Pakistan J. Zool., vol. 49(5), pp 1901-1907, 2017. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2017.49.5.1901.1907

New Genetic Evidence from Three Keel-Backed *Liza* Species Based on DNA Barcoding Confirms Morphology-Based Identification

Lu Liu[1], Sher Khan Panhwar[2], Tianxiang Gao[3], Zhiqiang Han[3], Chunhou Li[4], Dianrong Sun[4] and Na Song[1,*]

[1]*Fisheries College, Ocean University of China, Qingdao, 266003, People's Republic of China*
[2]*Center of Excellence in Marine Biology, University of Karachi, Karachi, 75270, Parkistan*
[3]*Fishery College, Zhejiang Ocean University, 266003, People's Republic of China*
[4]*South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, 510003, People's Republic of China*

Article Information
Received 14 February 2017
Revised 18 March 2017
Accepted 25 March 2017
Available online 22 September 2017

Authors' Contribution
LL designed this study, supervised the work and wrote the article. SKP, DS and CL collected samples and the data. ZH and NS analylyzed the data statistically. TXG, NS helped in preparation of manuscript.

Key words
DNA barcoding, *Liza affinis*, *Liza carinata*, *Liza klunzingeri*, Morphological identification.

ABSTRACT

Three *Liza* species exhibiting a dorsal keel have been identified based on morphology: *Liza affinis*, *Liza carinata*, and *Liza klunzingeri*. To confirm the validity of the species by molecular methods, we sequenced a fragment of the cytochrome oxidase subunit I (COI) gene of mitochondrial DNA of *Liza affinis* and *Liza klunzingeri* from coastal waters of China and Pakistan, respectively. Sequences of *L. carinata* were obtained from GenBank. A neighbor-joining tree showed the specimens to form a strong monophyletic group. The mean within-species genetic distances for *L. affinis*, *L. carinata*, and *L. klunzingeri* were 0.26%, 0.52%, and 0.47%, respectively. The mean genetic distances among *L. affinis*, *L. carinata*, and *L. klunzingeri* ranged from 12.22% to 14.74%, higher than the threshold for species delimitation. The results confirmed the taxonomic validity of the three *Liza* species and verified that the COI gene could provide effective DNA barcoding for identification.

INTRODUCTION

Fishes are the largest vertebrate group comprising more than 30000 species (Eschmeyer *et al*., 2010). Besides their importance in biodiversity, they also have a major impact on the economy of many nations through fisheries, providing a significant amount of world food supply. To provide sustainability, better control and management of fisheries should be implemented. Identification of fish species still stands high as one of the most basic but important issues in fisheries management (Walters and Holling, 1990). Most fish species are marketed under their common names, which vary among regions and even within regions (Keskin and Atar, 2013). The genus *Liza* belongs to the class Mugiliformes, family Mugilidae. They are widely distributed throughout tropical and temperate seas, and most are commercially harvested. Taxonomic differentiation among *Liza* species has traditionally been based on morphology. However, external characters may not be clearly differentiated among species, resulting in taxonomic uncertainty and imprecise nomenclature (Heras *et al*., 2009).

The *Liza carinata* complex (Jordan and Swain 1884) is a distinct group, characterized by a mid-dorsal keel anterior to the spiny dorsal fin. The members of this complex are irregularly distributed in the tropical and temperate Indo-West Pacific region of the Northern Hemisphere, inhabiting estuarine and shallow coastal waters, and have frequently been taxonomically confused (Okiyama, 1993; Shao *et al*., 1993; Xu *et al*., 1994; Randall, 1995).

The taxonomy of keel-backed *Liza* species was revised by Senou *et al*. (1987), based on morphology, to include three species: *Liza carinata* (Valenciennes 1836) distributed in the Red Sea and the eastern Mediterranean; *Liza klunzingeri* (Day 1888) distributed on the west coast of India, Pakistan, and the Arabian Gulf (Persian Gulf); and *Liza affinis* (Günther 1861) in China, Taiwan, and Japan.

Morphology, as a traditional taxonomic method, has been successful in describing diverse species, and is the foundation of classification and identification. However,

* Corresponding author: songna624@163.com
0030-9923/2017/0005-1901 $ 9.00/0

there are significant limitations to morphological diagnosis for routine identification. Phenotypic plasticity and genetic variability in distinguishing characters can lead to incorrect assignment (Herbert *et al*., 2003), and cryptic species may be overlooked (Knowlton and Weight, 1998; Jarman and Elliott, 2000). In addition, morphological characteristics may be limited to a life stage or sex.

DNA barcoding can distinguish species by detecting interspecific genetic variation that is higher than intraspecific variation. DNA barcoding is poised to contribute to taxonomic research, population genetics, and phylogenetics (Herbert *et al*., 2003; Puckridge *et al*., 2013). In taxonomy, DNA barcoding can be used for routine identification of specimens and for investigation of atypical specimens (Hajibabaei *et al*., 2007). Although the description and identification of a new species is ultimately achieved through comprehensive morphological study, DNA barcoding can significantly enhance this process. The workflow of traditional classification, involving collection of morphological and ecological data, can lead to differing classification results (Xiao *et al*., 2016), whereas barcode analysis can be applied in a standardized way to all life stages (Hajibabaei *et al*., 2007). In the present study, we used DNA barcoding to validate morphological identification of three keel-backed *Liza* species.

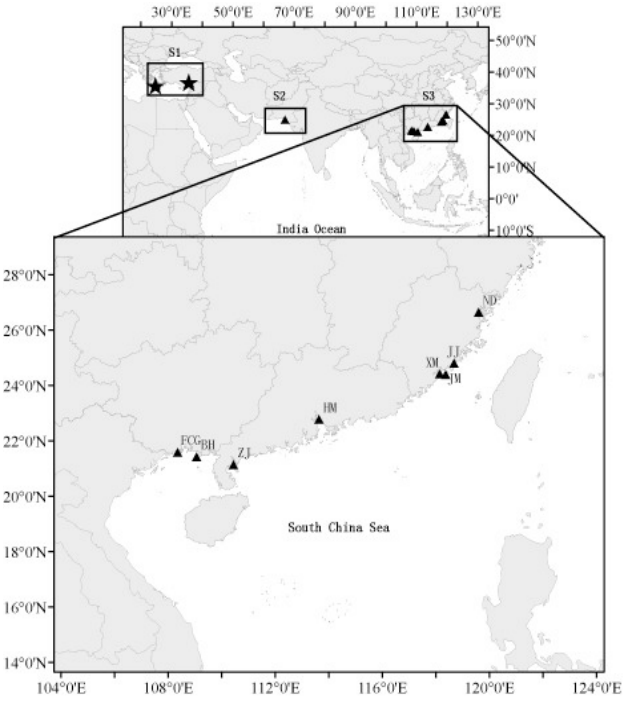

Fig. 1. Sampling sites in the present study; S1, Liza carinata; S2, Liza klunzingeri; S3, Liza affinis; *, sites indexed from GenBank and reference [2]

MATERIALS AND METHODS

Sampling

Fish were collected from coastal waters of China and Pakistan (Fig. 1). Pectoral fin length/body length, head length/body length, and the total number of gill rakers were used to identify the samples as *Liza affinis* (Fig. 2A) and *Liza klunzingeri* (Fig. 2B). Sequence of the COI gene of *Liza carinata* (Fig. 2C) was obtained from GenBank. *Mugil cephalus* was chosen as outgroup for genetic analysis. All specimens were frozen and preserved at the Fishery Ecology Laboratory, Fisheries College, Ocean University of China (Qingdao).

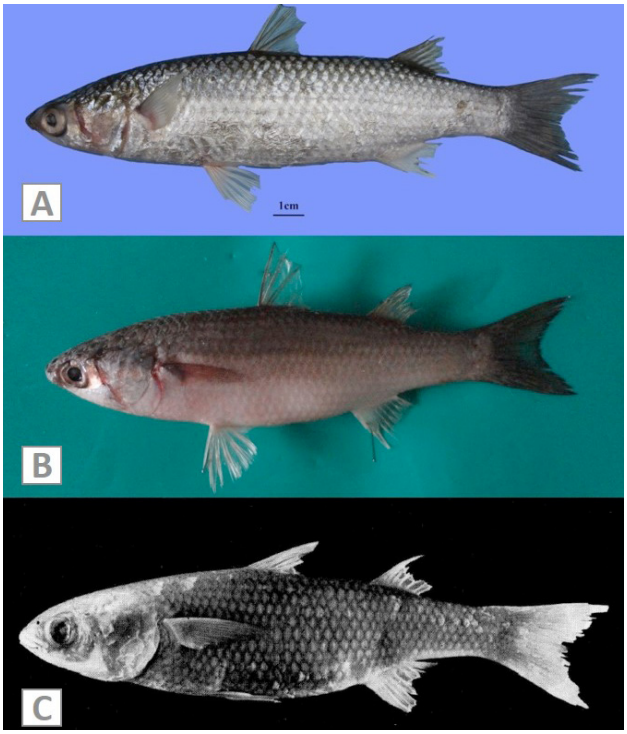

Fig. 2. A, Liza affinis (GÜnther, 1861); B, Liza klunzingeri (Day, 1888); C, Liza carinata (Valenciennes, 1836)

Morphological identification

Data were collected from 26 *Liza affinis* specimens from various locations off the coast of China and 50 *Liza klunzingeri* specimens collected off the coast of Karachi, Pakistan.

Meristic and morphometric characters were compared with previous records of *Liza affinis* and *Liza klunzingeri*. Counts and measurements were made according to Senou *et al*. (1987). The distinguishing characters included counts of gill rakers, pectoral fin length, body length, and head length. Measurements were made with calipers to the nearest 0.1mm.

Table I.- Sampling locations and sequence data.

Species	Sampling sites	Sampling time	Samples	Index accession No.
Liza affinis	Jinmen, China	October, 2013	3	KU884213-KU884238
	Ningde, China	October, 2014	3	
	Jinjiang, China	November, 2013	3	
	Xiamen, China	February, 2014	4	
	Humen, China	May, 2014	4	
	Zhanjiang, China	November, 2014	3	
	Beihai China	December, 2014	3	
	Fangchenggang, China	November, 2015	3	
Liza klunzingeri	Karachi, Pakistan	November, 2015	50	KU884239-KU884288
Liza carinata	Iskenderun Bay, Turkey	2011	21	JQ623947, KC500833-KC500852
	Red Sea, Egypt	December, 2009	1	FN600159
Mugil cephalus	Taiwan, China	2012	1	JX559532

KEY TO THE SPECIES OF KEELED-BACK *LIZA* (Senou *et al*., 1987):

1 dorsal midline keel

2(3) pectoral fin length 14.5-18.4% of body length; head length 22.1-26.9% of body length.....*Liza affinis*

3(2) pectoral fin length 19.8-23.9% of body length, head length 27.0-31.3% of body length

4(5) Number of gill rakers 79-96 (66.3-91.0 mm body length), 94-109 (110.1-138.6mm body length).....*Liza klunzingeri*

5(4) Number of gill rakers 69-82 (66.3-91.0mm body length), 83-93 (106.0-124.1mm body length).....*Liza carinata*

DNA extraction and sequencing

Pieces of muscle were removed and preserved in 95% ethanol. Total genomic DNA was extracted by proteinase K digestion followed by a standard phenol-chloroform method. A 514 bp fragment was amplified from the 5' region of the cytochrome oxidase subunit I (COI) gene of mitochondrial DNA using the following primers (Ward *et al*., 2005):

FishF1-5' - TCA ACC AAC CAC AAA GAC ATT GGC AC -3'

FishR1-5'- ACT TCA GGG TGA CCG AAG AAT CAG AA -3'

The PCR reactions were carried out in 25 µL reaction mixture containing 17.25 µL ultrapure water, 2.5 µL 10×PCR buffer, 2 µL dNTPs, 1 µL of each primer (5 µM), 0.25 µL Taq polymerase, and 1µL of DNA template. The thermal regime consisted of an initial step of 2 min at 95°C followed by 40 cycles of 30 s at 94°C, 45 s at 52°C, and 1 min at 72°C; with a final step of 10 min at 72°C, after which the reaction was held at 7°C. Negative controls were included in all PCR reactions to confirm the absence of contaminants. PCR products were purified with a Gel Extraction MiniKit (Watson Bio Technologies Inc., China). The purified products were sequenced on ABI Prism 3730 (Applied Biosystems) from both strands with the same primers used for PCR reactions.

Sequence analysis

For phylogenetic analysis, COI sequences of *Mugil cephalus*, of the same family as *Liza*, were obtained from GenBank. Species and GenBank accession numbers are listed in Table I. Sequences were aligned using DNASTAR software (DNASTAR, Madison, WI, USA), and a neighbor-joining tree (Saitou and Nei, 1987) was constructed in MEGA 5.0 (Tamura *et al*., 2011) with 1000 bootstrap replications, based on evolutionary distances calculated using the Kimura two parameter (K2P) model (Kimura, 1980).

RESULTS

Morphological characters

Liza affinis, weight 75.5-90.7 g. Meristic characters: dorsal fin IV, I-8; anal fin III, 9; pectoral fins 15-17; pelvic fins I-5; lateral line scales 35-41; gill rakers 71-77. Morphometric characters: head length 27.5-34.9 mm; pectoral fin length 20.0-26.7 mm; Body length 116.4-151.0 mm. body elongate, subcylindrical anteriorly, becoming compressed toward the tail. Mid-dorsal line keeled anterior to the first dorsal fin, the keel forming a sharp edge. Keel between the first and the second dorsal fins absent or insignificant. Head small, interorbital space slightly convex in frontal view. Adipose eyelid well developed anteriorly and posteriorly, posterior adipose eyelid thick.

Table II.- Comparison of meristic data for *Liza affinis*, *Liza klunzingeri*, and *Liza carinata* with those of Senou *et al.* (1987).

		Dorsal fin	Pectoral fin	Anal fin	Pelvic fin	Lateral line scales	Gill rakers
Liza affinis	Present study	IV, I-8	15-17	III, 9	I-5	35-41	71-77
	Senou *et al.* (1987)	IV, I-8-10	15-18	III, 9-10	I-5	35-43	69-89
Liza klunzingeri	Present study	IV, I-8	15-16	III, 9-10	I,5-6	32-38	(66.3-91.0 mm Body length) 82-94 (110.1-138.6mm Body length) 94-105
	Senou *et al.* (1987)	IV, I-9	15-17	III, 9	I-5	32-38	28-40+51-69
Liza carinata	Senou *et al.* (1987)	IV, I-8,9	15-18	III, 9	I-5	36-40	25-35+44-59

Liza klunzingeri, weight 62.5-106.7 g. Meristic characters: dorsal fin IV, I-8; anal fin III, 9-10; pectoral fins 15-16; pelvic fins I-5-6; lateral line scales 32-38; gill rakers 82-105. Morphometric characters: Head length 26.5-43.0 mm; pectoral fin length 18.1-32.1 mm; body length 91.2-155.0 mm. body short, well compressed. Mid-dorsal line keeled anterior to 1st dorsal fin. Keel well developed, forming sharp edge No keel between first and second dorsal fins. Head large, interorbital space slightly convex in frontal view. Adipose eyelid well developed anteriorly and posteriorly, relatively thin in posterior.

Data on selected morphological characters of *Liza affinis*, *Liza klunzingeri*, and *Liza carinata* in the present study and Senou *et al.* (1987) are shown in Table II. Pectoral fin length/body length and head length/body length of *Liza affinis*, *Liza klunzingeri*, and *Liza carinata* in the present study and Senou *et al.* (1987) are shown in Table III.

Table III.- Comparison of morphometric characters of *L. affinis*, *L. klunzingeri*, and *L. carinata* with those of Senou *et al.* (1987).

		Pectoral fin length/body length %	Head length/ body length %
Liza affinis	Present study	15.0-18.3	22.5-25.1
	Senou *et al.* (1987)	14.5-18.4	22.1-26.9
Liza klunzingeri	Present study	19.7-23.7	26.9-30.4
	Senou *et al.* (1987)	19.8-23.9	27.0- 31.3
Liza carinata	Senou *et al.* (1987)	19.8-23.9	27.0- 31.3

Sequence analysis of the COI gene

The COI gene fragments of 26 *Liza affinis* and 50 *Liza klunzingeri* specimens were amplified. COI sequences were submitted to GenBank under the accession numbers KU884213-KU884238 for *Liza affinis* and KU884239-KU884288 for *Liza klunzingeri*.

A neighbor-joining tree was constructed based on a K2P model with 1000 bootstrap replications. *Mugil cephalus* was chosen as outgroup to root the tree (Fig. 3). Three haplotypes were detected in the COI sequences of *Liza carinata* (GenBank) and in the COI sequences of *Liza affinis*. One haplotype (Hap 1) of *Liza affinis* was unique to a specimen from Beihai. A second haplotype (Hap 2) of *Liza affinis* was shared in two specimens from Fangchenggang and Humen. All remaining specimens of *Liza affinis* shared the third haplotype (Hap 3).

Seventeen haplotypes were detected in the COI sequences of *Liza klunzingeri*.

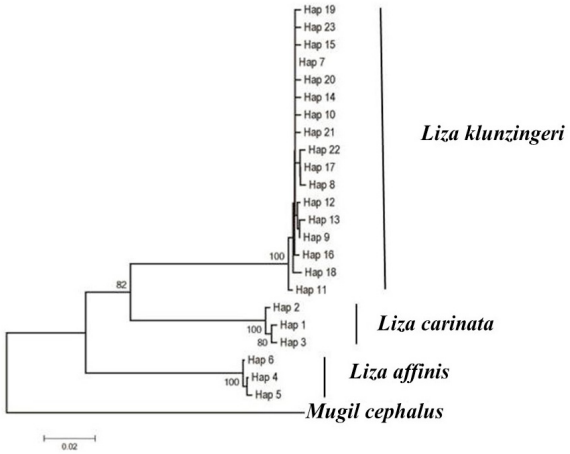

Fig. 3. Neighbor-joining tree constructed using the K2P model for COI gene sequences of three Liza species with a keel on the back. Bootstrap values of >50% from 1000 replicates are shown.

The phylogenetic tree consisted of three main branches, with *L. klunzingeri* in the upper branches clustering with *L. carinata* and *L. affinis* in the third branch. The mean within-group K2P distances for *L. affinis*, *L. klunzingeri*, and *L. carinata* were 0.26%, 0.47%, 0.52%, respectively, in contrast to the distances between *Liza carinata* and *Liza klunzingeri* (12.22%), *Liza affinis* and *Liza klunzingeri* (14.74%), *Liza affinis* and *Liza carinata* (13.01%). This exceeded the threshold of species

delimitation (3.5%) proposed and applied by Ward et al. (2005, 2009) based on 10× the mean intra-species genetic variation (Hebert et al., 2004).

Table IV.- Genetic distances of COI within (on the diagonal) and among (below the diagonal) the four species.

	Liza carinata	***Liza klunzingeri***	***Liza affinis***	***Mugil cephalus***
Liza carinata	0.0052			
Liza klunzingeri	0.1222	0.0047		
Liza affinis	0.1301	0.1474	0.0026	
Mugil cephalus	0.2274	0.2307	0.2096	0.0000

DISCUSSION

DNA barcoding using the COI gene is recognized as an effective and reliable molecular method for identification of species (Herbert *et al*., 2003). DNA barcoding can be useful in species diagnosis because sequence divergences are ordinarily much lower among individuals of a species than between closely related species. For example, congeneric species of moths show an average sequence divergence of 6.5% in the mitochondrial gene cytochrome c oxidase I (COI), whereas divergences among conspecific individuals average only 0.25% (Moore, 1995). Similar values were obtained in birds, with intraspecific divergences at COI averaging 0.27%, whereas congener divergences averaged 7.93% (Hebert *et al*., 2004).

Because of the advantages offered by molecular diagnostic tools, morphological methods are sometimes neglected. However, adequate identification by traditional taxonomic keys is usually an essential first step in the development and introduction of new molecular methods (Mehle and Trdan, 2012). In addition, identification by molecular methods may still require morphologically based confirmation of identification to genus (Rugman-Jones *et al*., 2006). The limitations of molecular methods, and their ineffectiveness in distinguishing cryptic species and larva, suggests that taxonomy should encompass both traditional and molecular methods.

Liza includes species inhabiting coastal, brackish, and fresh waters of all tropical and temperate regions worldwide. Within the genus, interspecific variability of meristic characters usually overlap, and some anatomical characters considered to be of taxonomic value undergo marked changes with growth, potentially leading to misidentification.

Senou *et al*. (1987) reviewed the *L. carinata* complex from most known localities, including the type materials, and identified three species, based on morphology. In their research, more than one hundred morphological indices were used to distinguish the three species and produce a taxonomic key. Due to the significant limitations of morphological diagnosis for routine identification, the results need to be confirmed.

The present study, based on morphological characters as well as DNA barcoding, documented the validity of the work of Senou *et al*. (1987). COI sequence analysis demonstrated significant differences among *L. affinis*, *L. klunzingeri*, and *L. carinata*, confirming the validity of the species at genetic level. Net evolutionary distances among the species ranged from 12.22% to 14.75%, greater than the threshold for species delimitation. The phylogenetic tree consisted of three main branches and showed morphologically similar *L. klunzingeri* and *L. carinata* to be clustered in one clade with *L. affinis* at the bottom of the tree. However, *L. catinata* is reported to be endemic to the Red Sea and the eastern Mediterranean, and *L. klunzingeri* occurs in the west coast of India and Pakistan, and in the Arabian Gulf. The results suggested a large evolutionary distance (12.22%) between *L. carinata* and *L. klunzingeri*. *Liza affinis*, reported to be endemic to China and Japan, formed a single clade and is easily distinguished from *L. carinata* and *L. klunzingeri* by its short pectoral fin and small head. The genetic distance between *Liza klunzingeri* and *Liza carinata* is smaller than the genetic distance of either from *Liza affinis*.Hence we inferred that *Liza affinis* may be more ancient than the other two species. The specimens of *L. affinis*, *L. carinata*, and *L. klunzing*eri formed a strong monophyletic group, with no indication of cryptic species.

Species ranges provide insight into past and present dispersal barriers. Many fish species distribution patterns suggest a past disjunction along the eastern side of the Sunda Shelf in Indonesia, which may be related to Holocene sea-level fluctuations. These results for *Liza* species are in agreement with the importance of the most recent sea-level drop as an important factor in determining current species ranges, and consequently for species diversity patterns (McMillan and Palumbi, 1995; Perrin and Borsa, 2001; Lourie and Vincent, 2004; Ovenden *et al*., 2004; Whitfield *et al*., 2012). Results also indicate the need for further investigation of the genetic structure of these three *Liza* species in order to better manage important marine resources.

ACKNOWLEDGEMENTS

The study was supported by the National Natural

Science Foundation of China (41506158) and the Special Fund for Agro-scientific Research in the Public Interest (201303048, 201303050). This study was also supported by the Scientific Research Starting Foundation of Zhejiang Ocean University (Q1505).

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Eschmeyer, W.N., Fricke, R., Fong, J.D. and Polack, D.A., 2010. Marine fish diversity: history of knowledge and discovery (Pisces). *Zootaxa*, **2525**: 19-50.

Hajibabaei, M., Singer, G.A.C., Hebert, P.D.N. and Hickey, D.A., 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends Genet.*, **4**: 168-172. https://doi.org/10.1016/j.tig.2007.02.001

Heras, S., Roldan, M.I. and Castro, M.G., 2009. Molecular phylogeny of Mugilidae fishes revised. *Rev. Fish Biol. Fisher.,* **19**: 217-231. https://doi.org/10.1007/s11160-008-9100-3

Herbert, P.D.N., Ratnasingham, S. and Dewaard, J.R., 2003. Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. *Trans. R. Soc. Lond. B. Biol. Sci.,* **270(Suppl.)**: 96-99. https://doi.org/10.1098/rsbl.2003.0025

Herbert, P.D.N., Cywinska, A., Ball, S. and Dewaard, J.R., 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B. Biol. Sci.,* **270**: 313-321. https://doi.org/10.1098/rspb.2002.2218

Herbert, P.D.N., Stoeckle, M.Y., Zemlak, T.S. and Francis, C.M., 2004. Identification of birds through DNA barcodes. *PLoS Biol.,* **2**: e312. https://doi.org/10.1371/journal.pbio.0020312

Jarman, S.N. and Elliott, N.G., 2000. DNA evidence for morphological and cryptic Cenozoic speciations in the Anaspididae, 'living fossils' from the Triassic. *J. Evol. Biol.,* **13**: 624–633. https://doi.org/10.1046/j.1420-9101.2000.00207.x

Keskin, E. and Atar, H.H., 2013. DNA barcoding commercially important fish species of Turkey. *Mol. Ecol. Res.,* **13**: 788–797. https://doi.org/10.1111/1755-0998.12120

Kimura, M., 1980. A simple method of estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. mol. Evol.,* **16**: 111–120. https://doi.org/10.1007/BF01731581

Knowlton, N. and Weight, L.A., 1998. New dates and new rates for divergence across the Isthmus of Panama. *Proc. R. Soc. B.,* **265**: 2257–2263. https://doi.org/10.1098/rspb.1998.0568

Lourie, S.A. and Vincent, A.C.J., 2004. A marine fish follows Wallace's Line: the phylogeography of the three-spot seahorse (*Hippocampus trimaculatus*, Syngnathidae, Teleostei) in Southeast Asia. *J. Biogeogr.,* **31**: 1975–1985. https://doi.org/10.1111/j.1365-2699.2004.01153.x

McMillan, W.O. and Palumbi, S.R., 1995. Concordant evolutionary patterns among Indo-West Pacific Butterflyfishes. *Proc. R. Soc. B.,* **260**: 229–236. https://doi.org/10.1098/rspb.1995.0085

Mehle, N. and Trdan, S., 2012. Traditional and modern methods for the identification of thrips (Thysanoptera) species. *J. Pestic. Sci.,* **85**: 179–190. https://doi.org/10.1007/s10340-012-0423-4

Moore, W.S., 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution*, **49**: 718-726. https://doi.org/10.2307/2410325

Okiyama, M., 1993. *An atlas of the early stage fishes in Japan*. Koeltz Scientific Books, Germany, pp. 1154

Ovenden, J.R., Salini, J., O'Connor, S. and Street, R., 2004. Pronounced genetic population structure in a potentially vagilefish species (*Pristipomoides multidens*, Teleostei: Perciformes: Lutjanidae) from the East Indies triangle. *Mol. Ecol.,* **13**: 1991–1999. https://doi.org/10.1111/j.1365-294X.2004.02210.x

Perrin, C. and Borsa, P., 2001. Mitochondrial DNA analysis of the geographic structure of Indian scad mackerel in the IndoMalay archipelago. *J. Fish Biol.,* **59**: 1421–1426. https://doi.org/10.1111/j.1095-8649.2001.tb00205.x

Puckridge, M., Andreakis, N., Appleyard, S.A. and Ward, R.D., 2013. Crypytic diversity in flathead fishes (Scorpaeniformes: Platycephalidae) across the Indo-west Pacific uncovered by DNA barcoding. *Mol. Ecol. Resour.,* **13**: 32–42. https://doi.org/10.1111/1755-0998.12022

Randall, J.E., 1995. *Coastal fishes of Oman*. University of Hawaii Press, Honolulu, Hawaii, pp. 439.

Rugman-Jones, P.F., Hoddl,e M.S., Mound, L.A. and Stouthamer, R., 2006. Molecular identification key for pest species of Scirtothrips (Thysanoptera: Thripidae). *J. econ. Ent.,* **99**: 1813–1819. https://doi.org/10.1093/jee/99.5.1813

Saitou, N. and Nei, M., 1987. The neighbor-joining method: a new method for reconstructing evolutionary trees. *Mol. Biol. Evol.,* **4**: 406–425.

Senou, H., Yoshino, T. and Okiyama, M., 1987. A review of the mullets with a keel on the back, *Liza carinata*

complex (Pisces: Mugilide). *Publ. Seto. Mar. Biol. Lab.,* **32**: 303–321.

Shao, K.T., Chen, J.P., Kao, P.H. and Wu, C.Y., 1993. Fish fauna and their geographical distribution along the western coast of Taiwan. *Acta Zool. Taiwan,* **4**: 113-140.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S., 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.,* **28**: 2731–2739. https://doi.org/10.1093/molbev/msr121

Walters, C.J. and Holling, C.S., 1990. Large scale management experiments and learning by doing. *Ecology*, **71**: 2060-2068. https://doi.org/10.2307/1938620

Ward, R.D., Hanner, R. and Hebert, P.D.N., .2009. The campaign to DNA barcode all fishes, FISH-BOL. *J. Fish Biol.,* **74**: 329–356. https://doi.org/10.1111/j.1095-8649.2008.02080.x

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. and Herbert, P.D.N., 2005. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.,* **360**: 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Whitfield, A.K., Panfili, J. and Durand, J.D., 2012. A global review of the cosmopolitan flathead mullet *Mugil cephalus* Linnaeus 1758 (Teleostei: Mugilidae), with emphasis on the biology, genetics, ecology and fisheries aspects of this apparent species complex. *Rev. Fish Biol. Fisher.,* **22**: 641–681. https://doi.org/10.1007/s11160-012-9263-9

Xiao, J.G., Song, N., Gao, T.X. and McKay, R.J., 2016. Redescription and DNA Barcoding of *Sillago indica* (Perciformes: Sillaginidae) from the coast of Pakistan. *Pakistan J. Zool.,* **48**: 317–323.

Xu, G., Zheng, W. and Huang, G., 1994. *Atlas of the fishes and their biology in Daya Bay*. Anhui Scientific and Technical Publishers, The People's Republic of China, pp. 311.