MITOCHONDRIAL DNA PART B: RESOURCES, 2018
VOL. 3, NO. 1, 405–408
https://doi.org/10.1080/23802359.2018.1456982

Taylor & Francis
Taylor & Francis Group

MITO COMMUNICATION

Species identification of juvenile fishes of the genus *Pseudoblennius* using mitochondrial DNA barcoding

Hyuck Joon Kwun

National Marine Biodiversity Institute of Korea, Seocheon, Korea

ABSTRACT

Species identification is important in natural science and should be precise. Six specimens of juvenile *Pseudoblennius* were collected from the eastern coastal waters of the Korean Peninsula and Jeju Island in 2016–2017, and identified for the first time using DNA barcoding based on mitochondrial DNA cytochrome oxidase subunit I sequences. DNA barcoding analysis supported three adult species of genus *Pseudoblennius* (*P. cottoides*, *P. marmoratus*, and *P. percoides*) being quite distinct from each other. Six juvenile specimens were completely identified: two as *P. cottoides*; two more as *P. marmoratus*; and the final two as *P. percoides*. Mitochondrial DNA COI can be effective as a means of species identification method for the genus *Pseudoblennius*.

ARTICLE HISTORY
Received 8 March 2018
Accepted 17 March 2018

KEYWORDS
Barcoding; *Pseudoblennius*; identification; Korea; juvenile

Accurate species identification in natural science is important because misidentification has a serious negative effect on scientific results or decisions (Austen et al. 2016), such as mistaken identification of endangered species (Hunt 2015) and errors in species monitoring (Culverhouse et al. 2003).

Identification of marine fish larvae or juveniles is a prerequisite for understanding a species' life history, for which morphological characters have traditionally been used (Blaxter 1984). However, a problem with morphology-based identification is that many fishes exhibit the same or duplicated characters (Victor et al. 2009; Ko et al. 2013). In particular, closely related taxa, such as congeneric species and cryptic species, can be difficult to identify on the basis of morphology (Taylor and Watson 2004; Matarese et al. 2011). Thus, various new methods have emerged to solve this problem, including DNA barcoding.

DNA barcoding is a fast and easy method of species identification for taxonomic experts or non-experts using a single gene sequence (Hebert et al. 2003). The greatest benefit of DNA barcoding is that it can fill lacunae in morphological identification, given an understanding of ecology and evolution (Ko et al. 2013; Kress et al. 2015; Bhattacharya et al. 2016). Therefore, DNA barcoding is widely used in the identification of larval and juvenile marine fishes (Victor 2007; Paine et al. 2008; Hubert et al. 2010; Ji et al. 2017).

The sculpins family Cottidae is represented by about 70 genera and 282 species on the coasts of the Pacific Ocean (Nelson et al. 2016). They exhibit great diversity in both morphology and ecology, and most species occur in the intertidal region to the continental slope in the North Pacific (Hastings et al. 2014). The genus *Pseudoblennius* (Temminck and Schlegel 1850) contains only six nominal species distributed throughout the coastal waters of Korea and Japan in the northwestern Pacific (Nakabo and Kai 2013; Echmeyer et al. 2017). Of these six species, four have been reported from Korea (Kim et al. 2005): *Pseudoblennius cottoides* (Richardson 1848), *Pseudoblennius marmoratus* (Steindachner and Döderlein 1884), *Pseudoblennius percoides* (Günther 1861), and *Pseudoblennius zonostigma* (Jordan and Starks 1904). Although previous studies have provided morphological descriptions of larvae and juveniles (Yoo et al. 2003; Okiyama 2014), species in this genus are difficult to identify because early growth stages show similar shape, colouration pattern, and meristic characters. Also, indoor-reared early stage specimens exhibit a different morphology from natural specimens of the same species (Leis and Carson-Ewart 2000). Therefore, biochemical methods are necessary for accurate species identification, particularly for wild-captured specimens. The aim of the present study was to identify wild-captured juvenile specimens of the genus *Pseudoblennius* using DNA barcoding of the mitochondrial DNA cytochrome oxidase subunit I (COI) region, and to provide barcode information for this genus for the first time.

Six juvenile specimens of the genus *Pseudoblennius* were collected from the eastern coastal waters of the Korean Peninsula and Jeju Island in 2016–2017 (Figure 1; Table 1). For comparison, three adult *Pseudoblennius* species (*P. cottoides*, *P. marmoratus*, and *P. percoides*) were collected from the eastern coast of Jeju Island, Korea (Table 1); identification of these taxa was based on morphology, following Nakabo and Kai (2013). *Furcina osimae* (family Cottidae) was

CONTACT Hyuck Joon Kwun kwunhj@hotmail.com 75, 101 Jangsan-ro, Janghang-eup, Seocheon-gun, Chungcheongnam-do 33662, Korea

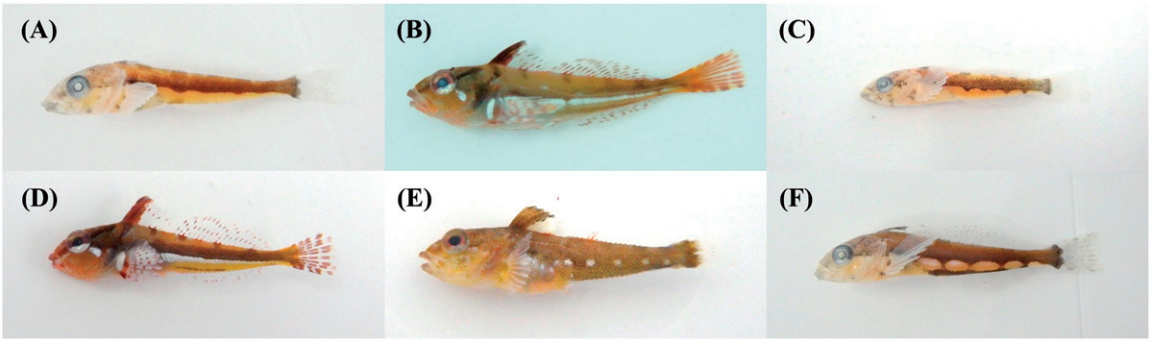

Figure 1. The photography of six juvenile *Pseudoblennius* specimens. (A) *Pseudoblennius* sp. 1, MFD-590; (B) *Pseudoblennius* sp. 2, MFD-673; (C) *Pseudoblennius* sp. 3, MFD-926; (D) *Pseudoblennius* sp. 4, MFD-927; (E) *Pseudoblennius* sp. 5, MFD-931; (F) *Pseudoblennius* sp. 6, MFD-949.

Table 1. List of specimens.

Species	Locality	Voucher no.	Accession no.
Juvenile			
Pseudoblennius sp. 1	Ulsan, Korea	MFD-590	MG922924
Pseudoblennius sp. 2	Jeju Island, Korea	MFD-673	MG922927
Pseudoblennius sp. 3	Jeju Island, Korea	MFD-926	MG922931
Pseudoblennius sp. 4	Jeju Island, Korea	MFD-927	MG922928
Pseudoblennius sp. 5	Jeju Island, Korea	MFD-931	MG922932
Pseudoblennius sp. 6	Samcheok, Korea	MFD-949	MG922926
Adult			
Pseudoblennius cottoides	Jeju Island, Korea	MFD-901	MG922925
Pseudoblennius marmoratus	Jeju Island, Korea	MFD-1024	MG922929
Pseudoblennius percoides	Jeju Island, Korea	MFD-672	MG922930
Outgroup			
Furcina osimae	Ulsan, Korea	MFD-607	MG922933

selected as an outgroup species. All specimens were fixed as whole-body specimens in 99% ethanol, and have been deposited at the Marine Fish Diversity (MFD) of the National Marine Biodiversity Institute of Korea.

The juvenile specimens were identified by means of the DNA barcoding protocol described by Ward et al. (2005). Genomic DNA was extracted from the right-side eye using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Mitochondrial DNA COI was amplified using a universal primer set (VF2_t1 and FishR2_t1) (Ward et al. 2005). The nucleotide sequences of all specimens (juvenile, adult, and outgroup specimens) have been deposited in the DDBJ/EMBL/GenBank databases (accession numbers: MG922924–922933). The sequences were aligned with ClustalW (Thompson et al. 1994) in BioEdit ver. 7 (Hall 1999). The genetic distances were calculated and a neighbour-joining (NJ) tree was produced using MEGA 7 (Kumar et al. 2016), based on the Kimura two-parameter model (Kimura 1980) and 10,000 bootstrap replications.

Mitochondrial DNA COI sequences for six juveniles and three adults of the genus *Pseudoblennius* were obtained. Based on an analysis of 618 base pairs (bp), interspecific genetic distances (*d*) calculated for three adult species of *Pseudoblennius* were between 0.012 and 0.125. Comparing juveniles and adults, *Pseudoblennius* sp. 1 corresponds to *P. cottoides* (genetic distance $d = 0.000$), but differs from *P. percoides* ($d = 0.077$) and *P. marmoratus* ($d = 0.125$). *Pseudoblennius* sp. 2 is similar to *P. marmoratus* ($d = 0.002$), but differs from *P. percoides* ($d = 0.117$) and *P. cottoides* ($d = 0.127$). *Pseudoblennius* sp. 3 almost corresponds to *P. percoides* ($d = 0.005$), but is distinct from *P. cottoides* ($d = 0.076$) and *P. marmoratus* ($d = 0.118$). *Pseudoblennius* sp. 4 can be identified as *P. marmoratus* ($d = 0.000$), but differs from *P. percoides* ($d = 0.119$) and *P. cottoides* ($d = 0.125$). *Pseudoblennius* sp. 5 almost corresponds to *P. percoides* ($d = 0.008$), but differs from *P. cottoides* ($d = 0.079$) and *P. marmoratus* ($d = 0.121$). *Pseudoblennius* sp. 6 be assigned to *P. cottoides* ($d = 0.000$), but differs from *P. percoides* ($d = 0.077$) and *P. marmoratus* ($d = 0.125$).

The result of the NJ analysis is shown in Figure 2. In the NJ tree, the three adult species of genus *Pseudoblennius* are well divided into three major clades, which are supported by high bootstrap values. *Pseudoblennius* sp. 1 and sp. 6 cluster with *P. cottoides*, *Pseudoblennius* sp. 2 and sp. 4 cluster with *P. marmoratus*, and *Pseudoblennius* sp. 3 and sp. 5 cluster with *P. percoides*, all of which are corroborated by 100% bootstrap value.

Overall, six juvenile specimens of *Pseudoblennius* are completely identified to the species level: *Pseudoblennius* sp. 1 and sp. 6 as *P. cottoides*, *Pseudoblennius* sp. 2 and sp. 4 as *P. marmoratus*, and *Pseudoblennius* sp. 3 and sp. 5 as *P. percoides*.

The present study provides mitochondrial DNA barcoding information for species identification within the genus *Pseudoblennius* for the first time. Mitochondrial DNA COI sequence data support the species-level distinction of three *Pseudoblennius* species. Therefore, mitochondrial DNA COI can be effective as a species identification method for the genus *Pseudoblennius*. In the future, further research could focus on validating previous morphological descriptions and identifying diagnostic morphological characters for use as taxonomic keys in juvenile *Pseudoblennius* species.

Disclosure statement

The author reports no conflicts of interest.

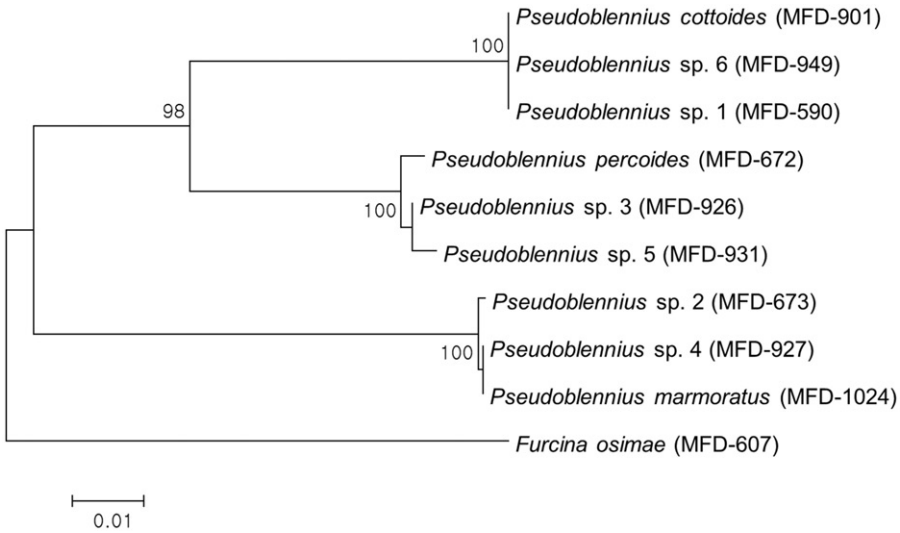

Figure 2. Neighbour-joining tree of mitochondrial DNA COI for three *Pseudoblennius* species including six juvenile specimens. Numbers of branches correspond to bootstrap probabilities in 10,000 bootstrap replications. Bar indicates genetic distance of 0.01.

Funding

This work was supported by National Marine Biodiversity Institute of Korea Program (2018M00100).

ORCID

Hyuck Joon Kwun http://orcid.org/0000-0003-2885-0042

References

Austen GE, Bindemann M, Griffiths RA, Roberts DL. 2016. Species identification by experts and non-experts: comparing images from field guides. Sci Rep. 6:33634.

Bhattacharya M, Sharma AR, Patra BC, Sharma G, Seo EM, Nam JS, Chakraborty C, Lee SS. 2016. DNA barcoding to fishes: current status and future directions. Mitochondrial DNA A DNA Mapp Seq Anal. 27:2744–2752.

Blaxter JHS. 1984. Ontogeny, systematic and fisheries. In: Moser HG, Richards WJ, Cohen DM, Fahay MP, Kendall Jr AW, Richardson SL, editors. Ontogeny and systematic of fishes based on an international symposium dedicated to the memory of Elbert Halvor Ahlstrom. Gainesville (FL): America Society of Ichthyologists and Herpetologists; p. 1–6.

Culverhouse PF, Williams R, Reguera B, Herry V, González-Gil S. 2003. Do experts make mistakes? A comparison of human and machine identification of dinoflagellates. Mar Ecol Prog Ser. 247:17–25.

Echmeyer WN, Fricke R, van der Laan R. 2017. Catalog of fishes: genera, species, references. San Francisco (CA): California Academy of Sciences; [accessed 2018 Jan 17]. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp

Günther A. 1861. Catalogue of the acanthopterygian fishes in the collection of the British Museum. Gobiidae, Discoboli, Pediculati, Blenniidae, Labyrinthici, Mugilidae, Notacanthi. London (UK): Order of the Trustees (Catalogue of the fishes in the British Museum; 3).

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser. 41:95–98.

Hastings PA, Walker HJ, Galland GR. 2014. Fishes: a guide to their diversity. Oakland (CA): University of California Press.

Hebert PD, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. Proc Biol Sci. 270:313–321.

Hubert N, Delrieu-Trottin E, Irisson JO, Meyer C, Planes S. 2010. Identifying early stages of coral reef fishes through DNA barcoding: a test case with the families Acanthuridae and Holocentridae. Mol Phylogenet Evol. 55:1195–1203.

Hunt E. 2015. New Zealand hunters apologise over accidental shooting of takahē. The Guardian. Environment [about 3 screens]; [accessed 2018 Jan 15]. https://www.theguardian.com/environment/2015/aug/21/new-zealand-conservationists-apologise-over-accidental-shooting-of-endangered-takahe

Ji JM, Yu HJ, Hwang KS, Park JH, Lee JH, Kim JK. 2017. Molecular identification and morphological development of larvae of *Psettina tosana* collected from southern sea of Korea. Korean J Ichthyol. 29:244–251 (in Korean).

Jordan DS, Starks EC. 1904. A review of the Cottidae or sculpins found in the waters of Japan. Proc US Natl Mus. 27:231–335.

Kim IS, Choi Y, Lee CL, Lee YJ, Kim BJ, Kim JH. 2005. Illustrated book of Korean fishes. Seoul (KR): Kyo-Hak Publishing Co. (in Korean).

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions though comparative studies of nucleotide sequences. J Mol Evol. 16:111–120.

Ko HL, Wang YT, Chiu TS, Lee MA, Leu MY, Chang KZ, Chen WY, Shao KT. 2013. Evaluating the accuracy of morphological identification of larval fishes by applying DNA barcoding. PLoS One. 8:e53451.

Kress WJ, García-Robledo C, Uriarte M, Erickson DL. 2015. DNA barcodes for ecology, evolution, and conservation. Trends Ecol Evol (Amst). 30:25–35.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 33:1870–1874.

Leis JM, Carson-Ewart BM. 2000. The larvae of Indo-Pacific coastal fishes: an identification guide to marine fish larvae. Leiden (NL): Brill.

Matarese AC, Spies IB, Busby MS, Orr JW. 2011. Early larvae of *Zesticelus profundorum* (family Cottidae) identified using DNA barcoding. Ichthyol Res. 58:170–174.

Nakabo T, Kai Y. 2013. Cottidae. In: Nakabo T, editor. Fishes of Japan with pictorial keys to species. 3th ed. Tokyo (JP): Tokai University Press; p. 1160–1188 (in Japanese).

Nelson JS, Grande T, Wilson MVH. 2016. Fishes of the world. 5th ed. Hoboken (NJ): John Wiley and Sons Inc.

Okiyama M. 2014. An atlas of early stage fishes in Japan. 2nd ed. Hadano (JP): Tokai University Press (in Japanese).

Paine MA, McDowell JR, Graves JE. 2008. Specific identification using COI sequence analysis of scombrid larvae collected off the Kona coast of Hawaii Island. Ichthyol Res. 55:7–16.

Richardson J. 1848. Fishes. In: Adams, editor. The zoology of the voyage of H. M. S. Samarang; under the command of Captain Sir Edward

Belcher, during the years 1843–1846. London (UK): Reeve and Benham; p. 1–28.
Steindachner F, Döderlein L. 1884. Beiträge zur Kenntniss der Fische Japan's. Denkschr Kaiserl Akad Wiss Wien Math-Naturwiss Kl. 49:171–212.
Taylor CA, Watson W. 2004. Utility of larval pigmentation to identify nearchore rockfishes of the *Sebastes* subgenus *pteropodus* from southern California. CalCOFI Rep. 45:113–117.
Temminck CJ, Schlegel H. 1850. Pisces. In: de Siebold PF, editor. Fauna Japonica sive descriptio animalium, quae in itinere per Japoniam, jussu et auspiciis superiorum, qui summum in India Batava imperium tenent, suscepto, annis 1825–1830 collegit, notis, observationibus et adumbrationibus illustravit Ph. Fr. de Siebold. Conjunctis studiis C. J. Temminck et H. Schlegel pro vertebratis atque W. de Haan pro invertebratis elaborata. Leiden (NL): Lugduni Batavorum; p. 270–324.
Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673–4680.
Victor BC. 2007. *Coryphopterus kuna*, a new goby (Perciformes: Gobiidae: Gobinae) from the western Caribbean, with the identification of the late larval stage and an estimate of the pelagic larval duration. Zootaxa. 1526:51–61.
Victor BC, Hanner R, Shivji M, Hyde J, Caldow C. 2009. Identification of the larval and juvenile stages of the cubera snapper, *Lutjanus cyanopterus*, using DNA barcoding. Zootaxa. 2215:24–36.
Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. Philos Trans R Soc Lond B Biol Sci. 360:1847–1857.
Yoo DJ, Han KH, Baek SR, Kim KS, Ha SC, Zang HC, Lee GS. 2003. Morphological development of eggs, larvae and juvenile of the sunrise sculpin, *Pseudoblennius cottoides* (Teleostei: Cottidae). J Kor Fish Soc. 36:263–269 (in Korean).