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Note

Molecular identification of three deepsea fish species of the genus *Chelidoperca* (Perciformes: Serranidae) from Indian waters

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ABSTRACT

The deepwater basslets of the genus *Chelidoperca* has eight nominal species and these are relatively small fishes caught in trawl nets operated at depths greater than 100 m. Mitochondrial cytochrome c oxidase subunit 1 (COI) and 16S rRNA gene sequence variation among three species under the genus *Chelidoperca viz*., *C. investigatoris*, *C. occipitalis* and *C. maculicauda* from Indian waters and their phylogenetic relationship with other representatives from same genus was studied. Fifteen individuals of *Chelidoperca* were sampled from different localities in the east and west coasts of India and further, four COI sequences from GenBank were used to reconstruct the phylogenetic relationship in the genus *Chelidoperca*. Based on COI sequence data analysis, the intraspecies genetic distance ranged from 0.000 to 0.005 while interspecies distance varied from 0.073 to 0.194. With respect to 16S rRNA sequences, the intraspecies genetic distance ranged from 0.000 to 0.002, while interspecies genetic distance varied from 0.062 to 0.118. The mean genetic difference observed between *C. investigatoris* and the other species used in this study was 11.53%. Results of the study revealed that the genus *Chelidoperca* is monophyletic.

Keywords: *Chelidoperca*, Deepsea fishes, Mitochondrial COI gene, 16S rRNA

The family Serranidae comprises carnivorous marine fishes inhabiting tropical and subtropical waters. Members of the genus *Chelidoperca* are usually found in muddy continental shelf and slope bottoms in the Indo-West Pacific (Bineesh *et al*., 2013). These fishes are relatively small, usually less than 200 mm in length and are generally caught in trawl nets at depths greater than 100 m. Hence, only little information is available on these perchlets and are poorly represented in the museum collections worldwide (Williams and Carpenter, 2015). The genus *Chelidoperca* has eight valid species: *Chelidoperca hirundinacea* (Valenciennes, 1831); *C. investigatoris* (Alcock, 1890); *C. lecromi* Fourmanoir, 1982; *C. margaritifera* Weber, 1913; *C. occipitalis* Kotthaus, 1973; *C. pleurospilus* (Gunther, 1880); *C. maculicauda* Bineesh & Akhilesh, 2013 and *C. santosi* Williams & Carpenter, 2015 (Eschmeyer and Fong, 2015). Three species of *Chelidoperca*, namely *C. investigatoris*, *C. occipitalis* and *C. maculicauda* are reported from Indian waters (Bineesh *et al*., 2013). Targeted trawl fishery for deepsea shrimp is being carried out at three major harbours in India at Saktikulangara, Thoothukudi and Chennai. Deepsea shrimp trawl bycatch has been estimated to comprise >40 species (Pillai *et al*., 2009; Akhilesh *et al*., 2011). *Chelidoperca* spp. are landed as bycatch in deepsea shrimp trawlers and sold in the local market (Bineesh *et al.,* 2014; Shanis *et al*., 2014).

Accurate identification of morphologically similar species is essential for sustainable management of the fishery. Species identification using traditional morphology based methods sometime result in misidentification due to the morphological plasticity and overlapping characters. In such cases, DNA based molecular markers have proven to resolve taxonomic ambiguity to a great extent (Hebert *et al*., 2003). Presently, DNA based species identification has become very popular due to its ease of use, application to all life stages including eggs and larvae and even cooked food items. In the present study, mitochondrial COI and 16S rRNA gene sequences of the three species of *Chelidoperca viz.*, *C. investigatoris*, *C. occipitalis* and *C. maculicauda* were analysed to assess the genetic relatedness and phylogenetic relationship.

Specimens of *Chelidoperca investigatoris* (4 nos.) *C. maculicauda* (3 nos.) and *C. occipitalis* (4 nos.) were collected from bycatch landings of commercial deepsea shrimp trawls operated in the Arabian Sea (off Kollam) and Bay of Bengal (Thoothukudi and Chennai) during 2009-2011(Fig. 1) and these individuals were used for partial sequence analysis of mitochondrial COI and 16S rRNA genes. The voucher specimens were deposited in the fish collections at ICAR-National Bureau of Fish Genetic Resources (ICAR-NBFGR), Kochi and ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), Kochi, India. DNA extraction for all samples followed the protocol of Miller *et al*. (1988). The extracted DNA was checked by agarose (0.8%) gel electrophoresis and DNA concentration was estimated by optical density (OD) measurement using a spectrophotometer (SPECORD 205) at 260 nm.

The partial sequence of COI gene was amplified using primers Fish F1 (5' – TCA ACC AAC CAC AAA GAC ATT GGC AC - 3') and Fish R1 (5' -TAG ACT TCT GGG TGG CCA AAG AAT CA - 3') (Ward *et al*., 2005) in 25 µl reaction volume containing 1x assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0) with 1.5 mM $MgCl_2$ (Genei, Bangalore, India), 5 p moles of each primer, 200 µM of each dNTP (Genei, Bangalore, India), 1.5 U Taq DNA polymerase and 20 ng of template DNA. The thermal cycling comprised an initial preheating at 95°C for 3 min, denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 35 sec, repeated for 29 cycles, followed by a final extension for 3 min at 72°C. The mitochondrial 16S rRNA gene was also amplified in a 25 µl reaction volume containing 10x assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0) with 1.5 mM $MgCl_2$ (Genei, Bangalore, India), 5 p moles of each primer, 200 µM of each dNTP (Genei, Bangalore, India), 1.5 U Taq DNA polymerase and 50 ng of template DNA. The primer used for the amplification of the partial 16S rRNA gene were 16SAR (5'-CGC CTGTTTATC AAAAA CAT-3')and 16SBR (5'-CC GGTC T GAA CTCAGATCACGT-3') (Palumbi *et al*., 1991). The amplification conditions were 95°C for 7 min followed by 30 cycles at 94°C for 1 min, annealing at 53°C for 1 min and 72°C for 1 min, with a final extension of 72°C for 10 min. For each sample, 3 µl of PCR product was electrophoresed through 1.5% agarose gel followed by ethidium bromide staining, and visualised under UV illumination in the Gel-Doc system (BIO-RAD, Molecular Imager, Gel DocTM XR). Products were labelled using Big DyeTerminator V.3.1 cycle sequencing kit (Applied Biosystems Inc.) and sequenced bidirectionally using ABI 3730 capillary sequencer following the manufacturer's instructions.

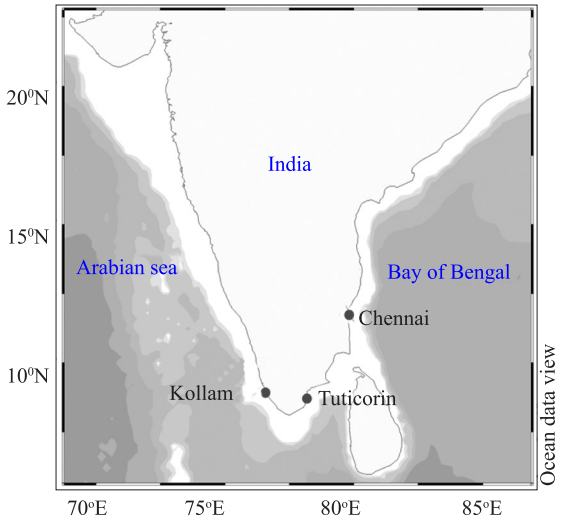

Fig. 1. Map showing the collection localities of *Chelidoperca* spp.

The raw DNA sequences were edited and aligned using BioEdit sequence alignment editor version 7.0.5.2 (Hall, 1999). The COI sequences of the individuals of each species were aligned to yield a final alignment of 675 bp. The 16S rRNA sequences of the individuals of each species were aligned to yield a final alignment of 510 bp. The edited sequences were submitted to GenBank (Table 1). The sequence divergence values within and between

Table 1. Details of *Chelidoperca* spp. used in the present study with collection locations and GenBank accession numbers

Species	Collection locality	GenBank Acc. no.		Voucher Reg. no.
		COI sequence	16S rRNA sequence	
Chelidoperca investigatoris	Thoothukudi	KP009557	KF814970	NBFGR CHN 3014
Chelidoperca investigatoris	Thoothukudi	KP009558		NBFGR CHN 3015
Chelidoperca investigatoris	Thoothukudi	KP009559	KF814973	NBFGR CHN 3016
Chelidoperca investigatoris	Chennai	JX185312	KF814971	NBFGR CHN 3020
Chelidoperca investigatoris	Chennai	JX185310	KF814972	NBFGR CHN 3021
Chelidoperca occipitalis	Kollam	JX185306	KF814974	GB31.139.16.1
Chelidoperca occipitalis	Mangaluru	JX185304		GB31.139.16.2
Chelidoperca occipitalis	Mangaluru	JX185311	KF814976	GB31.139.16.3
Chelidoperca occipitalis	Kollam	JX185313	KF814975	NBFGR CHN 3001
Chelidoperca maculicauda	Kollam	JX185308	KF814976	GB31.139.14.5

species were calculated using Kimura 2 Parameter (K2P) distance model implemented in MEGA V.6.0 (Tamura *et al*., 2013) software. The number of polymorphic sites and nucleotide diversity (Pi), nucleotide composition and number of transition and transversion between species were determined by DnaSp ver. 4.10.4 (Rozas *et al*., 2005). Neighbour Joining (NJ) trees of K2P distance were created to provide graphic representation of divergence with 1000 replications using MEGA V.6.0.

The COI sequences of all the individuals from different locations in Indian waters were used to estimate genetic divergence values and for constructing phylogenetic tree. A total of 675 base pairs of aligned sequences of COI gene were studied in *Chelidoperca*. Out of this 521 sites were constant, 154 sites exhibited variation of which 131 were parsimony informative and 21 were singletons. The DNA sequence information of COI gene was translated to amino acid sequences using MEGA 6.0 and all codon positions were identified. The estimated transition/ transversion bias (R) was 3.03. Most of the variability between the individuals and outgroup was observed at the third codon position. In the present study, all the codon positions were used for analysis. The observed nucleotide frequency in all the species was found to be G : 17.3%, T : 29.6%, A : 24.1% and C : 28.9%. The pairwise genetic distance values (K2P) based on COI sequences are given in Table 2. The highest distance was observed between *C. maculicauda* and *C. santosi* (0.194) and lowest distance between *C. investigatoris* and *C. pleurospilus* (0.071). Phylogenetic analysis using Maximum parsimony and Neighbour Joining identified three clades supported by high bootstrap values (Fig. 2).

Table 2. Pair-wise genetic distances (Kimura 2 parameter) based on COI sequences from *Chelidoperca* spp.

Species (with GenBank Acc. no.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C. maculicauda JX262929															
C. maculicauda JX185309	0.000														
C. maculicauda JX185308	0.000	0.000													
C. investigatoris KP009559	0.158	0.158	0.158												
C. investigatoris KP009558	0.158	0.158	0.158	0.000											
C. investigatoris KP009557	0.158	0.158	0.158	0.000	0.000										
C. investigatoris JX185312	0.158	0.158	0.158	0.000	0.000	0.000									
C. occipitalis JX185313	0.175	0.175	0.175	0.126	0.126	0.126	0.126								
C. occipitalis JX185311	0.177	0.177	0.177	0.128	0.128	0.128	0.128	0.001							
C. occipitalis JX185306	0.175	0.175	0.175	0.125	0.125	0.125	0.125	0.003	0.004						
C. occipitalis JX185304	0.177	0.177	0.177	0.124	0.124	0.124	0.124	0.001	0.003	0.004					
Chelidoperca sp. JQ681476	0.166	0.166	0.166	0.071	0.071	0.071	0.071	0.132	0.134	0.132	0.134				
C. pleurospilus JQ681449	0.164	0.164	0.164	0.073	0.073	0.073	0.073	0.130	0.132	0.130	0.132	0.001			
C. pleurospilus JQ681448	0.164	0.164	0.164	0.073	0.073	0.073	0.073	0.130	0.132	0.130	0.132	0.001	0.000		
C. santosi KP150308	0.194	0.194	0.194	0.137	0.137	0.137	0.137	0.148	0.150	0.148	0.150	0.126	0.128	0.128	0.00

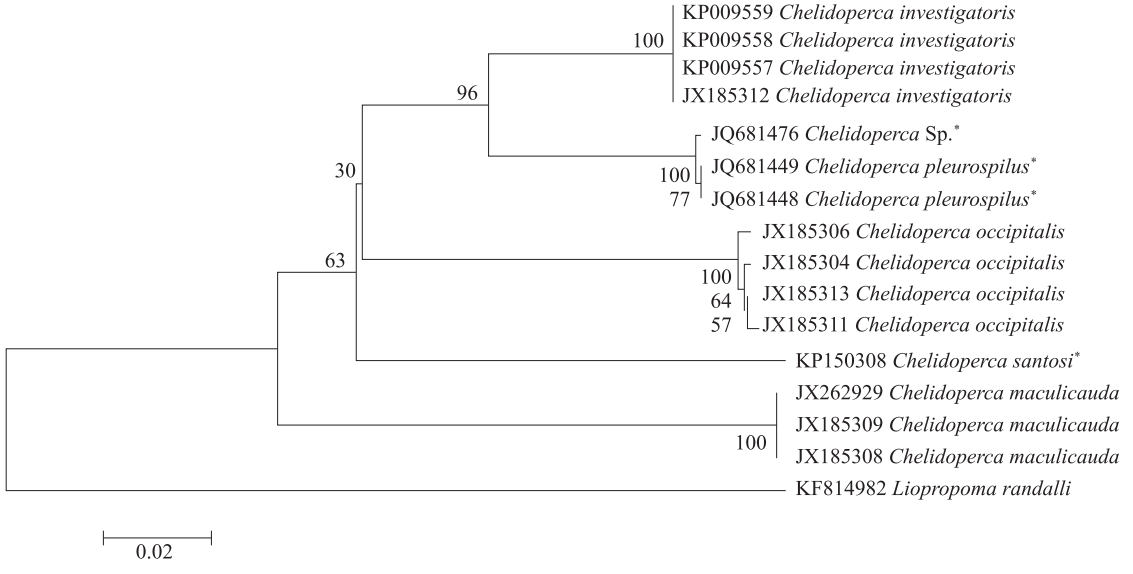

Fig. 2. Neighbor Joining (NJ) phylogenetic tree of *Chelidoperca* inferred from mitochondrial COI gene sequences (*indicates the sequences collected from NCBI)

16S rRNA gene sequencing produced an average of 511 nucleotide base pairs. The MEGA analysis showed nucleotide frequencies as A = 29.50%, T = 24.80%, G = 22.40% and C = 23.40%. The transition/transversion ratios obtained were: k1 = 18.339 (purines) and k2 = 68.152 (pyrimidines). The overall transition/transversion bias is R = 20.64. Of the 511 sites, 443, 56, 55 and 1 were invariable (monomorphic), variable (polymorphic), parsimony informative and singleton respectively. Pair-wise genetic distance values (K2P) based on 16S rRNA sequences using MEGA 6.1 are given in Table 3. Neighbour Joining (NJ) tree of Kimura two parameter (K2P) distances was also created to reveal identical phylogenetic relationship among the species (Fig. 3). Three well-supported highly divergent lineages were identified corresponding to all the three species in the genus *Chelidoperca*. The intraspecies genetic distance was found ranging from 0.000 to 0.002 while the interspecies distance ranged from 0.062 to 0.118.

Species identification through DNA barcoding technique is based on the principle that interspecific sequence divergence is more than intraspecific divergence and species can be separated by threshold value (Hebert *et al*., 2003). This is the first study of mt DNA sequences of the genus *Chelidoperca* in Indian waters to assess its taxonomic status and to develop species specific DNA barcodes for three species of the genus. From results of the present study, it is found that the genus *Chelidoperca* is monophyletic. The DNA barcode sequences based on partial sequence information of COI gene has been widely used in identification and validation of species (Ward *et al*., 2005; Lakra *et al*., 2009). The phylogenetic tree derived from two mitochondrial genes (COI and 16S rRNA) are identical and strongly suggest that all the individuals collected belong to three distinct species *viz*., *C. investigatoris*, *C. occipitalis* and *C. maculicauda* which are not sharing any haplotypes and there is no overlapping based on both COI and 16S rRNA genes.

The primer pairs for amplifying partial sequences of COI used in the present study was developed by Ward *et al.* (2005) mainly for invertebrates, but could successfully amplify an approximately 655 bp segment in all species. Within the mitochondrial gene, 16S rRNA gene is one among the extensively studied gene. Because of its slowest mutation rate compared to other mtDNA genes, it has been reported to be useful when analysing

Table 3. Pair-wise genetic distances (Kimura 2 parameter) based on 16S rRNA sequences from *Chelidoperca* spp.

	1	2	3	4	5	6	7	8	9
C. investigatoris	0.000								
C. investigatoris	0.002								
C. investigatoris	0.002	0.000							
C. investigatoris	0.002	0.000	0.000						
C. occipitalis	0.064	0.062	0.062	0.062					
C. occipitalis	0.064	0.062	0.062	0.062	0.000				
C. occipitalis	0.064	0.062	0.062	0.062	0.000	0.000			
C. maculicauda	0.118	0.116	0.116	0.116	0.091	0.091	0.091		
C. maculicauda	0.118	0.116	0.116	0.116	0.091	0.091	0.091	0.000	0.000

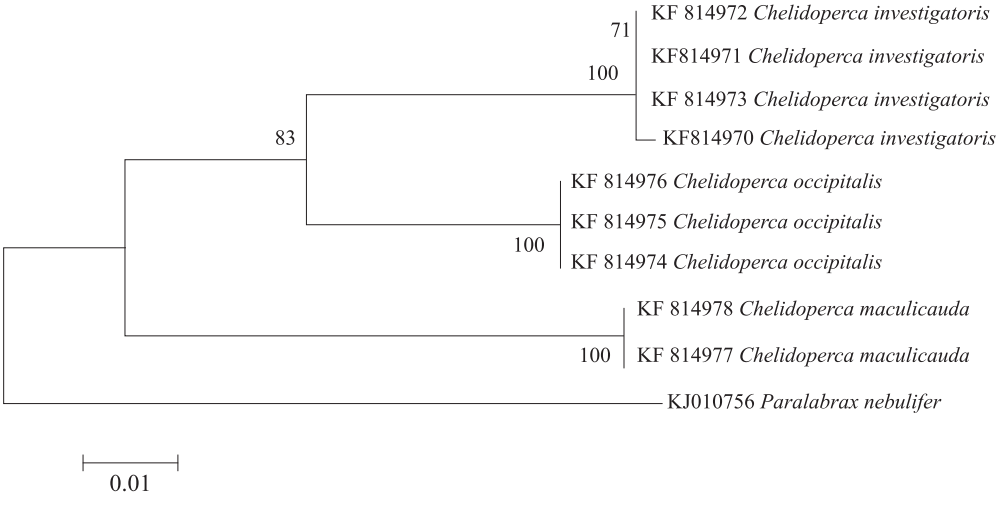

Fig. 3. Neighbor Joining (NJ) phylogenetic tree of *Chelidoperca* inferred from mitochondrial 16S rRNA gene sequences

families, species and population. The genetic divergence values obtained in the current study are comparable with the same obtained in other fish species using COI and 16S rRNA sequence data (Lakra *et al*., 2009; Basheer *et al*., 2015). The lowest distance between *C. investigatoris* and *C. pleurospilus* based on COI sequences was 7.1% and this value is above the threshold value proposed (Ward *et al.,* 2005). The GC content of 675 bp COI (46.2%) and 511 bp 16S rRNA (45.7%) was comparatively high in all the three species. The observed transition versus transversion ratios in *Chelidoperca* is also comparable to those of many serranid fishes (Craig and Hastings, 2007). This transition transversion ratio will develop estimates of divergence and phylogenetic relationships and also work as an indirect measure of saturation (loss of phylogenetic information). Phylogenetic analysis using NJ tree method with the COI and 16S rRNA data identified three major clades supported by high bootstrap values.

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References

Akhilesh, K. V., Ganga, U., Pillai, N. G. K., Vivekanandan, E., Bineesh, K. K., Rajool Shanis, C. P. and Manjebrayakath, H. 2011. Deepsea fishing for chondrichthyan resources and sustainability concerns - a case study from south-west coast of India. *Indian J. Geo. Mar. Sci.*, 40(3): 347-355.

Basheer, V. S., Mohitha, C., Vineesh. N., Divya, P. R., Gopalakrishnan, A. and Jena, J. K. 2015. Molecular phylogenetics of three species of the genus *Rastrelliger* using mitochondrial DNA markers. *Mol. Biol. Rep.,* 42(4): 873-879.

Bineesh, K. K., Akhilesh, K. V., Abdussamad, E. M. and Pillai, N. G. K. 2013. *Chelidoperca maculicauda*, a new species of perchlet (Teleostei: Serranidae) from the Arabian Sea. *Aqua Int. J. Ichthyol*., 19(2): 71-78.

Bineesh, K. K., Akhilesh, K. V., Abdussamad, E. M., Pillai, N. G. K., Ralf Thiel, Jena, J. K. and Gopalakrishnan, A. 2014. Redescriptions of *Chelidoperca investigatoris* (Alcock, 1890) and *Chelidoperca occipitalis* Kotthaus, 1973 (Perciformes: Serranidae) from the south-west coast of India. *Indian J. Fish*, 61(4): 117-122.

Craig, M. T. and Hastings, P. A. 2007. Molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. *Ichthyol. Res.*, 54(1): 1-17.

Eschmeyer, W. N. and Fong, J. D. 2015. Species of fishes by family/subfamily. On-line version dated 04/05.2015 *http://research.calacademy.org/research/ichthyology/catalog* (Accessed November 2015).

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser*., 41: 95-98.

Hebert, P. D. N., Cywinska, A., Ball, S. L. and deWaard, J. R. 2003. Biological identifications through DNA barcodes. *P. Roy. Soc. Lond. B. Bio.*, 270: 313-321.

Lakra, W. S., Goswami, M. and Gopalakrishnan, A. 2009. Molecular identification and phylogenetic relationships of seven Indian sciaenids (Pisces: Perciformes, Sciaenidae) based on 16S rRNA and cytochrome C oxidase subunit I mitochondrial genes. *Mol. Biol. Rep.,* 36(5): 831-839.

Miller, S. A., Dykes, D. D. and Polesky, H. F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*., 16(3): 1215.

Palumbi, S., Martin, A., Romano, S., McMillan, W. O., Stice, L. and Grabowski, G. 1991. *The simple fool's guide to PCR, version 2.0.* Honolulu: Department of Zoology and Kewalo Marine Laboratory, University of Hawaii.

Pillai, N. G. K., Bineesh, K. K., Manju. S. and Akhilesh. K. V. 2009. Lanternfishes (Myctophids): bycatch of deepsea shrimp trawlers operated off Kollam, south-west coast of India. *Mar. Fish. Inf. Serv. T&E Ser.,* 202: 1-4.

Shanis, C. P. R., Radhakrishnan, E. V., Ganga, U. and Pillai, N. G. K. 2014. Misidentification in fishery: the case of deep-sea pandalid shrimp *Plesionika spinipes* (Spence Bate, 1888) from Indian waters. *Int. J. Mar. Sci.,* 4(50): 1-4.

Rozas, J., Sanchez-DelBarrio, J. C., Messeguer, X. and Rozas, R. 2005. *DNA sequence polymorphism version 4.10.4* DNAsp4 computer software, Bareelena University, Barcelera, Spain.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: Molecular evolutionary genetics analysis, version 6.0. *Mol. Biol. Evol*., 30(12): 2725-2729.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R. and Hebert, P. D. N. 2005. DNA barcoding of Australia's fish species. *Philos. T. Roy. Soc. B.,* 360: 1847-1857.

Williams, J. T. and Carpenter, K. E. 2015. A new fish species of the subfamily Serraninae (Perciformes, Serranidae) from the Philippines. *Zootaxa*, 3911(2): 287-293.

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