Zoological Studies **55:** 31 (2016)
doi:10.6620/ZS.2016.55-31

Zoological Studies

Smilosicyopus leprurus (Teleostei: Gobiidae) is a Fin-eater

Ying-Tzu Lu[1], Min-Yun Liu[2], You He[3], and Te-Yu Liao[1,*]

[1]*Department of Oceanography, National Sun Yat-sen University, Kaohsiung 80424, Taiwan. E-mail: spnphy@gmail.com (YTL)*
[2]*Taiwan Ocean Research Institute, National Applied Research Laboratories, Kaohsiung 852, Taiwan. E-mail: mylalex@gmail.com*
[3]*Shanghai Synchrotron Radiation Facility, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201204, China. E-mail: heyou@sinap.ac.cn*

(Received 28 January 2016; Accepted 14 April 2016)

Ying-Tzu Lu, Min-Yun Liu, You He, and Te-Yu Liao (2016) *Smilosicyopus leprurus* is a small goby distributed in the Ishigaki Island and Taiwan. Hobbyists found *S. leprurus* attacked on the caudal fin of fishes kept together. In this study we investigate whether this fin-eating behavior occurs in the field and whether *S. leprurus* can be considered a fin-eater. Behavior observation in a tank showed *S. leprurus* snapped off a piece of fins and swallowed. Among *S. leprurus* preserved immediately after capture, a fin fragment and a few scales were recovered in the gut of a specimen and DNA barcoding shows the fin fragment belonging to *Sicyopterus japonicas*. The fin-eating behavior of *S. leprurus* occurs in captivity and in the wild. In addition, the 3D renderings of oral jaws of *S. leprurus* were provided. Oral teeth are numerous and arranged in close proximity to each other all together like a bladed dentition, which may provide the capability to shear the fin of its prey. The present study shows *S. leprurus* a fin-eater despite not feeding exclusively on fins.

Key words: Pterygophagy, DNA barcoding, *Sicyopterus japonicas*, *Sicyopus zosterophorum*, Dentition.

BACKGROUND

Teleosts are a highly diverse fish group in terms of morphology and behavior, broadly inhabiting all over the world except for the Antarctica. Morphology includes outwards appearance that can be detected easily. However, behavior of fish is innate or learned response to internal and/or external stimuli, which needs long term observations and sometimes some luck to reveal. Although most species are polyphagous, feeding behavior is diverse among teleosts, including detritivory, herbivory, insectivory, piscivory as well as parasitic behaviors such as pterygophagy, lepidophagy and even endoparasitic candirus. Pterygophagy is a fin-eating behavior, either obligatory or facultative (Stiassny et al. 2013), only documented in the blennid genus *Aspidontus* (Eibl-Eibesfeldt 1959; Wickler 1966), cichlid genera *Docimodus* (Eccles and Lewis 1976; Ribbink 1984) and *Genyochromis* (Ribbink et al. 1983), distichodontid genera *Belonophago*, *Eugnathichthys*, *Ichthyborus* and *Phago* (Matthes 1961; Stiassny et al. 2013) as well as serrasalmid genus *Serrasalmus* (Northcote et al. 1986). However, no goby is known as a fin-eater to date.

Smilosicyopus leprurus (Fig. 1) is a small goby species distributed in the Ishigaki Island and Taiwan (Sakai and Nakamura 1979; Chen et al. 2012), inhabiting in stream pool's head with moderate running water. This species is considered critically endangered in Japan (Okinawa Prefecture 2005), but relatively common in Taiwan despite still a rare species. Hobbyists observed that caudal fins of fishes kept in aquariums with *S. leprurus* were frequently damaged (Chao and Zhou pers. Comm. and our own observation). In the present study, we show that *S. leprurus* is a facultative fin-eater based on behavior observation on the fish in captivity and stomach contents analysis of

*Correspondence: E-mail: swp0117@gmail.com; tyliao@mail.nsysu.edu.tw

specimens collected from the field. In addition, the relation of dentition to fin-eating of *S. leprurus* is discussed.

MATERIALS AND METHODS

Fin-eating filming and stomach contents

Due to the rareness, it's not possible and/or appropriate to collect a huge amount of *Smilosicyopus leprurus*. In total, 16 specimens were collected from three streams in the southeastern Taiwan. Two of the specimens obtained from hobbyists were kept in aquariums for several months before donation and they were used to film the attack on other fish in order to show the fin-eating behavior. These two individuals were kept in a small tank (24L × 14W × 18H cm) with a small stone inside and starved for two days. Six individuals of *Poecilia reticulata*, two males and four females, were placed into the tank and the fishes were filmed for 24 hrs. Fourteen specimens collected in the wild were euthanized using anesthetics and fixed in 95% alcohol immediately after captures in order to cease the digestion. The 14 ethanol-preserved specimens were dissected. Aquatic insects of ten specimens were identified to the family level and counted only when the head is attached to the remnant. Fin fragments found in the stomachs were rinsed to avoid contamination with *S. leprurus*' tissue (Arroyave and Stiassny 2014). All specimens are deposited in the collection of the Department of Oceanography, National Sun Yat-sen University (DOS), Kaohsiung.

Barcoding and cloning of prey items in stomachs

Fin fragments obtained in stomachs were identified using DNA barcoding (Hebert et al. 2003). Mitochondrial DNA of fin fragments was extracted using Easy Tissue & cell Genomic DNA Purification Kit (GeneMark) with recommended protocol. The primer set, FishF1 and FishR1 (Ward et al. 2005), was used to amplify the fragment of COI gene. The PCR protocol was as follows: PCR cycling: 94°C 2'; 40* (94°C 25"; 52°C 25"; 72°C 1'); 72°C 7'. PCR products were checked on minigel and sequenced in the commercial lab of BioKit (Hsinchu, Taiwan). Assemblage of the sequence fragments was conducted using the Lasergene software package (DNASTAR). Sequences were blasted in NCBI (Johnson et al. 2008) and species identifications were based on the similarity between query and reference sequences with the criteria set as 98% (Arroyave and Stiassny 2014).

To avoid that fin fragments were too small to detect, cloning of residues other than fin fragments and aquatic insects collected from the specimens was carried out. Mitochondrial DNA extraction and COI fragments amplifications followed the

Fig. 1. *Smilosicyopus leprurus*. Approximately 5 cm TL; not preserved. Collected in the Daliu Creek in southeastern Taiwan. Photographed by M.T. Zhou.

procedures described above. PCR products were checked on minigel and bands of approximately 600 bps were cut and purified using a Clean/Gel Extraction Kit (BioKit) and cloned with the pGM-T cloning Kit (BioKit) following the manufacturer's instructions. Colonies with ampicillin resistance were sequenced with the primer set, FishF1 and FishR1. Ten to 30 positive colonies were sequenced and blasted in NCBI for species identification.

Synchrotron X-ray microtomography

X-ray microtomography is a powerful tool to generate a high-quality virtual model inside a specimen without destruction (Flannery et al. 1987; Metscher 2009). In the present study, synchrotron X-ray microtomography was applied to show the differences of dentition between *S. leprurus* and the most aggressive relative, *Sicyopus zosterophorum*. The synchrotron radiation X-ray microtomography (SR-μCT) was performed at BL13W1 beamline of Shanghai Synchrotron Radiation Facility (SSRF), Shanghai, China. The gobies' heads were vertically held in a plastic tube filled with formalin solution which mounted on the sample stage, and imaged by 24.0 keV monochromatic X-ray. A dedicated detector was used, and the X-rays penetrating through the specimen were recorded by a YAG:Ce scintillator screen coupled via visible light microscope optics to a digital CMOS camera (ORCA-Flash4.0 C11440-22CU, Hamamatsu Photonics KK, Japan) with final 3.25 μm pixel size. Total 720 projections (radiographs), with 500 ms exposure time for each, were recorded during specimen rotating over 180°. Flat-field and dark-field images were collected to correct the electronic noise and variations in the X-ray source brightness during each acquisition procedure. As the vertical size of synchrotron X-ray beam (about 5 mm) is less than the head length, the specimen was moved upward 4.8 mm after first scanning to enable two consecutive scanning. The data set were reconstructed into corresponding slices using the filtered back-projection algorithm (X-TRACT software, CSIRO). The three-dimensional rendering (3D rendering) of each goby head was created from two consecutive stacks of the slices, and further manipulated and analyzed in the VG Studio Max (v2.1) software. During the visualization process, the soft tissues with low grey value were virtually removed, but the bones with high grey value remained (He et al. 2013).

RESULTS

Fin-eating filming and stomach content barcoding

Despite of two-day starvation, all fins of *Smilosicyopus leprurus* remained intact. The two individuals of *S. leprurus* didn't attack each other. Only male *P. reticulate* was attacked. *Smilosicyopus leprurus* followed the victims soon after they were placed in the tank and attacked from the rear to snap off a piece of fins. All fins were damaged except for the anal fin in both male *P. reticulate*. Video clips of fin-biting and fin-eating by *S. leprurus* are available at https://youtu.be/rg28ZT4-ls8 and https://youtu.be/QgEDp5t3GkU, respectively.

Among 14 specimens sacrificed immediately after captures, aquatic insects were recovered in all specimens except for one individual (DOS01814) with an empty stomach. Identification and numbers of aquatic insects of 10 specimens were listed in table 1. The entire stomach contents of the other

Table 1. Identification and numbers of aquatic insects in the stomach contents of *Smilosicyopus leprurus*. Only ten specimens are listed because the stomach contents of the other specimens are used for cloning without identification and count

	Catalog numbers									
Family	DOS 01807	DOS 01808	DOS 01809	DOS 01810	DOS 01811	DOS 01812	DOS 01813	DOS 01814	DOS 01815	DOS 01816
Baetidae	1		1		4		1		2	2
Heptageniidae				1	3					
Hydropsychidae									1	
Limnephilidae	1									
Philopotamidae	1		3							
Psychomyiidae	1	1				1	2			
Rhyacophilidae	1									

specimens (DOS 01801, DOS 01804-01806) were also used in cloning, so their aquatic insects were not identified and counted. Only one specimen (DOS01804) found to contain a fin fragment and a few scales in the gut close to the gill arches after dissection. DNA barcoding shows the fin fragment belonging to *Sicyopterus japonicus* (accession No. JX628620; max score: 793; total score: 793; query cover: 100%; e value: 0.0; ident: 99%) and the second most similar sequence is *Sicyopterus lagocephalus* (accession No. KF668858; max score: 693; total score: 693; query cover: 100%; e value: 0.0; ident: 96%). Both species are syntopic with *Smilosicyopus leprurus* and the former is more abundant. A specimen of *Sicyopus zosterophorum* (Fig. 2; DOS02097) with damage on the caudal fin was collected with a *Smilosicyopus leprurus* (one of the specimen cataloged as DOS01810-01816, not denoted) in the same net, but no fin fragment was recovered in the gut of *S. leprurus*.

Cloning of prey items in stomachs of 13 specimens failed to recover any COI sequences of fish other than species of *Smilosicyopus*. Two sequences of NADH dehydrogenase subunit 4L (ND4L) gene of *Sicyopus* sp. (both most similar to HQ005355) were retrieved. However, the query coverage values were both 66. They were probably obtained by chance.

Dentition

The 3D renderings of *Smilosicyopus leprurus* and *Sicyopus zosterophorum* are shown in figure 3. In general, the bones around the jaws, including maxilla, premaxilla, dentary, palatine and ectopterygoid appear stouter in *Smilosicyopus leprurus* than those in *Sicyopus zosterophorum*. In *Smilosicyopus leprurus*, the premaxilla possesses an ascending process with broad base and tapering point end. The length of the ascending process is similar with the shaft of premaxilla. The maxilla is stubby with an expanded posterior flange. The ectopterygoid is short, deep, and articulates the anterior ventral corner of the quadrate. In *Sicyopus zosterophorum*, the premaxilla is similar to that in *Smilosicyopus leprurus*, but more slender. The length of ascending process is less than half of the shaft. The maxilla and dentary are more slender.

(a)

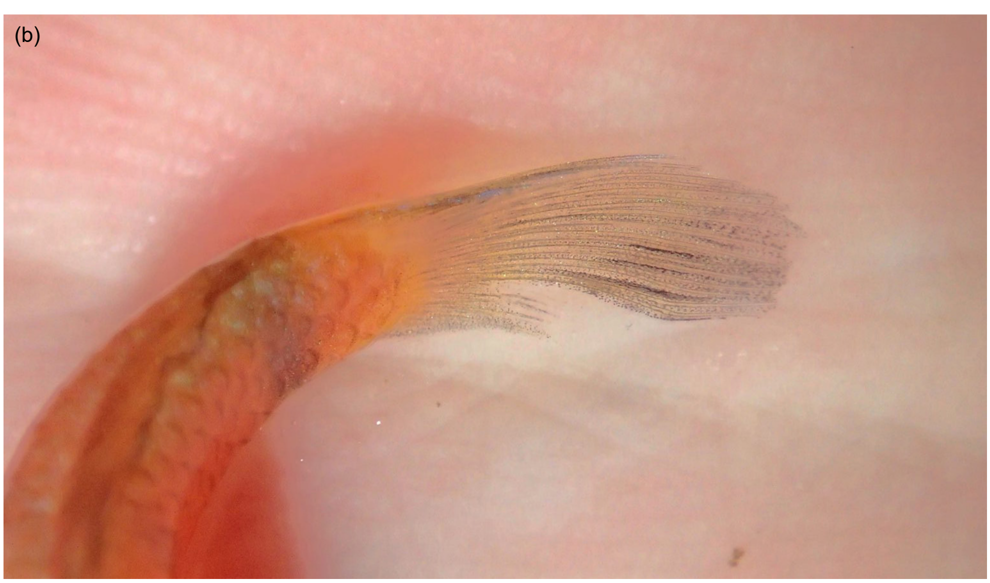

Fig. 2. *Sicyopus zosterophorum* with damage on caudal fin. 27.4 mm SL; DOS02097 (a); the same specimen photographed right after capture in the Xianantian Creek in southeastern Taiwan (b).

The ectopterygoid is thin and longer.

In *Smilosicyopus leprurus*, the premaxilla bears one row of closely crowded conical teeth in anterior segment followed by two larger canine-like teeth and 4-5 smaller curved conical teeth in posterior segment. The dentition is sturdy and bears 16 teeth on the left premaxilla (Fig. 3a). In *Sicyopus zosterophorum*, the premaxilla bears one row of spaced curved conical teeth increasing in size posteriorly. The dentary is slender and bears 8 (Fig. 3c; only seven are shown; one fell off during processing) teeth on the left premaxilla. The difference between the oral dentition is evident in the frontal view.

DISCUSSION

The Gobiidae are a diverse fish group, comprising more than 200 genera and two thousand species found in various habitats (Nelson 2006). Despite rich in morphology, feeding habits of goby are not various. Most species are carnivorous and only some are known as cleaner or algae grazers (Böhlke and Robins 1968). Special feeding behavior such as pterygophagy and lepidophagy are never documented. Based on the behavior observed in tanks (*in vitro*; personal observations and this study) and the fin fragment recovered from a specimen collected in the wild

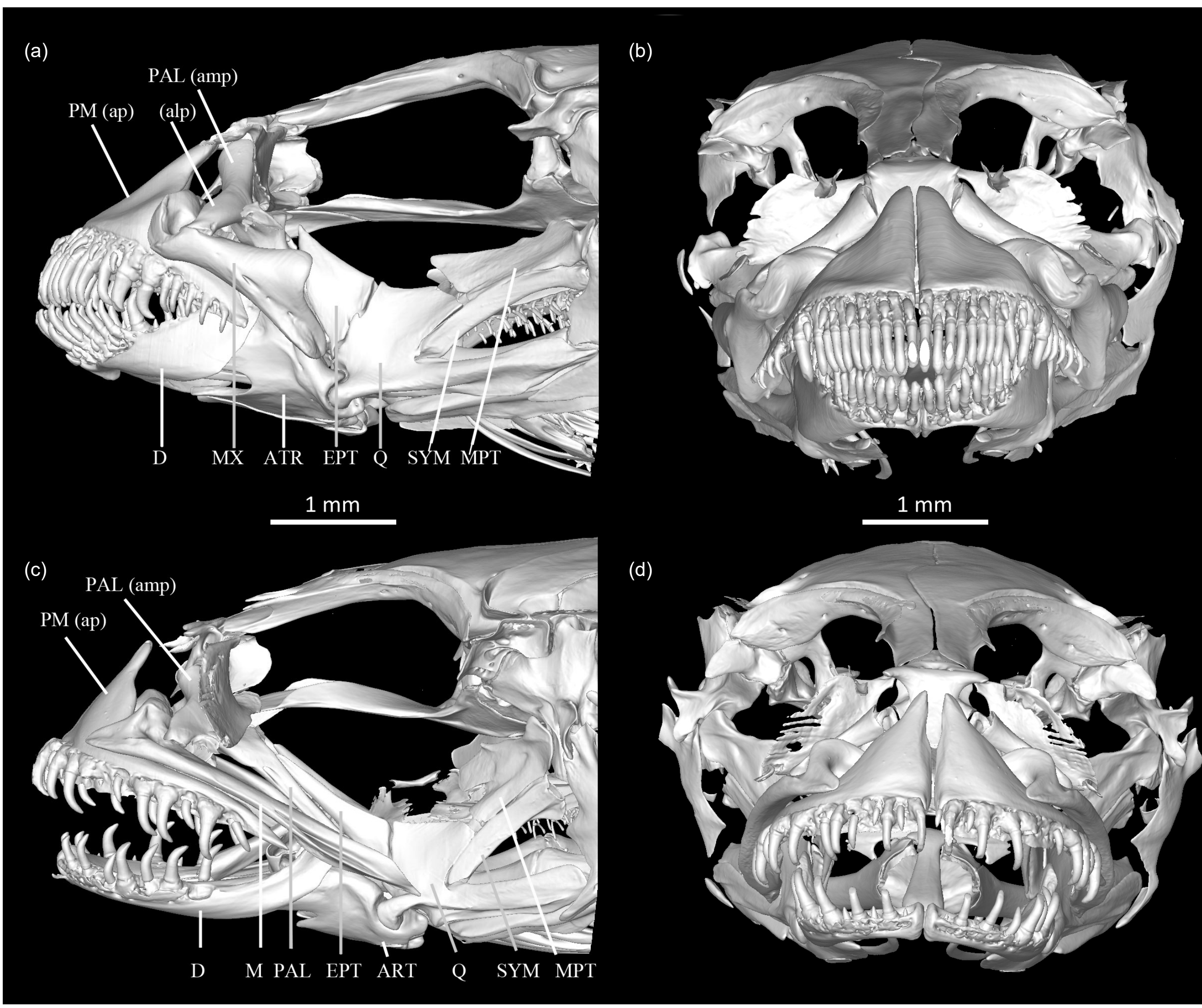

Fig. 3. 3D renderings of *Smilosicyopus leprurus* in (a) latera and (b) front views as well as *Sicyopus zosterophorum* in (c) lateral and (d) front views. Abbrevations: ART for anguloarticular; D for dentary; EPT for ectopterygoid; ME for mesethmoid; MPT for metapterygoid; M for maxilla; PAL for palatine; PM for premaxilla; Q for quadrate; SYM for symplectic; alp for anterior lateral process; amp for anterior media process; ap for ascending process.

(*in vivo*), we show that *Smilosicyopus leprurus* the first documented pterygophagy in the Gobiidae, despite a facultative fin-eater (Table 1), in addition to the Blennidae, Cichlidae, Distichodontidae and Serrasalmidae (Eccles and Lewis 1976; Eibl-Eibesfeldt 1959; Matthes 1961; Northcote et al. 1986; Ribbink et al. 1983).

Most gobies are territorial, for example *Smilosicyopus leprurus* and its relative *Sicyopus zosterophorum*. Fighting or expelling behavior is common and sometimes the defensive activity may result in broken and filamentous inter-radial membrane. However, the fin-eating behavior of *Smilosicyopus leprurus* is not a consequence of territorial behavior while expelling the intruders. Instead of broken membrane, *Smilosicyopus leprurus* shear a piece of fin off, including fin rays and inter-radial membrane, and the cut is sharp without any filamentous edge. The sharp cut is probably due to the bladed dentition. Compared to *Sicyopus zosterophorum*, the teeth of *Smilosicyopus leprurus* are numerous (16 vs. 8 on the left upper jaw) and arranged in close proximity to each other, all together like a bladed dentition providing the capability to shear the fin of its prey (Fig. 3). The difference of dentition may explain that although both *Smilosicyopus leprurus* and *Sicyopus zosterophorum* are aggressively terrestrial, fin-cutting behavior of *Sicyopus zosterophorum* is never observed. The bladed dentition is also present in other fin-eaters such as *Phago*, *Eugnathichthys*, and *Ichthyborus* (Arroyave and Stiassny 2014: fig. 1). This implies the correlation of the specialized teeth to the fin-eating behavior and further supports *Smilosicyopus leprurus* a fin-eater.

Sakai and Nakamura (1979) had shown the difference of dentition between *Sicyopus zosterophorum* and *Smilosicyopus leprurus*. However, their figure was just a line drawing and only shown in the lateral view. We noticed the dentition is more blade-like in the front view and the teeth at the front probably contribute more to the fin-cutting. Therefore, 3D renderings are recreated and the bladed dentition is shown clearly in the front view.

Although there are not many documented records, cannibalism is considered a common behavior in teleosts (Smith and Reay 1991). However, among fin-eater fishes, Arroyave and Stiassny (2014) showed cannibalistic behavior is infrequent. Only two out of 45 fin fragments were conspecific to the fin eaters, *Ichthyborus quadrilineatus* and *Phago boulengeri*. Our aquarium observation shows that the two individuals of *S. leprurus* didn't attack each other, even after two-day starvation. This is also supported by our own and hobbyists' observation (Chao, Zhou and Sun, pers. comm.) that *S. leprurus*' fins are always intact when several individuals kept in a tank. The peaceful coexistence implies *S. leprurus* not cannibalism. Instead of opportunistic prey selection strategy, *S. leprurus* selectively avoid its own kind.

Acknowledgment: We are grateful to Y.H. Chao, M.T. Zhou, T.W. Lin, I.H. Huang, Z.H. Zheng and W.C. Sun for their assistance in collecting fish, providing specimens and personal observation on the behavior of *Smilosicyopus leprurus*. This study was supported by grants to TYL from the Ministry of Science and Technology (NSC 103-2611-M-110 -007) and Asia-Pacific Ocean Research Center, National Sun Yat-sen University. YH is supported by Shanghai Municipal Natural Science Foundation (15ZR1448100).

REFERENCES

Arroyave J, Stiassny MLJ. 2014. DNA barcoding reveals novel insights into pterygophagy and prey selection in distichodontid fishes (Characiformes: Distichodontidae). Ecol Evol **4:**4534-4542.

Böhlke JE, Robins CR. 1968. Western Atlantic seven-spined gobies, with descriptions of ten new species and a new genus, and comments on Pacific relatives. Proc Acad Nat Sci Philadelphia **120:**45-174.

Chen IS, Tzeng CS, Shao KT. 2012. Red Data Book of Freshwater Fishes in Taiwan. Forestry Bureau, Council of Agriculture, Taipei.

Eccles DH, Lewis D. 1976. A revision of the genus *Docimodus* Boulenger (Pisces: Cichlidae), a group of fishes with unusual feeding habits from Lake Malawi. Zool J Linnean Soc **58:**165-172.

Eibl-Eibesfeldt I. 1959. Der fisch Aspidontus taeniatus als nachahmer des putzers *Labroides dimidiatus*. Z Tierpsychol **16(1):**19-25.

Flannery BP, Deckman HD, Roberge WG, D'Amico KL. 1987. Three-Dimensional X-ray Microtomography. Science **237:**1439-1444.

He Y, Chen XY, Xiao TQ, Yang JX. 2013. Three-dimensional morphology of the *Sinocyclocheilus hyalinus* (Cypriniformes: Cyprinidae) horn based on synchrotron X-ray microtomography. Zool Res **34:**E128-134.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. Proc R Soc Lond B **270:**313-321.

Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL. 2008. NCBI BLAST: a better web interface. Nucleic Acids Res **36 (Web Server issue):**5-9.

Matthes H. 1961. Feeding habit of some Central African fresh-water fishes. Nature **192:**78-80.

Metscher BD. 2009. MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues. BMC Physiol **9:**11.

Nelson JS. 2006. Fishes of the World. 4th eds.. Wiley, New York.

Northcote T, Northcote R, Arcifa M. 1986. Differential cropping of the caudal fin lobes of prey fishes by the piranha, *Serrasalmus spilopleura* Kner. Hydrobiologia **141:**199-205.

Sakai H, Nakamura M. 1979. Two new spesies of freshwater gobies (Gobiidae: Sicydiaphiinae) from Ishigaki Island, Japan. Jpn J Ichthyol **26:**43-54.

Smith C, Reay P. 1991. Cannibalism in teleost fish. Rev Fish Biol Fisher **1:**41-64.

Stiassny ML, Denton JS, Iyaba RJM. 2013. A new ecto-parasitic distichodontid of the genus *Eugnathichthys* (Characiformes: Citharinoidei) from the Congo basin of central Africa, with a molecular phylogeny for the genus. Zootaxa **3693:**479-490.

Okinawa Prefecture. 2005. Threatened Wildlife in Okinawa, 2nd Edition (Animals). Okinawa Prefecture, Naha.

Ribbink AJ. 1984. The feeding behaviour of a cleaner and scale, skin and fin eater from Lake Malawi (*Docimodus evelynae*; Pisces, Cichlidae). Netherlands J Zool **34:**182-196.

Ribbink AJ, Marsh BA, Marsh AC, Ribbink AC, Sharp BJ. 1983. A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. S Afr J Zool **18:**149-309.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. Phil Trans R Soc B **360:**1847-1857.

Wickler W. 1966. Mimicry in tropical fishes. Phil Trans R Soc B **251:**473-474.