69A1BC57C8CD.

ETYMOLOGY: Dedicated to the first author's best friend and support Chloë Vervoort.

AUTHORSHIP: Note that the author of the new taxon is different from the authors of this paper; Article 50.1 and Recommendation 50A of the International Code of Zoological Nomenclature (ICZN, 1999).

DIAGNOSIS: Species of *Cichlidogyrus* with small uncinuli I (length ±20 μm) and long uncinuli III to VII (length ±38 μm), large ventral anchors (total length ±54 μm) with an asymmetrical base (guard length ±22 μm, shaft length ±11 μm) and large dorsal anchors (total length ±51 μm) with an asymmetrical base (guard length ±27 μm, shaft length ±13 μm). Dorsal transverse bar with large auricles (length ±42 μm) and ventral bar with distinct wing-shaped attachments. Penis stylet tubular (length ±90 μm) and broad (maximum width ±6 μm) and accessory piece (axial length ±87 μm, maximum width ±8 μm) with a triangular shaped cap at distal end.

Description (Fig. 3; Fig. 4a–b)

[Based on 31 specimens; metrical data in **Table 3**]

HAPTOR: Anchors 2 pairs. Ventral anchors large with massive asymmetrical base; guard and shaft broad with guard approximately 2 times as long as shaft. Dorsal anchors of about same total length as ventral anchors; base asymmetrical with guard and shaft narrower than those of ventral anchor; guard approximately 2 times as long as shaft. Blades of both ventral and dorsal anchors arched. Ventral transverse bar V-shaped, with 2 long branches with distinct wing-shaped attachments along distal half. Dorsal transverse bar large and made up of thick midsection, tapering towards its extremities, and 2 pronounced auricles inserted at its dorsal face. Uncinuli 7 pairs; uncinuli I short with small round secondary shaft; uncinuli III to VII long; uncinuli III on average shorter than uncinuli IV–VII (Uncinuli are called short or long based on their standardised length i.e. the division of their total length by the total length of the second uncinuli, which retain their larval size (Pariselle & Euzet, 2009)).

MALE GENITALIA: MCO consisting of a long penis stylet, accessory piece and heel. Penis stylet broad and tubular with constant width along its length and an enlarged proximal irregularly shaped basal bulb. Accessory piece about the same axial length as penis stylet and proximally connected to base of stylet; broader than penis stylet with an elongated triangular shaped cap at distal end. Heel pronounced and attached to base of penis stylet, narrower than basal bulb of penis stylet.

FEMALE GENITALIA: No sclerotised vagina visible.

Morphological discussion and morphometric evaluation of interspecific variation between *C. chloeae* sp. nov. and *C. halli*

The described species is classified as belonging to *Cichlidogyrus* because it shows all diagnostic features of the genus: two pairs of anchors (one dorsal and one ventral), two transverse bars

(ventral transverse bar V-shaped, dorsal transverse bar with two auricles, 14 uncinuli, and an MCO consisting of a penis stylet and, most often, an accessory piece (Paperna, 1960; Pariselle & Euzet, 2009; Vanhove et al., 2011). It belongs to the group of species of *Cichlidogyrus* with small uncinuli I and long uncinuli III to VII (Pariselle & Euzet, 2009). Based on the morphology of the sclerotised structures, *C. chloeae* sp. nov. resembles *C. halli*: both species have small uncinuli I and long uncinuli III to VII, large anchors with an asymmetrical base, a broad tubular stylet and a triangular shaped cap at the distal end of the accessory piece. However, *C. chloeae* sp. nov. differs from *C. halli* in the length of the auricles, which are almost twice as long in *C. chloeae* sp. nov. Also, the penis stylet is slightly wider, the accessory piece longer with more elongated and less pronounced triangular cap, and the heel narrower in *C. chloeae* sp. nov. compared to *C. halli,* in which the heel engulfs the entire basal bulb of the penis stylet (**Fig. 3**; **Fig. 4**).

In the PCA including measurements on both haptor and MCO, the first three principal components explain respectively 42.7%, 21.1% and 9.7% of the variation (**Fig. 5a**; **Fig. S1a**). The linear (*Ap*) and axial length of the accessory piece (*Apl*), and the length of the auricles of the dorsal transverse bar (*DBh*) have the highest contribution to PC1 as well as to PC2. The first three principal components of the PCA including only measurements on the haptor explain respectively 39.5%, 22.9% and 8.7% of the variation (**Fig. 5b**; **Fig. S1b**). The total length of the dorsal transverse bar (*DBx*), the length of the auricles of the dorsal transverse bar (*DBh*) and the length of the branches of the ventral bar (*VBx*) contribute most to PC1, while the total length of the dorsal transverse bar (*DBx*), the length of the auricles of the dorsal transverse bar (*DBh*) and the length of the secondary shaft of uncinuli III (*IIIus*) contribute most to PC2. Finally, in the PCA including only measurements on the MCO, the first three principal components explain 73.8%, 10.3%, and 7.6% of the variation, respectively (**Fig. 5c**; **Fig. S1c**). In this PCA, the linear (*Ap*) and axial length (*Apl*) of the accessory piece, and the length of the penis stylet

contribute most to both PC1 and PC2. Each biplot shows two clusters: one including specimens of *C. chloeae* sp. nov., the other including specimens of *C. halli*. The measurement contributing to this clustering (i.e. pointing in the direction perpendicular to the clusters) is the length of the auricles of the dorsal transverse bar (DBh) and the length of the accessory piece (**Fig. 5**).

Phylogenetic position of *C. chloeae* sp. nov. within the *Cichlidogyrus-Scutogyrus* monophylum

In the Bayesian phylogenetic tree inferred from the 18S-ITS1 fragment, specimens of *C. chloeae* sp. nov. fall in a well-supported monophyletic clade together with specimens of *C. halli*, with the '*C. chloeae* sp. nov.' clade being the sister group of part of the '*C. halli'* clade, save its Nilo-Sudanic (from Egypt and Senegal) and Upper Guinean representatives (from Ivory Coast) (**Fig. 6**). The same sister group relationship is suggested by the topology of the ML phylogenetic tree inferred from the same gene fragment albeit with weaker support (bootstrap value <0.85) (**Fig. S2**). Also, in the Bayesian (**Fig. 6**) and ML phylogenetic tree (**Fig. S2**) inferred from the *COI* fragment, specimens of *C. chloeae* sp. nov. fall in the same clade as specimens of *C. halli* with one specimen of *C. halli* (MG970255.1) forming the sister group of the clade including the specimens of *C. chloeae* sp. nov., though with low support values (bootstrap value <0.85). The barcoding gap between the intraspecific genetic distances of specimens of *C. chloeae* sp. nov. and the intraspecific distances between specimens of *C. chloeae* sp. nov. and *C. halli* ranged from 0.131 to 0.161 based on the *COI* fragment, and from 0 to 0.028 based on the 18S-ITS1 fragment.

Discussion

In the present study we describe *C. chloeae* sp. nov. and make a morphological and morphometric comparison of *C. chloeae* sp. nov. with *C. halli*. Based on the measurements and

drawings, *C. chloeae* sp. nov. can easily be distinguished from *C. halli* by longer auricles of the dorsal transverse bar and a longer accessory piece, a slightly wider penis stylet, a narrower heel, and a triangular cap of the accessory piece being more elongated and less pronounced in *C. chloeae* sp. nov. Therefore, it is clear that *C. chloeae* sp. nov. is a previously undescribed species.

C. chloeae sp. nov. and *C. halli* are closely related, falling in the same monophyletic clade based on both the nuclear and mitochondrial gene fragment. The sequences from Ivory Coast strongly cluster together forming an early diverging Upper Guinean lineage of *C. halli*. These findings add to the growing evidence of the presence of a '*C. halli* complex', encompassing several (undescribed) species as proposed by Jorissen et al. (2018b), Jorissen et al. (2022) and Geraerts et al. (2022a). A barcoding gap between 13 and 16% is found based on the *COI* fragment which is in accordance to the findings of Jorissen et al. (2022) (barcoding gap at 15% for the *COI* gene) and Geraerts et al. (2022a). The gap we found between the intra- and interspecific genetic distances based on the 18S-ITS1 fragment is much smaller (between 0 and 3%), which is consistent with the findings of Jorissen et al. (2022) and Geraerts et al. (2022b).

Previous mix-up of *C. chloeae* sp. nov. with *C. halli*?

The sole study collecting gill parasites of cichlids in Lake Kariba was carried out in the 1990s (Douëllou, 1993). The species of *Cichlidogyrus* found on *O. mortimeri* were *C. halli*, C. *dossoui* Douëllou, 1993, *C. karibae* Douëllou, 1993*, C. tilapiae* Paperna, 1960, *C. sclerosus* Paperna & Thurston, 1969*,* and *C. zambezensis* Douëllou, 1993. The species of *Cichlidogyrus* found on *Coptodon rendalli* were *C. dossoui*, *C. quaestio* Douëllou, 1993, and *C. tiberianus* Paperna, 1960. In Douëllou's research, the morphometrics of specimens of *C. halli* were compared with

those made by Price & Kirk (1967) in the original species description. She already reported a difference in the auricle length between the specimens of *C. halli* found in her study and those from the original description (Douëllou, 1993), but did not recognise it as a new species. In the present study, the auricle length of *C. chloeae* sp. nov. overlaps with '*C. halli*' found in the study of Douëllou (1993), while the auricle length of *C. halli* found in our study overlaps with that of *C. halli* described in the original species description by Price & Kirk (1967) (**Table 5**). We are, therefore, convinced of the fact that the specimens of '*C. halli*', found by Douëllou (1993), are actually specimens of *C. chloeae* sp. nov. This hypothesis is further supported by the fact that *C. halli* is abundant on farmed *O. niloticus*, but is not found on *O.* cf. *mortimeri* in the present study. Furthermore, *C. chloeae* sp. nov. is found on *O.* cf. *mortimeri* and feral *O. niloticus*, but not on farmed *O. niloticus* (**Table 2**).

Feral Nile tilapia as reservoir for C. *chloeae* sp. nov.

The absence of *C. chloeae* sp. nov. on farmed *O. niloticus* and its presence on feral *O. niloticus* suggests a host-switch from *O.* cf. *mortimeri* to *O. niloticus*. The few specimens of *C. chloeae* sp. nov. that were found on *Coptodon rendalli* also suggest a host switch from *O.* cf. *mortimeri*, as it was not yet detected on *Coptodon rendalli* by earlier research. Indeed, Douëllou (1993) did not find '*C. halli'* (now *C. chloeae* sp. nov.) on *Coptodon rendalli* either.

Invasion ecology often focuses on spillover of introduced parasites to native hosts, though infection of non-indigenous hosts by native parasites can also pose a potential threat to native species. Non-indigenous hosts can act as a new reservoir for native parasites, providing an additional habitat in which the parasite can persist and reproduce. Ultimately, this can lead to an expansion of the parasite population and potentially increase the prevalence and intensity of this parasite on its native host by spillback (Goedknegt et al., 2016; Kelly et al., 2009; Poulin et al., 2011).

Species of *Cichlidogyrus* are regarded as being highly host specific. However, *C. chloeae* sp. nov. is classified as an intermediate generalist (using the terminology proposed by Mendlová & Šimková 2014), infecting non-congeneric cichlids of different tribes, i.e. Oreochromini and Coptodonini. This, together with their one-host life cycle (Řehulková et al., 2018) and the fact that different species of tilapia are morphologically and ecologically similar (Vignon et al., 2011), could facilitate host switches between introduced and native tilapias in Lake Kariba.

Acknowledgements

We appreciate the help of the people involved in the field work and sampling procedure, especially Maxwell Barson (University of Zimbabwe/University of Botswana) and Shandré van der Merwe (Royal Museum for Central Africa/KU Leuven, Belgium). Furthermore, we would like to thank Christopher Laumer (EMBL-European Bioinformatics Institute, UK) and Gontran Sonet (Royal Belgian Institute of Natural Sciences, Belgium) for providing us with the extraction protocol and R scripts, respectively. Finally, we would like to thank Ria Vanderspikken (Hasselt University, Belgium) and Natascha Steffanie (Hasselt University, Belgium) for their technical support in the laboratory, and Timo Pajunen (Finnish Museum of Natural History, University of Helsinki, Finland) and Christophe Allard (Royal Museum for Central Africa, Belgium) for curatorial services.

Competing interests

The authors report there are no competing interests to declare.

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Funding

This work was supported by the Special Research Fund of Hasselt University (Grants BOF17NI02 and BOF20TT06); the Research Foundation—Flanders (FWO-Vlaanderen; Grant N 1513419). The research leading to results presented in this publication was carried out with infrastructure funded by EMBRC Belgium—FWO project GOH3817N.

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Associate Editor: Dr Peter D. Olson

Tables

Table 1. Sampling locations in Lake Kariba and in farms near Lake Kariba with the location label, details of the sampling location, and coordinates. Location labels correspond to the ones in **Fig. 1**.

Location label	Details	Longitude	Latitude
1	Nicholson Bream Farm	−16.5304	28.8608
2	Lake Harvest Aquaculture	−16.5259	28.8524
3	Lake Kariba at discharge channel of crocodile farm	−16.5495	28.8616
4	Green Water in Lake Kariba	−16.5386	28.8500
5	Fishermen village Gache Gache Cooperation at Lake Kariba	−16.5956	28.9198
6	Gatche River Bay in Lake Kariba	−16.6437	28.9369
7	Gatche River Bay in Lake Kariba	−16.6502	28.9290
8	Gatche River Bay in Lake Kariba	−16.6672	28.8809
9	Open water in Lake Kariba	−16.6643	28.8758
10	Gatche River Bay in Lake Kariba	−16.7088	28.9121
11	Open water in Lake Kariba	−16.7051	28.8291
12	Fishermen at Gache Gache	−16.7680	28.8599
13	Sayati Gorge	−16.8248	28.7592

Table 2. Number of specimens of *C. halli* and *C. chloeae* sp. nov. found on the fish species studied; number of fish specimens (n) between brackets.

Parasite species	***O.* cf. *mortimeri* (n = 27)**	***Coptodon rendalli* (Boulenger, 1897) (n = 29)**	***Oreochromis niloticus* (Linnaeus, 1758) (n = 58)**	
			Farmed (n = 27)	**Feral (n = 31)**
C. chloeae sp. nov	63	3	/	40
C. halli	/	1	203	16

Table 3. Measurements (in µm) on *C. halli* and *C. chloeae* sp. nov. Note: Measurements are given as the mean followed by the range and number of measured specimens (n) in parentheses.

Species	***C. halli***	***C. chloeae* sp. nov.**	***C. chloeae* sp. nov.**	***C. chloeae* sp. nov.**	***C. chloeae* sp. nov.**
Host	***Oreochromis niloticus* (Linnaeus, 1758)**	***Oreochromis* cf. *mortimeri***	***O. niloticus* (Linnaeus, 1758)**	***Coptodon rendalli* (Boulenger, 1897)**	***O. niloticus, O.* cf. *mortimeri*, and *C. rendalli***
Locality	Lake Kariba and surrounding fish farms	Lake Kariba	Lake Kariba	Lake Kariba	Lake Kariba
Number of specimens	n = 21	n = 10	n = 18	n = 3	n = 31
Ventral anchor					
Total length, *a*	52.56 (47.80–58.59, n = 21)	53.61 (51.09–58.54, n = 10)	53.76 (51.32–59.39, n = 16)	54.99 (53.17–56.29, n = 3)	53.84 (51.09–59.39, n = 29)
Blade length, *b*	43.19 (39.12–47.99, n = 21)	41.15 (37.39–43.33, n = 10)	41.81 (38.46–44.86, n = 16)	41.17 (40.69–41.84, n = 3)	41.52 (37.39–44.86, n = 29)
Shaft length, *c*	9.37 (6.42–15.27, n = 21)	10.88 (9.01–13.02, n = 10)	11.18 (8.99–14.38, n = 17)	9.57 (9.56–9.57, n = 2)	10.96 (8.99–14.38, n = 29)
Guard length, *d*	19.02 (15.10–24.98, n = 21)	21.72 (18.36–25.81, n = 10)	22.18 (19.45–25.83, n = 17)	19.63 (17.11–20.96, n = 3)	21.77 (17.11–25.83, n = 30)
Point length, *e*	14.06 (10.43–17.26, n = 21)	15.18 (14.03–16.95, n = 10)	15.35 (12.05–20.40, n = 17)	15.69 (15.24–16.31, n = 3)	15.33 (12.05–20.40, n = 30)

Dorsal anchor					
Total length, *a*	46.94 (39.27–50.66, n = 17)	50.25 (48.20–52.39, n = 6)	51.31 (46.30–56.57, n = 16)	46.05 (46.05–46.05, n = 1)	50.80 (46.05–56.57, n = 23)
Blade length, *b*	32.36 (28.68–38.26, n = 17)	28.64 (27.24–30.30, n = 6)	29.58 (26.79–31.84, n = 15)	25.99 (25.99–25.99, n = 1)	29.16 (25.99–31.84, n = 22)
Shaft length, *c*	8.41 (5.93–11.04, n = 21)	13.20 (11.9–14.96, n = 9)	12.46 (9.33–15.90, n = 16)	11.93 (11.07–13.55, n = 3)	12.64 (9.33–15.90, n = 28)
Guard length, *d*	19.57 (13.72–27.35, n = 21)	26.49 (23.22–29.51, n = 9)	26.88 (22.34–31.52, n = 16)	25.23 (24.04–26.11, n = 3)	26.58 (22.34–31.52, n = 28)
Point length, *e*	10.72 (9.39–13.05, n = 17)	12.11 (10.58–13.89, n = 7)	12.02 (7.02–14.12, n = 17)	15.08 (15.08–15.08, n = 1)	12.17 (7.02–15.08, n = 25)
Ventral transverse bar					
Branch length, *x*	78.17 (59.57–94.13, n = 21)	83.15 (75.36–93.65, n = 10)	85.52 (73.89–108.50, n = 17)	76.11 (69.16–81.70, n = 3)	83,79 (69.16–108.50, n = 30)
Maximum width, *w*	12.68 (8.92–16.43, n = 21)	13.23 (10.26–16.99, n = 10)	12.90 (9.10–16.44, n = 17)	13.30 (12.29–14.71, n = 3)	13.05 (9.10–16.99, n = 30)
Branch length as in Douëllou (1993), *V*	76.36 (62.44–91.11, n = 15)	79.48 (71.15–88.79, n = 9)	81.42 (66.99–102.69, n = 12)	71.46 (68.51–74.41, n = 2)	79.79 (66.99–102.69, n = 23)
Dorsal transverse bar					

Total length, *x*	78.23 (61.57–107.64, n = 21)	91.50 (74.25–104.21, n = 10)	85.33 (64.33–112.94, n = 16)	72.59 (69.06–77.47, n = 3)	86.14 (64.33–112.94, n = 29)
Distance between auricles, *y*	13.11 (7.98–17.84, n = 21)	17.93 (16.37–20.33, n = 10)	16.81 (13.72–20.33, n = 16)	19.35 (19.25–19.53, n = 3)	17.46 (13.72–20.33, n = 29)
Maximum width, *w*	31.74 (16.13–47.85, n = 21)	38.42 (31.47–42.34, n = 10)	36.96 (28.27–51.67, n = 16)	31.24 (27.72–33.64, n = 3)	36.87 (27.72–51.67, n = 29)
Auricle length, *h*	23.43 (17.85–29.06, n = 21)	40.67 (35.75–47.71, n = 8)	42.98 (32.25–53.50, n = 17)	39.83 (37.59–43.81, n = 3)	41.98 (32.25–53.50, n = 28)
Auricle length as in Douëllou (1993), *l*	15.61 (8.62–24.22, n = 20)	28.55 (23.68–36.33, n = 8)	31.73 (24.09–39.62, n = 15)	26.37 (23.50–28.38, n = 3)	30.14 (23.50–39.62, n = 26)
Distance between auricles as in Douëllou (1993), *d*	23.32 (12.64–34.25, n = 20)	28.29 (23.23–38.98, n = 10)	25.56 (17.38–43.15, n = 16)	19.98 (18.21–22.50, n = 3)	25.93 (17.38–43.15, n = 29)
Uncinuli					
Length I, U_I	18.68 (14.98–25.08, n = 21)	20.17 (19.05–21.88, n = 10)	20.69 (17.11–24.12, n = 17)	19.36 (18.37–20.17, n = 3)	20.38 (17.11–24.12, n = 30)
Maximum width I, *Uw*	4.11 (3.09–5.69, n = 21)	2.71 (2.31–3.26, n = 10)	2.63 (2.05–3.24, n = 17)	3.02 (2.76–3.40, n = 3)	2.69 (2.05–3.40, n = 30)
Length II, U_{II}	15.26 (13.53–18.82, n = 20)	17.85 (15.37–19.63, n = 10)	18.37 (16.21–22.41, n = 17)	16.92 (15.31–18.03, n = 3)	18.05 (15.31–22.41, n = 30)

Length III, U_{III}	31.58 (18.89–37.74, n = 19)	31.25 (25.38–36.66, n = 10)	31.91 (28.08–39.34, n = 16)	30.52 (28.99–32.40, n = 3)	31.54 (25.38–39.34, n = 29)
Length IV, U_{IV}	39.78 (33.02–47.74, n = 21)	39.16 (35.22–45.42, n = 10)	38.80 (35.71–43.16, n = 16)	38.41 (33.43–43.00, n = 3)	38.88 (33.43–45.42, n = 29)
Length V, U_V	40.08 (26.65–46.43, n = 20)	41.58 (36.83–49.48, n = 10)	41.38 (36.14–52.41, n = 17)	38.29 (36.18–40.73, n = 3)	41.14 (36.14–52.41, n = 30)
Length VI, U_{VI}	39.10 (33.77–51.11, n = 21)	37.46 (32.52–42.99, n = 8)	39.07 (34.44–46.93, n = 17)	37.85 (36.39–39.31, n = 2)	38.50 (32.52–46.93, n = 27)
Length VII, U_{VII}	37.93 (33.26–48.06, n = 21)	36.50 (33.07–39.44, n = 10)	38.14 (34.47–44.95, n = 16)	36.44 (35.73–37.15, n = 2)	37.43 (33.07–44.95, n = 28)
Length III–VII	37.79 (18.89–51.11, n = 102)	37.18 (25.38–49.48, n = 48)	37.91 (28.08–52.41, n = 82)	37.64 (25.38–52.41, n = 130)	37.64 (25.38–52.41, n = 130)
Length secondary shaft I, Us_I	4.89 (3.77–7.05, n = 21)	3.74 (2.81–5.35, n = 10)	3.59 (2.69–5.32, n = 17)	3.67 (2.90–4.69, n = 3)	3.65 (2.69–5.35, n = 30)
Length secondary shaft III, Us_{III}	18.99 (14.31–24.19, n = 19)	12.98 (10.04–19.50, n = 10)	13.23 (9.76–18.85, n = 17)	12.42 (9.98–16.13, n = 3)	13.07 (9.76–19.50, n = 30)
Length secondary shaft IV, Us_{IV}	25.24 (18.94–29.33, n = 20)	20.97 (16.74–25.61, n = 10)	19.85 (16.75–25.64, n = 16)	19.80 (15.87–21.84, n = 3)	20.23 (15.87–25.64, n = 29)
Length secondary shaft V, Us_V	26.29 (14.48–31.29, n = 20)	23.36 (19.48–31.41, n = 10)	22.75 (19.58–32.52, n = 17)	19.59 (18.38–20.57, n = 3)	22.64 (18.38–32.52, n = 30)

Length secondary shaft VI, *Us*$_{VI}$	24.66 (16.04–33.31, n = 21)	20.69 (15.14–26.08, n = 9)	20.20 (13.81–26.58, n = 17)	16.95 (14.83–18.50, n = 3)	20.01 (13.81–26.58, n = 29)
Length secondary shaft VII, *Us*$_{VII}$	23.77 (19.33–31.96, n = 21)	19.11 (16.67–22.59, n = 10)	20.48 (16.48–25.71, n = 17)	17.35 (14.66–19.59, n = 3)	19.71 (14.66–25.71, n = 30)
Length secondary shaft III–VII	23.75 (14.31–33.31, n = 101)	19.39 (10.04–31.41, n = 49)	19.30 (9.76–32.52, n = 84)	19.33 (9.76–32.52, n = 133)	19.33 (9.76–32.52, n = 133)
MCO					
Length stylet, *Stl*	86.67 (71.54–99.58, n = 19)	88.15 (84.25–90.97, n = 9)	91.04 (84.64–103.84, n = 18)	89.17 (86.57–92.12, n = 3)	89.99 (84.25–103.84, n = 30)
Maximum width stylet, *Stw*	8.52 (7.31–9.79, n = 21)	6.16 (5.15–7.88, n = 10)	6.36 (5.38–7.17, n = 18)	6.89 (6.74–7.19, n = 3)	6.34 (5.15–7.88, n = 31)
Linear length accessory piece, *Ap*	60.11 (44.63–71.46, n = 21)	68.56 (59.42–74.20, n = 10)	74.38 (61.30–89.54, n = 18)	58.75 (53.39–64.11, n = 2)	71.39 (53.39–89.54, n = 30)
Axial length accessory piece, *Apl*	71.20 (59.70–79.94, n = 21)	85.52 (79.09–95.00, n = 10)	88.60 (80.26–104.37, n = 18)	79.49 (79.11–79.86, n = 2)	86.76 (79.09–104.37, n = 30)
Maximum width accessory piece, *Apw*	8.41 (7.61–10.57, n = 21)	7.98 (6.60–8.99, n = 10)	8.26 (6.19–10.52, n = 18)	7.74 (6.80–8.53, n = 3)	8.12 (6.19–10.52, n = 31)
Length heel, *Hel*	29.83 (25.43–35.09, n = 21)	27.45 (22.53–31.36, n = 10)	28.91 (22.62–35.86, n = 18)	27.49 (26.62–29.16, n = 3)	28.30 (22.53–35.86, n = 31)

Partial length heel, *He*	7.62 (4.42–11.46, n = 21)	9.46 (5.66–11.78, n = 10)	9.46 (6.33–13.90, n = 18)	8.38 (6.35–11.10, n = 3)	9.35 (5.66–13.90, n = 31)
Width heel, *Hew*	15.13 (8.58–22.02, n = 21)	9.21 (5.77–13.96, n = 10)	9.34 (4.91–13.89, n = 18)	7.66 (7.22–7.98, n = 3)	9.14 (4.91–13.96, n = 31)
Body					
Length	696.07 (538.48–870.18, n = 17)	774.77 (637.26–916.25, n = 9)	807.73 (568.78–976.14, n = 15)	625.14 (606.28–645.42, n = 3)	776.46 (568.78–976.14, n = 27)
Width	294.07 (224.09–432.38, n = 19)	297.32 (257.35–322.38, n = 10)	331.76 (233.93–485.85, n = 18)	307.26 (283.05–323.95, n = 3)	318.28 (233.93–485.85, n = 31)

Table 4. Number of infected hosts and intensity as defined by Bush et al. (1997) of *C. chloeae* sp. nov. on the studied hosts with the intensity expressed as 'number of host specimens x number of parasites infecting these hosts'.

Infection parameters	***Oreochromis* cf. *mortimeri***	***Oreochromis niloticus* (Linnaeus, 1758)**	***Coptodon rendalli* (Boulenger, 1897)**
Number of hosts studied	27	58	56
Number of hosts infected	16	18	1
Intensity	1 x 1 2 x 4 6 x 3 3 x 2 3 x 1 1 x 6	6 x 2 12 x 1	1 x 3

Table 5. Measurements (in μm) on *C. halli* from Price & Kirk (1967), Douëllou (1993) and the present study, and measurements on *C. chloeae* sp. nov. from the present study.

Species	***C. halli***	***C. halli***	***C. halli***	***C. chloeae* sp. nov.**
Host	*Oreochromis shiranus* Boulenger, 1897	*Oreochromis* cf. *mortimeri*	*Oreochromis niloticus* (Linnaeus, 1758)	*O. niloticus, O. mortimeri, and C. rendalli*
Locality	Shire River, Malawi	Lake Kariba, Zimbabwe	Lake Kariba, Zimbabwe	Lake Kariba, Zimbabwe
Number of specimens	n = 8	n = 15	n = 21	n = 31
Reference	Price & Kirk, 1967	Douëllou, 1993	This study	This study
Ventral anchor				
Total length, *a*	54–62	49–60	52.56 (47.80–58.59, n = 21)	53.84 (51.09–59.39, n = 29)
Dorsal anchor				
Total length, *a*	53–60	42–56	46.94 (39.27–50.66, n = 17)	50.80 (46.05–56.57, n = 23)
Ventral transverse bar				
Branch length, *V*	104–122	104–144	76.36 (62.44–91.11, n = 15)	79.79 (66.99–102.69, n = 23)
Dorsal transverse bar				
Total length, *x*	68–79	51–73	78.23 (61.57–107.64, n = 21)	86.14 (64.33–112.94, n = 29)
Auricle length, *l*	14	20–25	15.61 (8.62–24.22, n = 20)	30.14 (23.50–39.62, n = 26)
Uncinuli				
Length I, U_1	20–22	17–20	18.68 (14.98–25.08, n = 21)	20.38 (17.11–24.12, n = 30)

Length II, U_{II}	20–22	16–18	15.26 (13.53–18.82, n = 20)	18.05 (15.31–22.41, n = 30)
Length III–VII	35–44	29–43	37.79 (18.89–51.11, n = 102)	37.64 (25.38–52.41, n = 130)
MCO				
Length stylet, *Stl*	82–86	66–96	86.67 (71.54–99.58, n = 19)	89.99 (84.25–103.84, n = 30)
Axial length accessory piece, *Apl*	61–67	54–66	71.20 (59.70–79.94, n = 21)	86.76 (79.09–104.37, n = 30)
Body				
Length	525–721	700–1400	696.07 (538.48–870.18, n = 17)	776.46 (568.78–976.14, n = 27)
Width	160–205	220–340	294.07 (224.09–432.38, n = 19)	318.28 (233.93–485.85, n = 31)

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Legends for figures

Fig. 1. Map of Zimbabwe on the left with the framed region expanded on the right. Red dots indicate different sampling localities. The numbers of the localities correspond with those in **Table 1.** At the bottom the three tilapia species that were collected in the present study.

Fig. 2. Additional measurements taken on **a** the ventral and **b** the dorsal transverse bar. Abbreviations: V, branch length ; l, auricle length; d, distance between auricles; all as in Douëllou (1993)

Fig. 3. Drawings of the sclerotised structures of *Cichlidogyrus chloeae* sp. nov. (top) and *C. halli* (bottom). Drawings of *C. chloeae* sp. nov. are based on two specimens: the holotype KN.28605 for the MCO, and paratype KN.28607 for the haptor. Drawings of *C. halli* are based on three specimens: voucher PZIM252 for the ventral bar and anchors, voucher PZIM292 for the dorsal bar, and voucher PZIM248 for the uncinuli and MCO. Abbreviations: I–VII, uncinuli; VA, ventral anchor (g, guard; s, shaft; b, blade); VB, ventral transverse bar; DA, dorsal anchor; DB, dorsal transverse bar (a, auricle); MCO, male copulatory organ with penis stylet (s) in white, and accessory piece (ap) and heel (h) in grey. Scale bar: 20 µm.

Fig. 4. Micrographs of the MCO and dorsal transverse bar of *C. chloeae* sp. nov. and *C. halli.* **a** MCO and **b** dorsal transverse bar of *C. chloeae* sp. nov. **c** MCO and **d** dorsal transverse bar of *C. halli*. The arrows in **a** and **c** indicate the penis stylet, the circle the

accessory piece, and the square the heel. Arrows in **b** and **d** indicate the auricles of the dorsal bar. Scale bar: 20 μm.

Fig. 5. Biplots of the PCAs plotting the first two principal components PC1 and PC2: **a** PCA based on all measurements, **b** measurements on the haptor only, and **c** measurements on the MCO only. Each dot represents one specimen. Different colours represent different species i.e. *C. halli* and *C. chloeae* **sp. nov.** Ellipses are drawn at a confidence interval of 0.95. The contribution of the different measurements to the principal components are shown by arrows.

Fig. 6. Bayesian phylogenetic trees of specimens of *Cichlidogyrus* and *Scutogyrus* inferred from the 18S-ITS1 fragment (left) and *COI* fragment (right). Only well supported nodes (bootstrap values ≥ 0.85) are indicated by support values (in red). Scale bar indicates number of substitutions per site. Specimens of *C. chloeae* sp. nov. framed in green, specimens of *C. halli* framed in orange. Parasite labels and GenBank accession numbers of the included specimens can be found in **Table S1**.

Figures

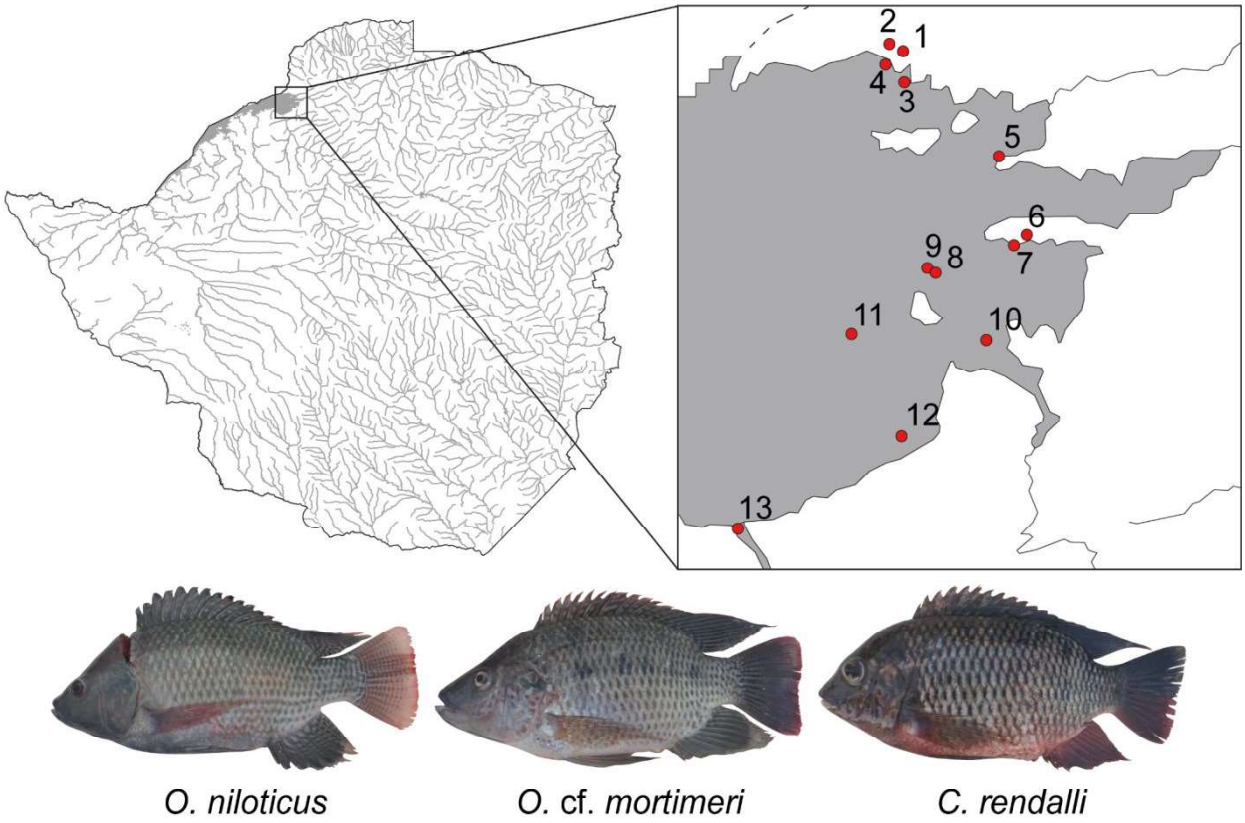


Fig. 1

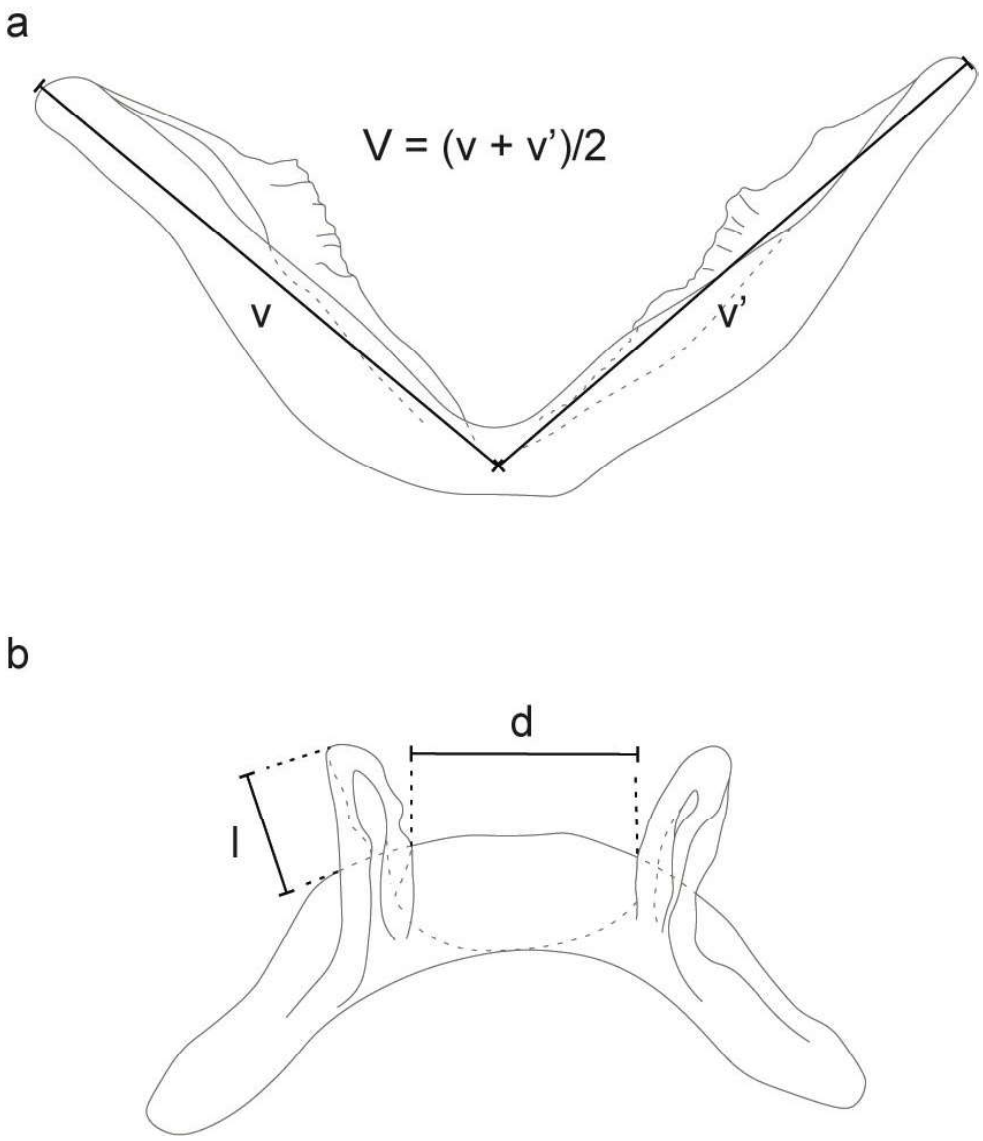


Fig. 2

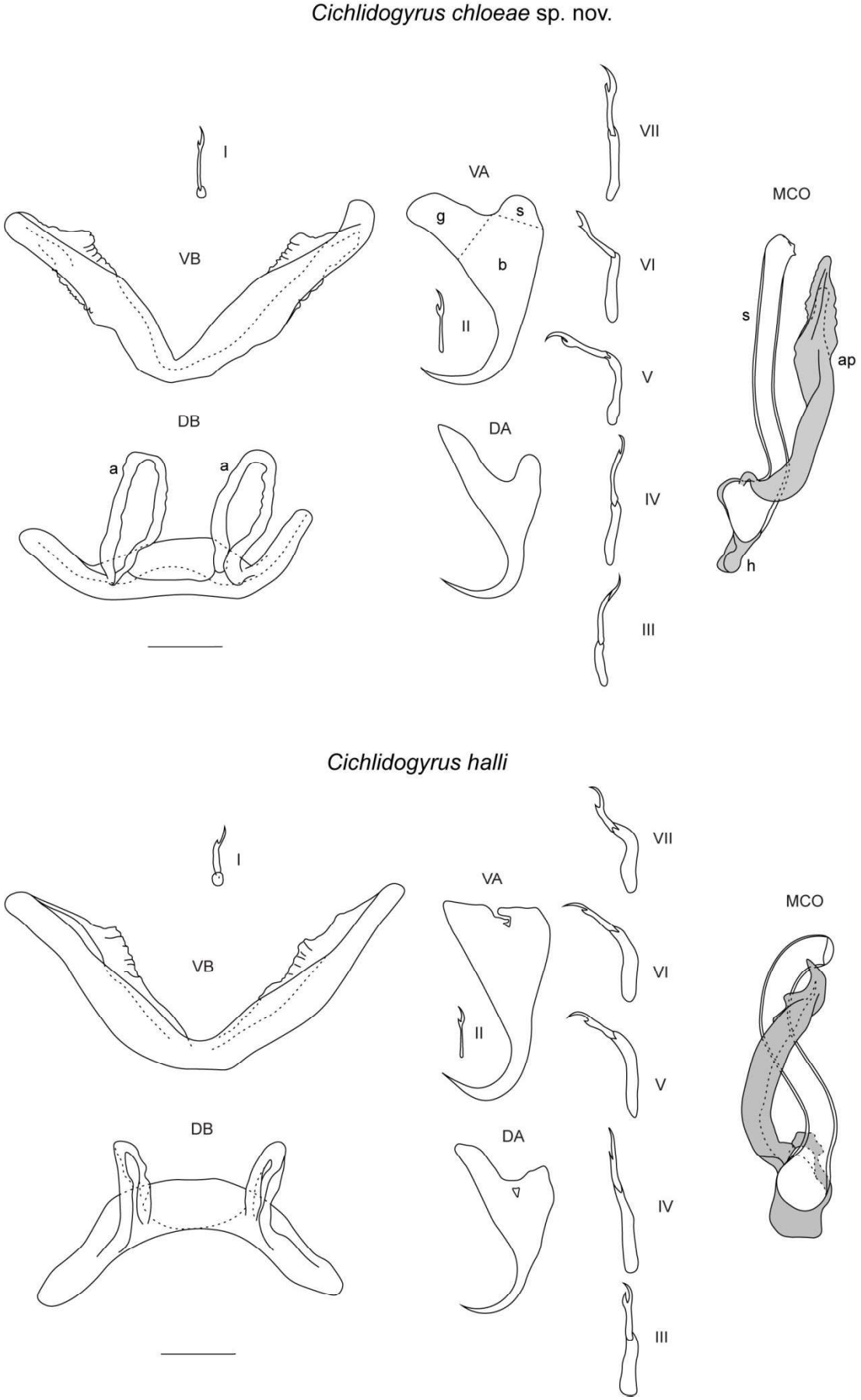

Cichlidogyrus chloeae sp. nov.
I
VB
VA
g
s
b
II
VII
VI
V
IV
III
MCO
s
ap
h
DB
a
a
DA
Cichlidogyrus halli
I
VB
VA
II
VII
VI
V
IV
III
MCO
DB
DA

Fig. 3

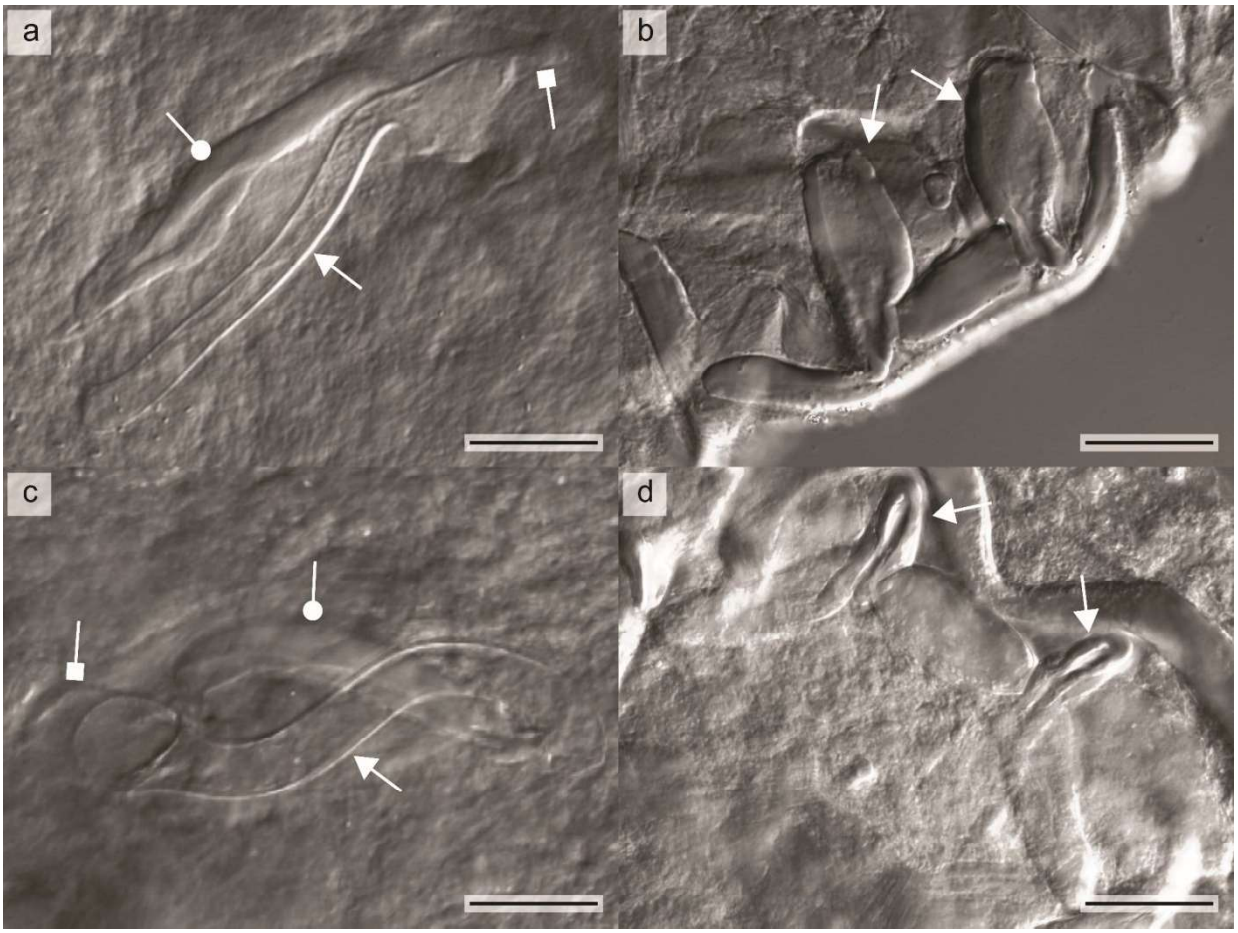


Fig. 4

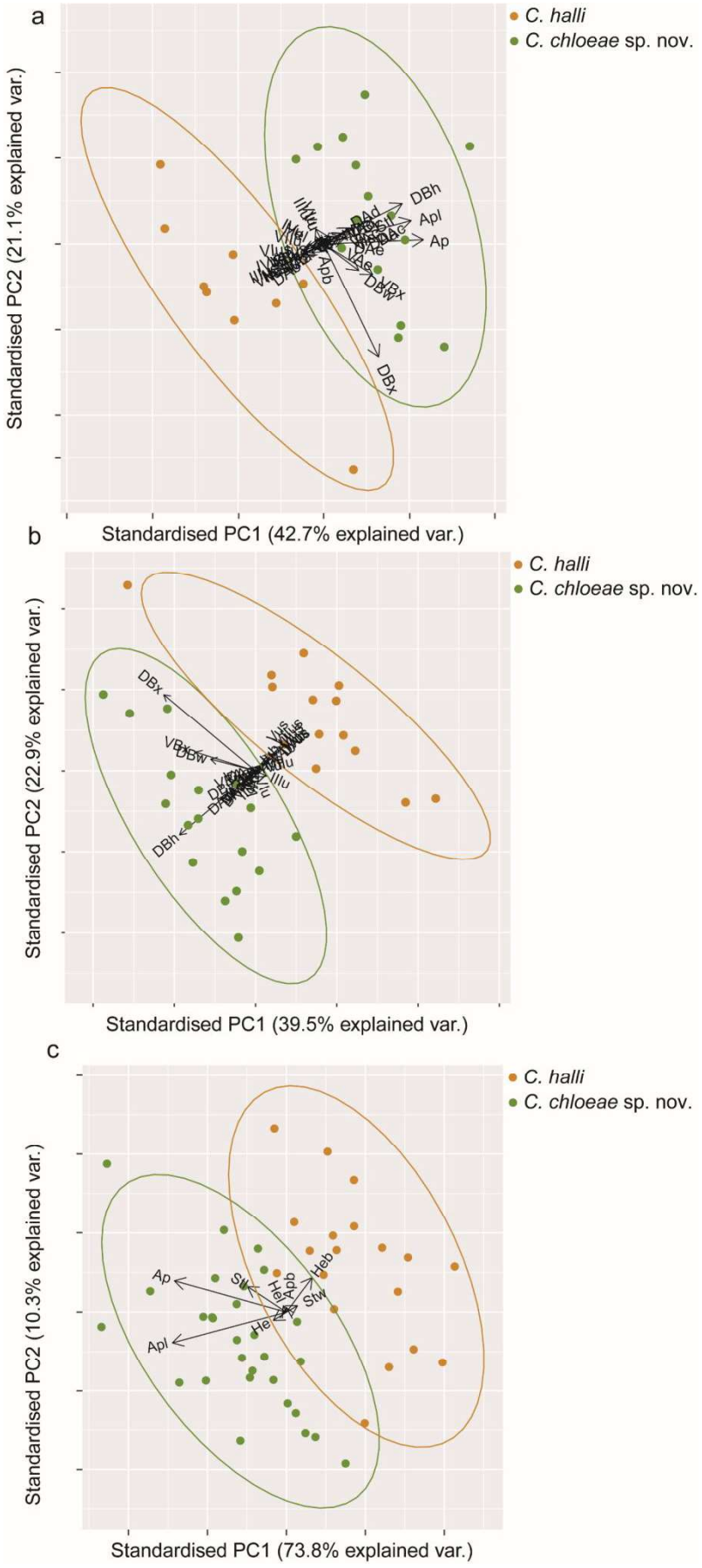
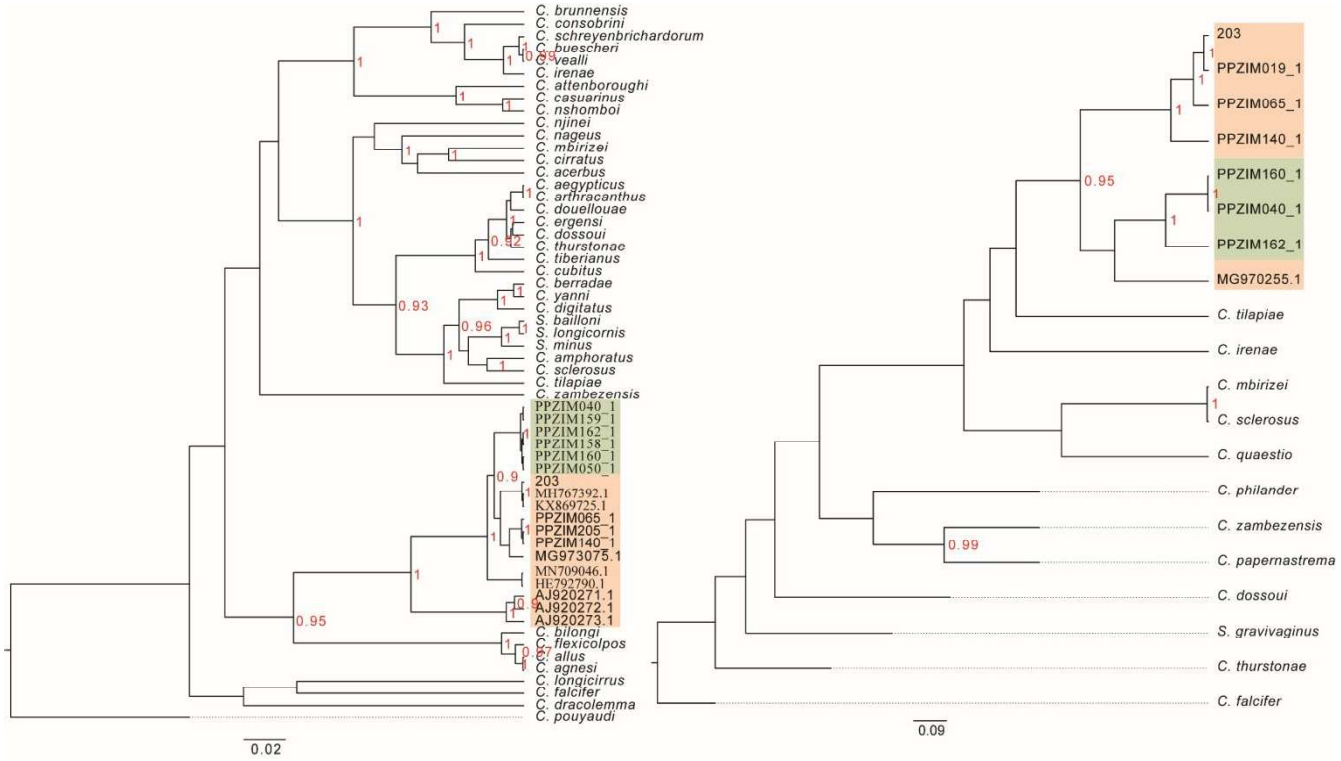

a
C. halli
C. chloeae sp. nov.
Standardised PC2 (21.1% explained var.)
Standardised PC1 (42.7% explained var.)
DBh
Apl
Ap
Apb
DBw
VBx
DBx
b
C. halli
C. chloeae sp. nov.
Standardised PC2 (22.9% explained var.)
Standardised PC1 (39.5% explained var.)
DBx
VBx
DBw
DBh
c
C. halli
C. chloeae sp. nov.
Standardised PC2 (10.3% explained var.)
Standardised PC1 (73.8% explained var.)
Ap
Sw
Heb
Apb
Stw
Apl

Fig. 5


C. brunnensis
C. consobrini
C. schreyenbrichardorum
C. buescheri
C. vealli
C. irenae
C. attenboroughi
C. casuarinus
C. nshomboi
C. njinei
C. nageus
C. mbirizei
C. cirratus
C. acerbus
C. aegypticus
C. arthracanthus
C. douellouae
C. ergensi
C. dossoui
C. thurstonae
C. tiberianus
C. cubitus
C. berradae
C. yanni
C. digitatus
S. bailloni
S. longicornis
S. minus
C. amphoratus
C. sclerosus
C. tilapiae
C. zambezensis
PPZIM040_1
PPZIM159_1
PPZIM162_1
PPZIM158_1
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AJ920272.1
AJ920273.1
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C. flexicolpos
C. allus
C. agnesi
C. longicirrus
C. falcifer
C. dracolemma
C. pouyaudi
0.02
203
PPZIM019_1
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PPZIM140_1
PPZIM160_1
PPZIM040_1
PPZIM162_1
MG970255.1
C. tilapiae
C. irenae
C. mbirizei
C. sclerosus
C. quaestio
C. philander
C. zambezensis
C. papernastrema
C. dossoui
S. gravivaginus
C. thurstonae
C. falcifer
0.09

Fig. 6

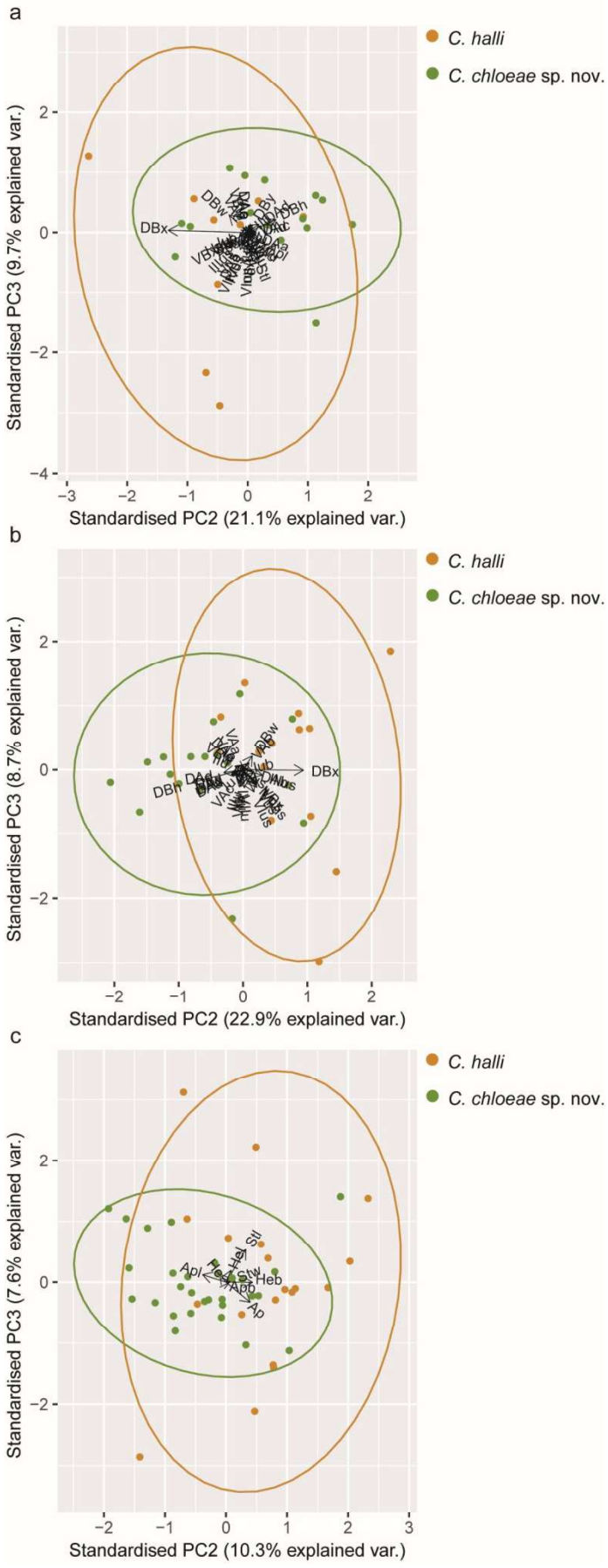
a
C. halli
C. chloeae sp. nov.
Standardised PC3 (9.7% explained var.)
Standardised PC2 (21.1% explained var.)
DBx
b
C. halli
C. chloeae sp. nov.
Standardised PC3 (8.7% explained var.)
Standardised PC2 (22.9% explained var.)
DBx
c
C. halli
C. chloeae sp. nov.
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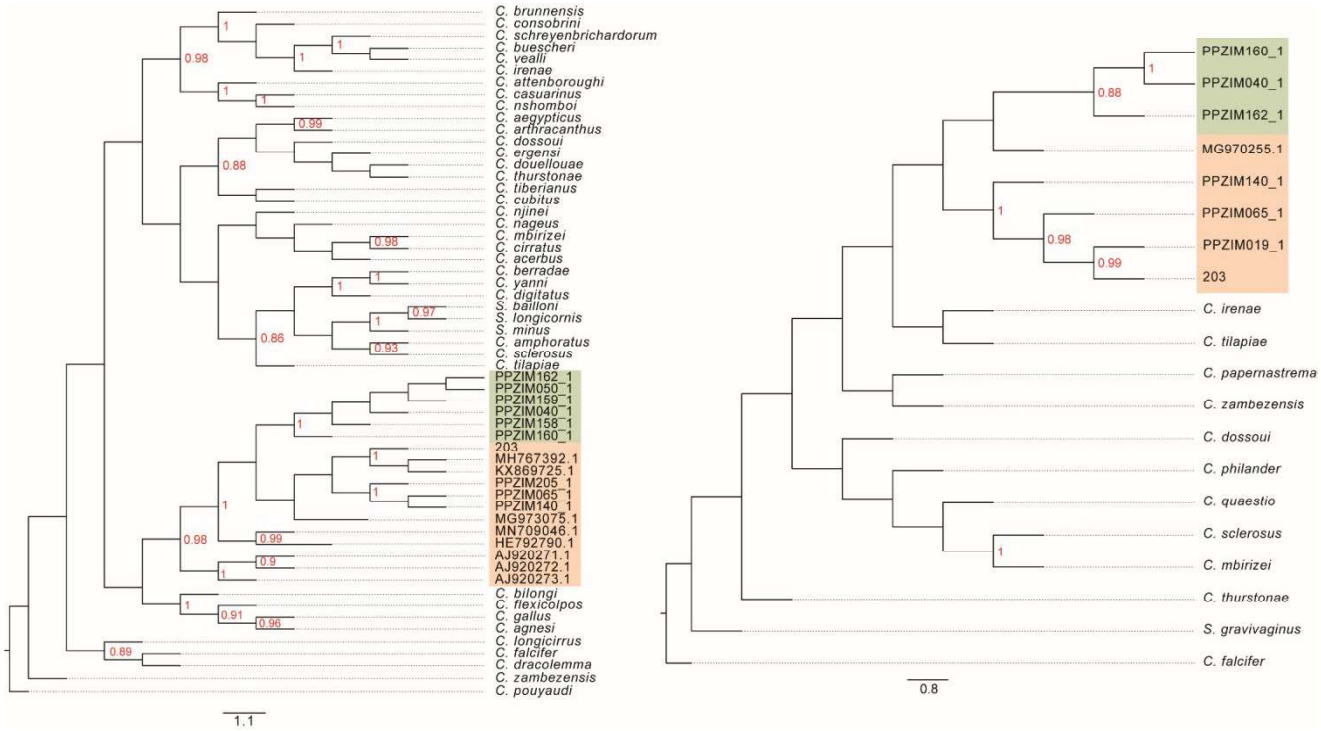
Fig. S1


C. brunnensis
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C. schreyenbrichardorum
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C. vealli
C. irenae
C. attenboroughi
C. casuarinus
C. nshomboi
C. aegypticus
C. arthracanthus
C. dossoui
C. ergensi
C. douellouae
C. thurstonae
C. tiberianus
C. cubitus
C. njinei
C. nageus
C. mbirizei
C. cirratus
C. acerbus
C. berradae
C. yanni
C. digitatus
S. bailloni
S. longicornis
S. minus
C. amphoratus
C. sclerosus
C. tilapiae
PPZIM162_1
PPZIM050_1
PPZIM159_1
PPZIM040_1
PPZIM158_1
PPZIM160_1
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MH767392.1
KX869725.1
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PPZIM065_1
PPZIM140_1
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HE792790.1
AJ920271.1
AJ920272.1
AJ920273.1
C. bilongi
C. flexicolpos
C. gallus
C. agnesi
C. longicirrus
C. falcifer
C. dracolemma
C. zambezensis
C. pouyaudi
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PPZIM040_1
PPZIM162_1
MG970255.1
PPZIM140_1
PPZIM065_1
PPZIM019_1
203
C. irenae
C. tilapiae
C. papernastrema
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C. dossoui
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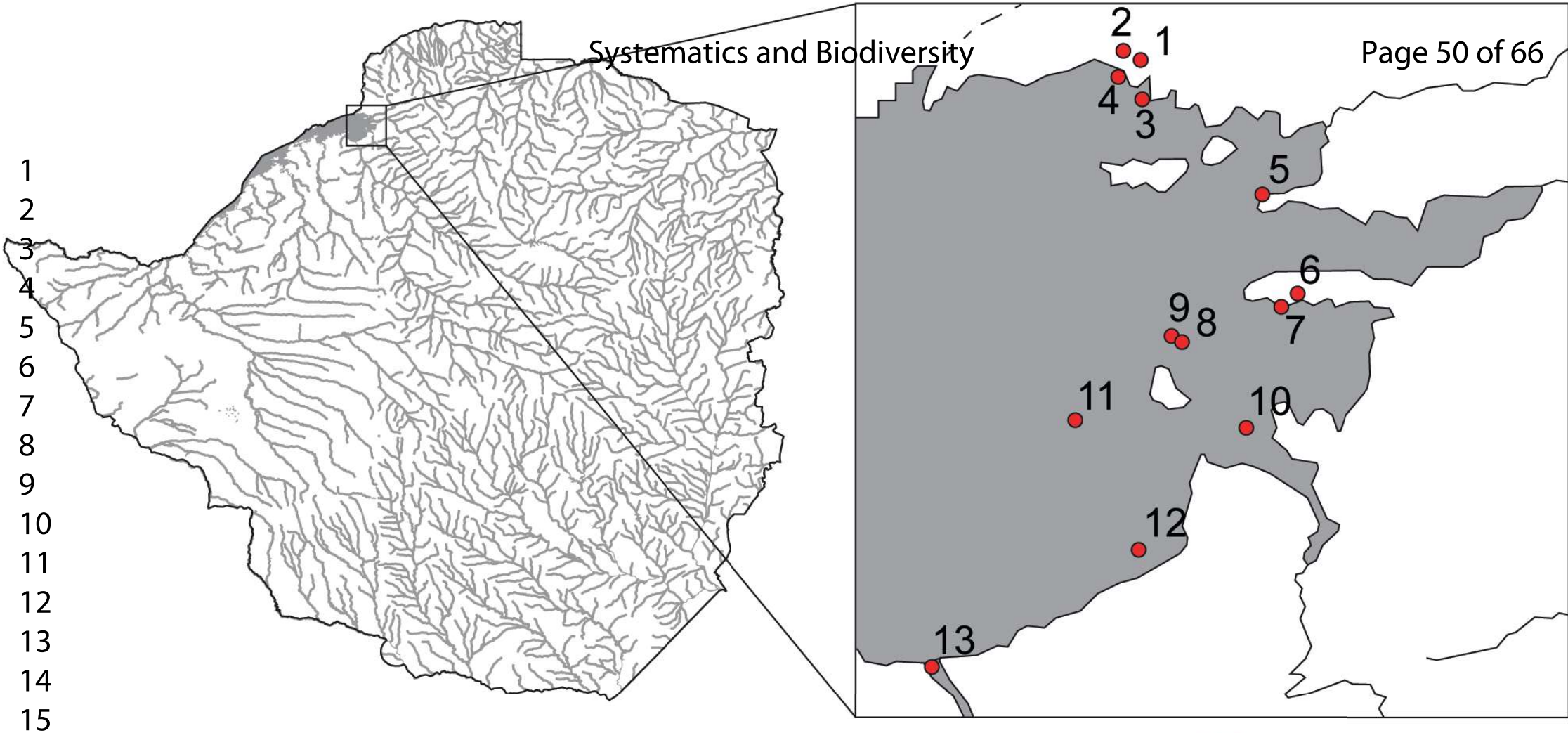
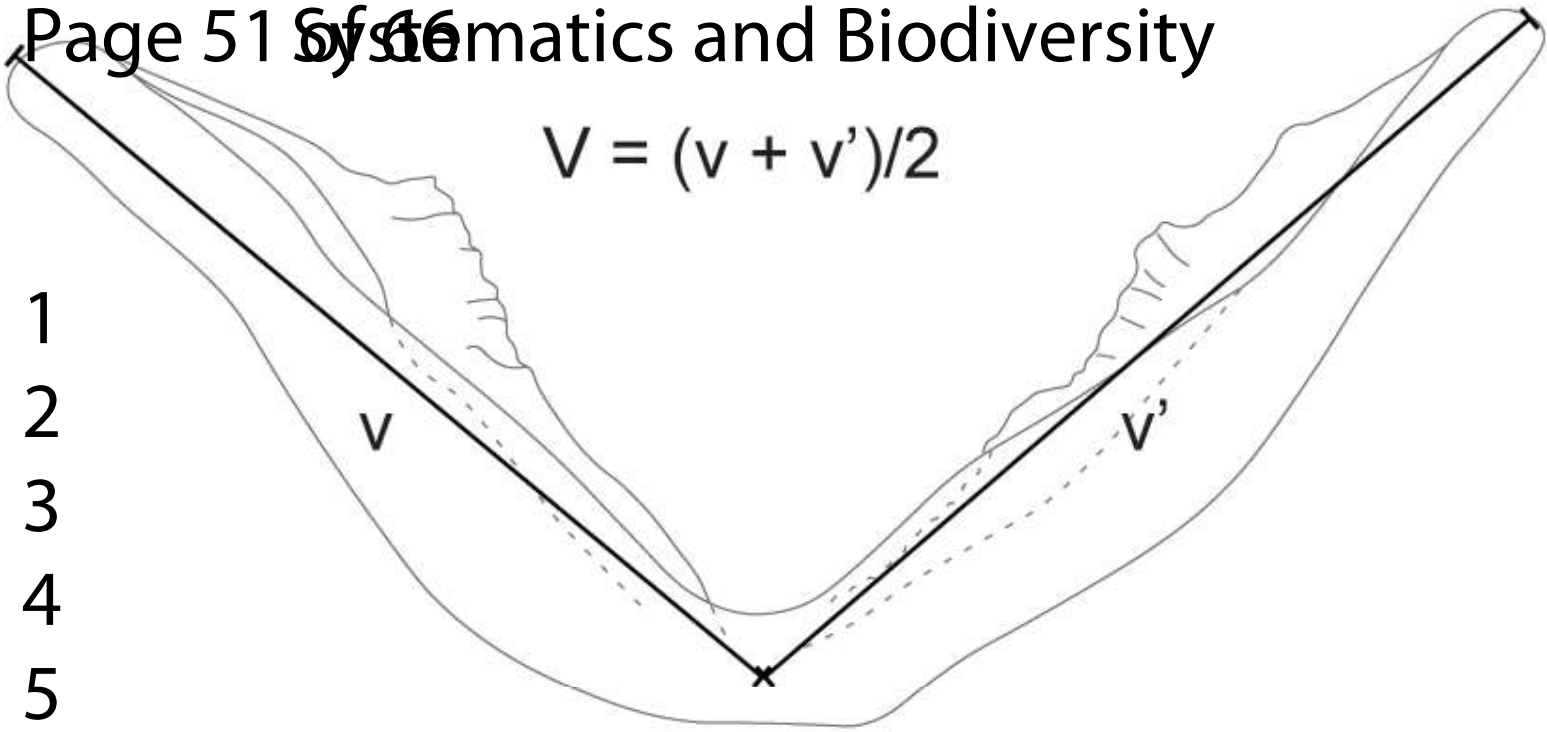
C. mbirizei
C. thurstonae
S. gravivaginus
C. falcifer
0.8

Fig. S2



O. niloticus *O. cf. mortimeri* *C. rendalli*

a



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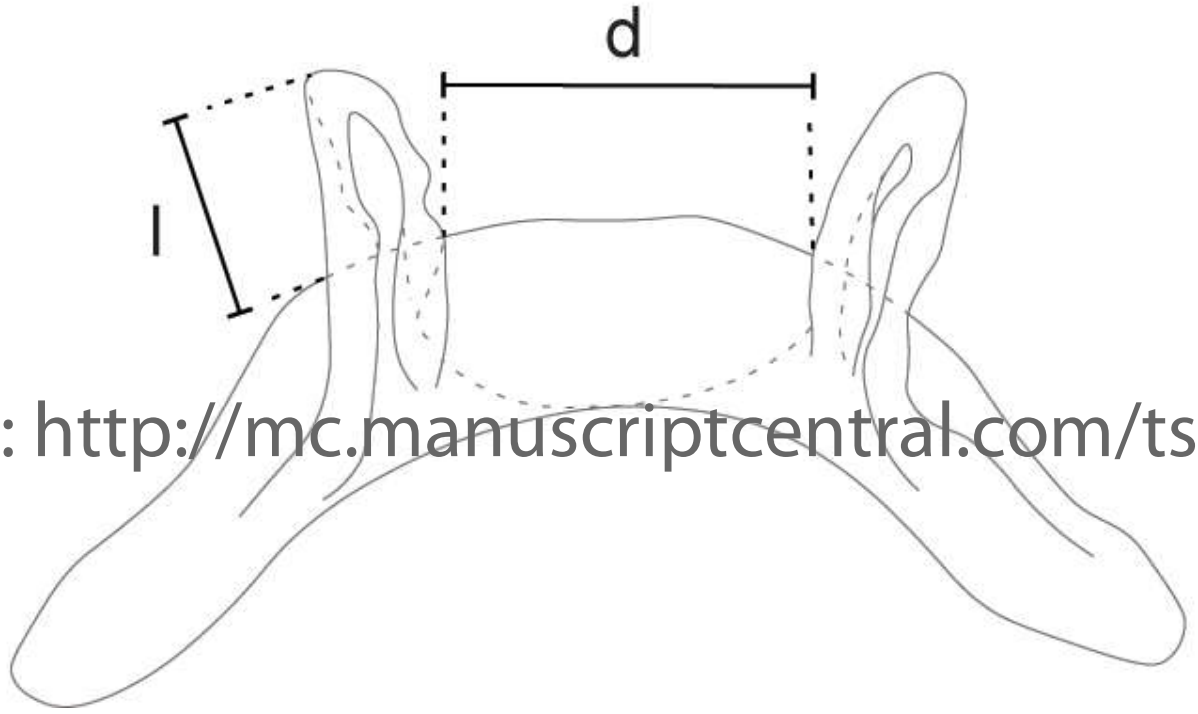
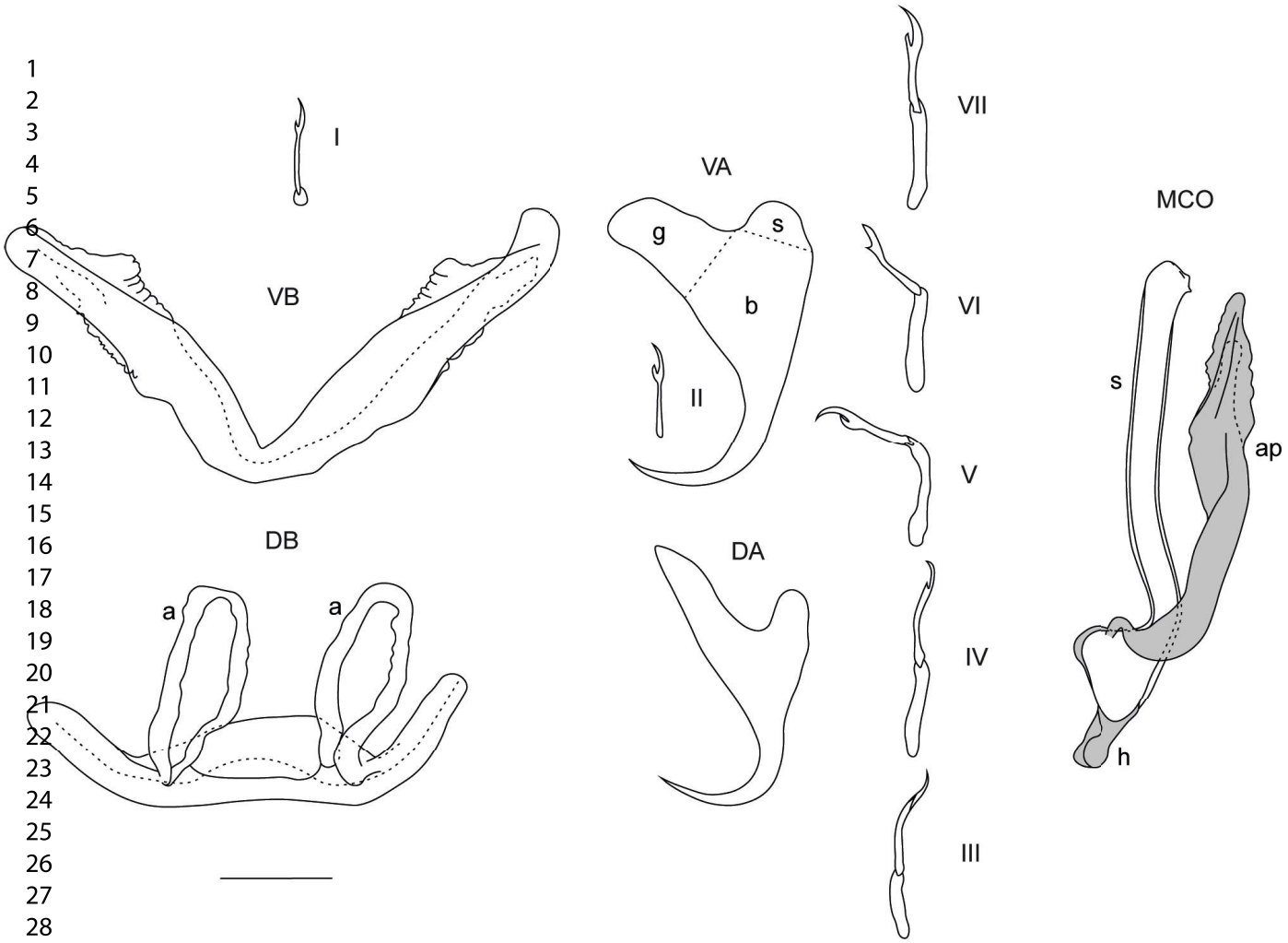
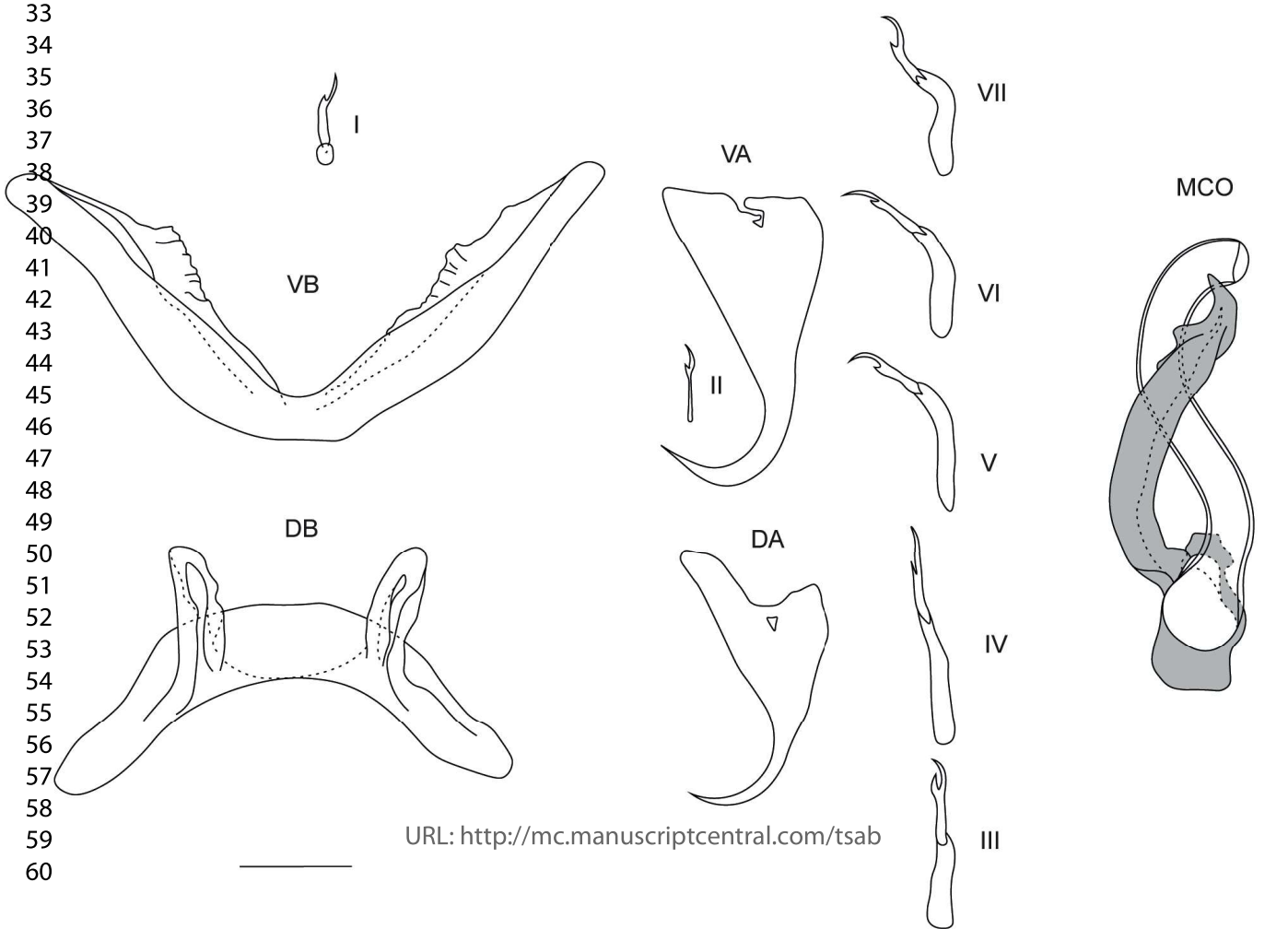
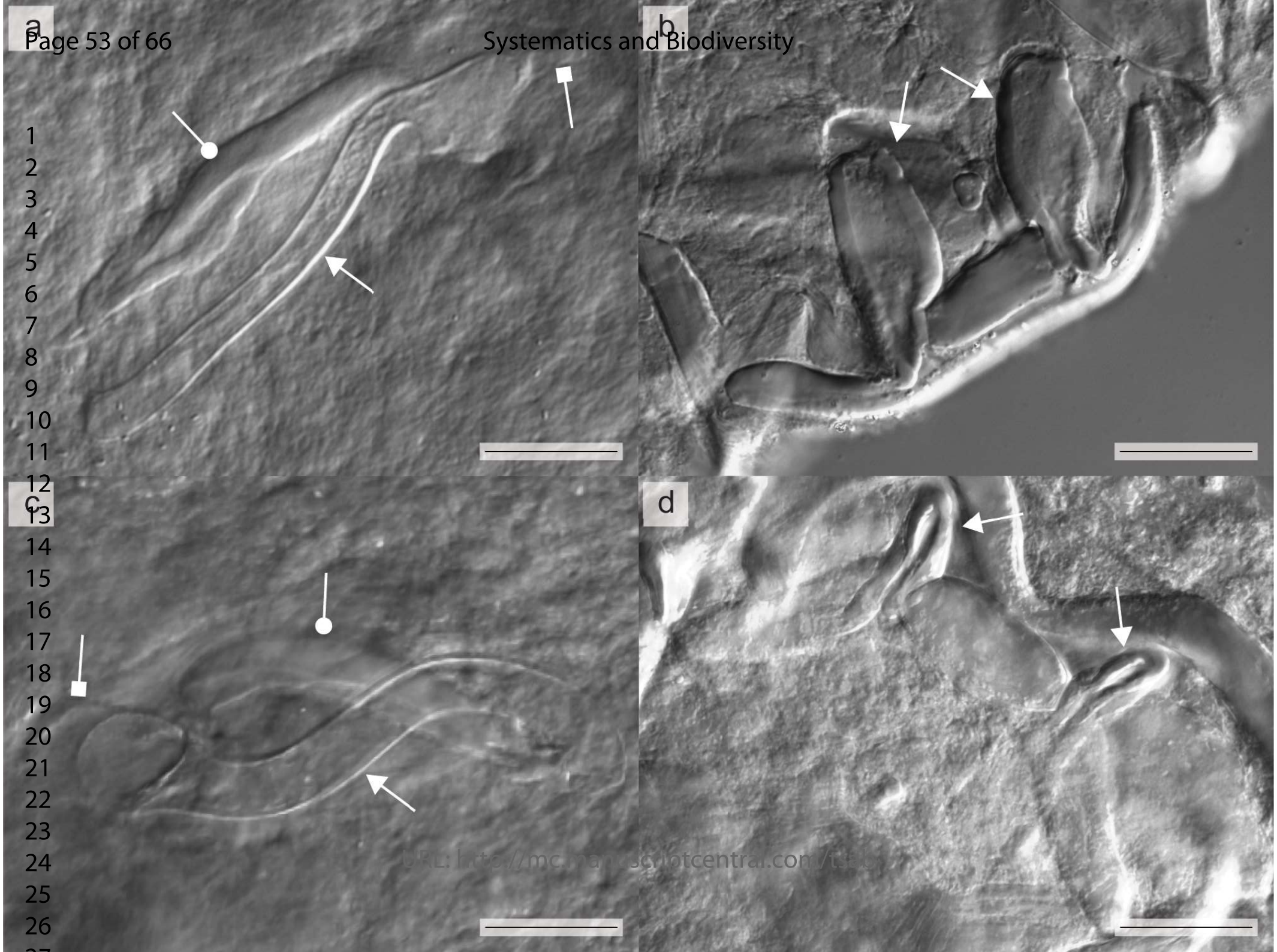
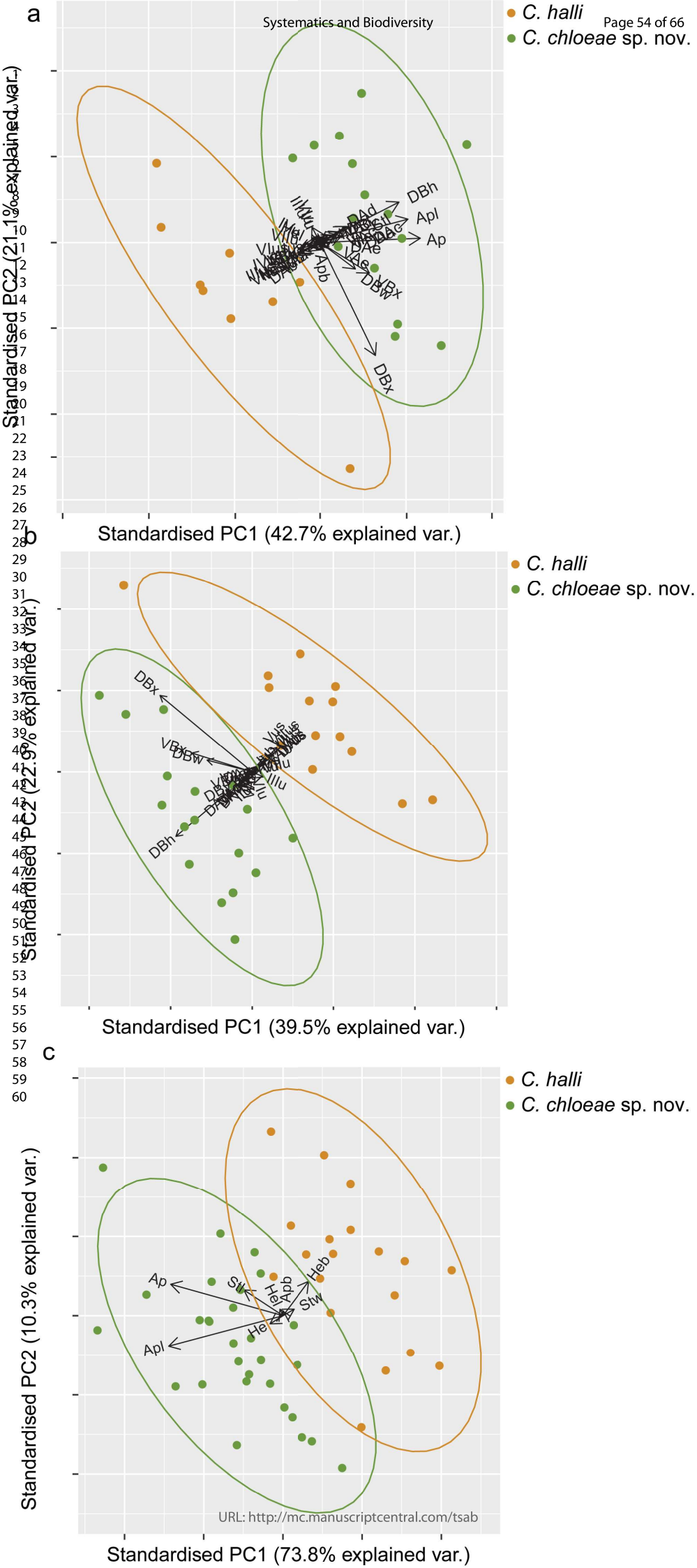


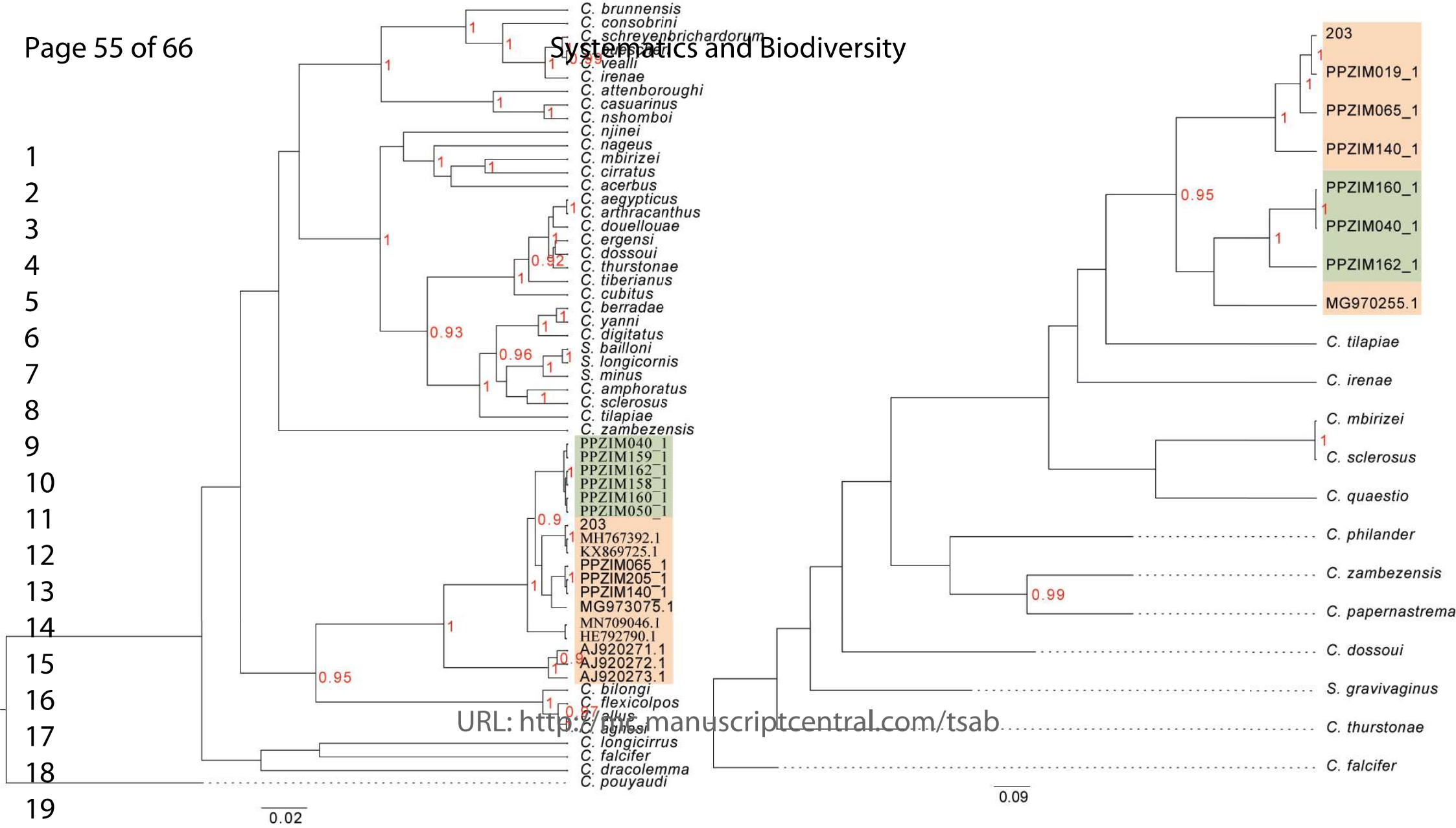


Cichlidogyrus halli









A new species of Cichlidogyrus Paperna, 1960 (Platyhelminthes: Monogenea: Dactylogyridae) infecting tilapias in Lake Kariba (Zimbabwe), with a discussion on its phylogenetic position

Mare Geraerts, Tine Huyse, Maarten P. M. Vanhove, and Tom Artois

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Table S1

Specimens of *Cichlidogyrus* Paperna, 1960 and *Scutogyrus* Pariselle & Euzet, 1995 selected for genetic analyses with details about the parasite species, host species, host label, parasite label, GenBank accession number, country (for *C. halli* and *C. chloeae* sp. nov.) and basin of sampling, and location label (L). Location labels correspond to the ones in **Fig. 1**. Parasites with labels starting with 'PPZIM' are the specimens collected in the present study.

Parasite species	Host species	Host label	Parasite label	Genbank accession number	Country	Basin	L
18S-ITS1							
C. chloeae **sp. nov.**	*Oreochromis* cf. *mortimeri*	ZIM046	PPZIM158.1	ON819324~~XXXX~~	Zimbabwe	Middle Zambezi	5
	Oreochromis cf. *mortimeri*	ZIM049	PPZIM159.1	ON819325~~XXXX~~	Zimbabwe	Middle Zambezi	5
	Oreochromis cf. *mortimeri*	ZIM050	PPZIM160.1	ON819326~~XXXX~~	Zimbabwe	Middle Zambezi	5
	Oreochromis cf. *mortimeri*	ZIM067	PPZIM162.1	ON819327~~XXXX~~	Zimbabwe	Middle Zambezi	5
C. halli (Price & Kirk, 1967)	*Oreochromis niloticus* (Linnaeus, 1758)	ZIM083	PPZIM040.1	ON819294~~XXXX~~	Zimbabwe	Middle Zambezi	11
	Oreochromis niloticus (Linnaeus, 1758)	ZIM059	PPZIM050.1	ON819296~~XXXX~~	Zimbabwe	Middle Zambezi	5
	Coptodon rendalli (Boulenger, 1897)	ZIM051	PPZIM205.1	ON819341~~XXXX~~	Zimbabwe	Middle Zambezi	5
	Oreochromis niloticus (Linnaeus, 1758)	ZIM021	PPZIM065_1	ON819300~~XXXX~~	Zimbabwe	Middle Zambezi	1
	Oreochromis niloticus (Linnaeus, 1758)	ZIM026	PPZIM140_1	ON819320~~XXXX~~	Zimbabwe	Middle Zambezi	1
	Oreochromis niloticus (Linnaeus, 1758)			203 (1)	DRC		
	Oreochromis niloticus x *mweruensis*			MG973075.1 (2)	DRC		
	Oreochromis niloticus (Linnaeus, 1758)			AJ920272.1 (3)	Ivory Coast		

Cichlidogyrus acerbus Dossou, 1982	*Sarotherodon melanotheron* Rüppel, 1852	AJ920271.1 (3)	Ivory Coast
Cichlidogyrus aegypticus Ergens, 1981	*Sarotherodon galilaeus* (Linnaeus, 1758)	AJ920273.1 (3)	Ivory Coast
Cichlidogyrus agnesi Pariselle & Euzet, 1995	?	KX869725.1 (4)	?
Cichlidogyrus arthracanthus Paperna, 1960	*Oreochromis niloticus* (Linnaeus, 1758)	MH767392.1 (5)	Madagascar
Cichlidogyrus attenboroughi Kmentová, Gelnar, Koblmüller & Vanhove, 2016	*Sarotherodon galilaeus* (Linnaeus, 1758)	HE792790.1 (6)	Senegal
Cichlidogyrus berradae Pariselle & Euzet, 2003	*Oreochromis niloticus* (Linnaeus, 1758)	MN709046.1 (7)	Egypt
Cichlidogyrus bilongi Pariselle & Euzet, 1995	*Sarotherodon galilaeus* (Linnaeus, 1758)	HE792780.2 (6)	
Cichlidogyrus brunnensis Kmentová, Gelnar, Koblmüller & Vanhove, 2016	*Coptodon guineensis* (Günther, 1862)	HE792781.1 (6)	
Cichlidogyrus buescheri Pariselle & Vanhove, 2015	*Coptodon guineensis* (Günther, 1862)	AJ920286.1 (3)	
Cichlidogyrus casuarinus Pariselle, Muterezi Bukinga & Vanhove, 2015	*Coptodon guineensis* (Günther, 1862)	HE792783.1 (6)	
Cichlidogyrus cirratus Paperna, 1964	*Benthochromis tricoti* (Poll, 1948)	MH708153.1 (8)	
Cichlidogyrus consobrini Jorissen, Pariselle & Vanhove in Jorissen, Pariselle, Huyse, Vreven, Snoeks, Volckaert, Chocha Manda, Kapepula Kasembele, Artois & Vanhove, 2017	*Coptodon rendalli* (Boulenger, 1897)	182 (1)	
Cichlidogyrus cubitus Dossou, 1982	*Coptodon guineensis* (Günther, 1862)	AJ920287.1 (3)	
Cichlidogyrus digitatus Dossou, 1982	*Trematocara unimaculatum* Boulenger, 1901	MH708152.1 (8)	
	Interochromis loocki (Poll, 1949)	363 (9)	
	Bathybates minor Boulenger, 1905	KX007775.1 (10)	
	Oreochromis niloticus (Linnaeus, 1758)	HE792784.1 (6)	
	Sargochromis mellandi (Boulenger, 1905)	144 (1)	
	Coptodon guineensis (Günther, 1862)	HE792785.1 (6)	
	Coptodon guineensis (Günther, 1862)	HE792786.1 (6)	

Cichlidogyrus dossoui Douëllou, 1993	*Coptodon rendalli* (Boulenger, 1897)	85 (1)
Cichlidogyrus douellouae Pariselle, Bilong Bilong & Euzet, 2003	*Sarotherodon galilaeus* (Linnaeus, 1758)	HE792787.1 (6)
Cichlidogyrus dracolemma Řehulková, Mendlová & Šimková, 2013	*Hemichromis letournaeuxi* Sauvage, 1880	HE792794.1 (6)
Cichlidogyrus ergensi Dossou, 1982	*Coptodon guineensis* (Günther, 1862)	HE792788.1 (6)
Cichlidogyrus falcifer Dossou & Birgi, 1984	*Hemichromis fasciatus* Peters, 1857	HE792789.1 (6)
Cichlidogyrus flexicolpos Pariselle & Euzet, 1995	*Coptodon guineensis* (Günther, 1862)	AJ920283.1 (3)
Cichlidogyrus gallus Pariselle & Euzet, 1995	*Coptodon guineensis* (Günther, 1862)	AJ920285.1 (3)
Cichlidogyrus irenae Gillardin, Vanhove, Pariselle, Huyse & Volckaert, 2012	Gnathochromis pfefferi (Boulenger, 1898)	KT692939.1 (10)
Cichlidogyrus longicirrus Paperna, 1965	*Hemichromis fasciatus* Peters, 1857	HE792791.1 (6)
Cichlidogyrus mbirizei Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012	*Oreochromis niloticus* x *mweruensis*	MG973076.1 (2)
Cichlidogyrus nageus Řehulková, Mendlová & Šimková, 2013	*Sarotherodon galilaeus* (Linnaeus, 1758)	HE792795.1 (6)
Cichlidogyrus njinei Pariselle, Bilong Bilong & Euzet, 2003	*Sarotherodon galilaeus* (Linnaeus, 1758)	HE792792.1 (6)
Cichlidogyrus nshomboi Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012	*Boulengerochromis microlepis* (Boulenger, 1899)	368 (9)
Cichlidogyrus philander Douëllou, 1993	*Pseudocrenilabrus philander* (Weber, 1897)	MG250200.1 (11)
Cichlidogyrus pouyaudi Pariselle & Euzet, 1994	*Tylochromis intermedius* (Boulenger, 1916)	HE792793.1 (6)
Cichlidogyrus schreyenbrichardorum Pariselle & Vanhove, 2015	*Interochromis loocki* (Poll, 1949)	362 (9)
Cichlidogyrus sclerosus Paperna & Thurston, 1969	*Oreochromis niloticus* (Linnaeus, 1758)	DQ537359.1 (12)
Cichlidogyrus thurstonae Ergens, 1981	*Paretroplus lamenabe* Sparks 2008	MH767395.1 (5)
Cichlidogyrus tiberianus Paperna, 1960	*Coptodon guineensis* (Günther, 1862)	HE792796.1 (6)
Cichlidogyrus tilapiae Paperna, 1960	*Hemichromis fasciatus* Peters, 1857	HE792797.1 (6)
Cichlidogyrus vealli Pariselle & Vanhove, 2015	*Interochromis loocki* (Poll, 1949)	356 (9)
Cichlidogyrus yanni Pariselle & Euzet, 1996	*Coptodon guineensis* (Günther, 1862)	HE792798.1 (6)

Cichlidogyrus zambezensis Douëllou, 1993	*Serranochromis macrocephalus* (Boulenger, 1899)			375 (9)			
Scutogyrus bailloni Pariselle & Euzet, 1995	*Sarotherodon galilaeus* (Linnaeus, 1758)			HE792799.1 (6)			
Scutogyrus longicornis (Paperna & Thurston, 1969)	*Oreochromis niloticus* (Linnaeus, 1758)			HE792800.1 (6)			
Scutogyrus minus (Dossou, 1982)	*Sarotherodon melanotheron* Rüppel, 1852			HE792801.1 (6)			
COI							
Cichlidogyrus chloeae **sp. nov.**	*Oreochromis* cf. *mortimeri*	ZIM050	PPZIM160.1	ON827403~~XXXX~~	Zimbabwe	Middle Zambezi	5
	Oreochromis cf. *mortimeri*	ZIM067	PPZIM162.1	ON827404~~XXXX~~	Zimbabwe	Middle Zambezi	5
Cichlidogyrus halli (Price & Kirk, 1967)	*Oreochromis niloticus (*Linnaeus, 1758)	ZIM083	PPZIM040.1	ON827384~~XXXX~~	Zimbabwe	Middle Zambezi	11
	*Oreochromis niloticus (*Linnaeus, 1758)	ZIM029	PPZIM019_1	ON827382~~XXXX~~	Zimbabwe	Middle Zambezi	2
	*Oreochromis niloticus (*Linnaeus, 1758)	ZIM021	PPZIM065_1	ON827389~~XXXX~~	Zimbabwe	Middle Zambezi	1
	*Oreochromis niloticus (*Linnaeus, 1758)	ZIM026	PPZIM140_1	ON827400~~XXXX~~	Zimbabwe	Middle Zambezi	1
Cichlidogyrus halli (Price & Kirk, 1967)	*Oreochromis niloticus* x *mweruensis*			MG970255.1 (2)	DRC		
	Oreochromis niloticus (Linnaeus, 1758)			203 (1)	DRC		
Cichlidogyrus sclerosus Paperna & Thurston, 1969	?			JQ038226.1 (13)			
Cichlidogyrus zambezensis Douëllou, 1993	*Serranochromis jallae* (Boulenger 1896)			KT037411.1 (14)			
Cichlidogyrus philander Douëllou, 1993	*Pseudocrenilabrus philander* (Weber, 1897)			MG288503.1 (15)			
Cichlidogyrus mbirizei Muterezi Bukinga, Vanhove, Van Steenberge & Pariselle, 2012	?			MN905506.1 (16)			
Cichlidogyrus irenae Gillardin, Vanhove, Pariselle, Huyse & Volckaert, 2012	*Gnathochromis pfefferi* (Boulenger, 1898)			KT037339.1 (14)			
Cichlidogyrus dossoui Douëllou, 1993	*Coptodon rendalli* (Boulenger, 1897)			85 (1)			
Cichlidogyrus falcifer Dossou & Birgi, 1984	*Hemichromis stellifer* Loiselle, 1979			193 (1)			
Cichlidogyrus papernastrema Price, Peebles & Bamford, 1969	*Tilapia sparrmanii* Smith, 1840			80 (9)			
Cichlidogyrus quaestio Douëllou, 1993	*Coptodon rendalli* (Boulenger, 1897)			83 (1)			

Cichlidogyrus thurstonae Ergens, 1981	*Oreochromis niloticus* (Linnaeus, 1758)	214 (1)
Cichlidogyrus tilapiae Paperna, 1960	*Oreochromis niloticus* (Linnaeus, 1758)	208 (1)
Scutogyrus gravivaginus (Paperna & Thurston, 1969)	*Oreochromis mweruensis* Trewavas, 1983	65 (9)

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Fig. S1

Biplots of the PCAs plotting the second and third principal components PC2 and PC3: a PCA based on all measurements, b only measurements of the haptor, and c only measurements of the MCO. Each dot represents one specimen. Different colours represent different species i.e. *C. halli* and *C. chloeae* sp. nov. Ellipses are drawn at a confidence interval of 0.95. The contribution of the different measurements to the principal components are shown by arrows.

Fig. S2

Maximum likelihood phylogenetic trees of specimens of *Cichlidogyrus* and *Scutogyrus* inferred from the 18S-ITS1 fragment (left) and *COI* fragment (right). Only well supported nodes (bootstrap values ≥ 0.85) are indicated by support values (in red). Scale bar indicates number of substitutions per site. Specimens of *C. chloeae* sp. nov. framed in green, specimens of *C. halli* framed in orange. Parasite labels and Genbank accession numbers of the included specimens can be found in Table S1.

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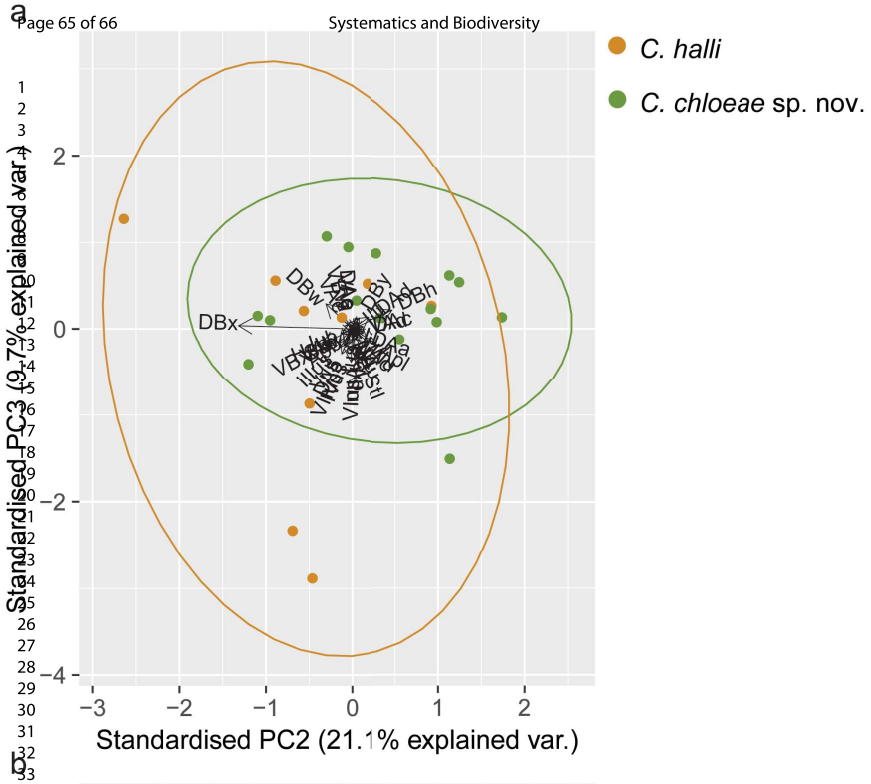
C. halli
C. chloeae sp. nov.
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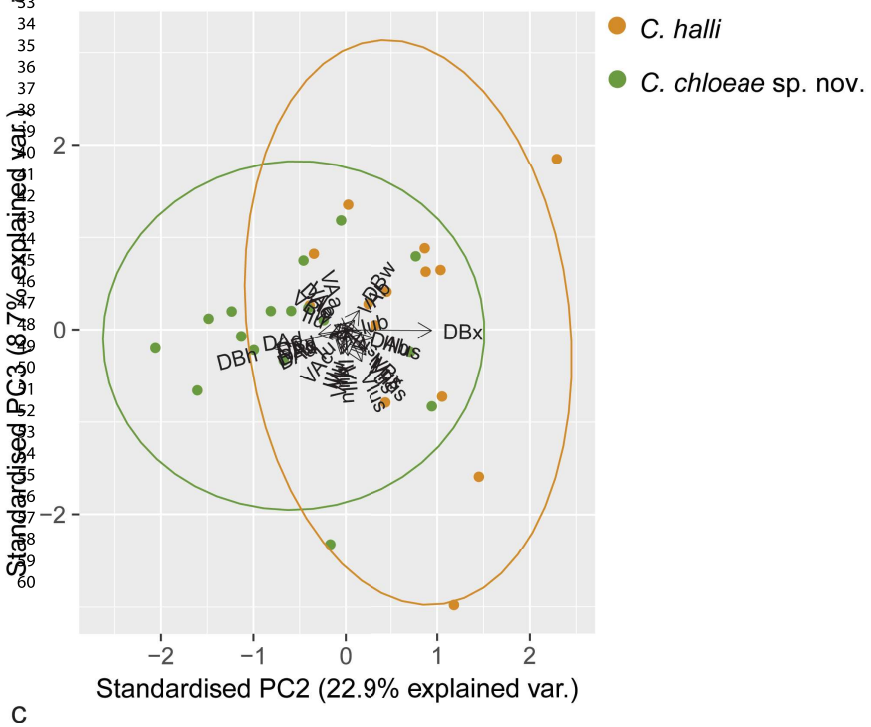
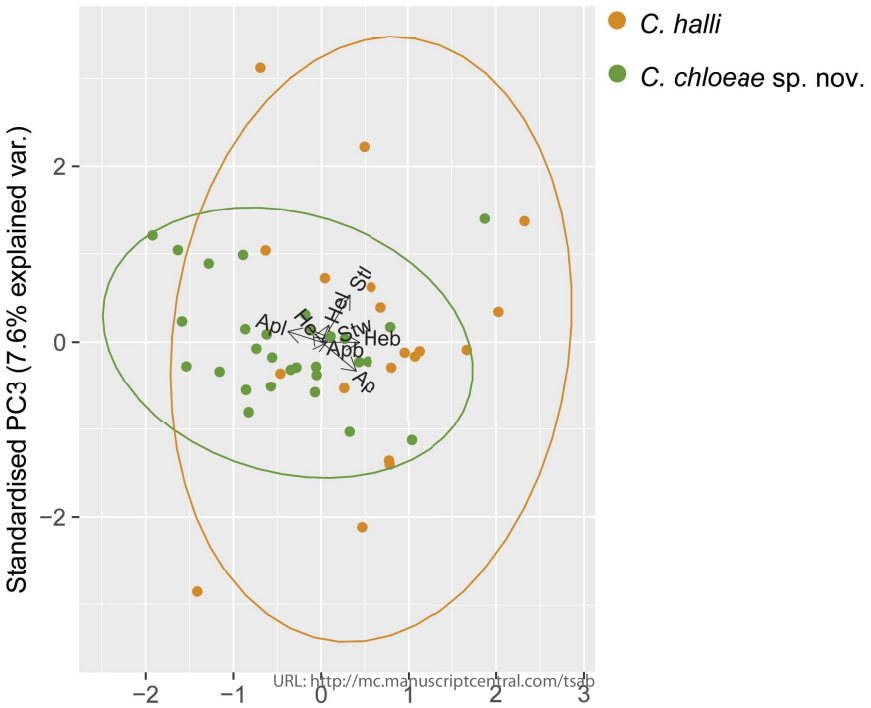
Standardised PC2 (21.1% explained var.)
Standardised PC3 (9.7% explained var.)

b


C. halli
C. chloeae sp. nov.
DBx
Standardised PC2 (22.9% explained var.)
Standardised PC3 (8.7% explained var.)

c


C. halli
C. chloeae sp. nov.
Standardised PC2 (10.3% explained var.)
Standardised PC3 (7.6% explained var.)