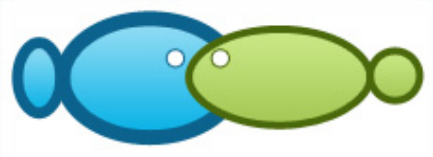

Levels of COI divergence in Family Leiognathidae using sequences available in GenBank and BOLD Systems: A review on the accuracy of public databases

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Abstract. DNA Barcoding is increasingly recognized as a new approach for the recognition and identification of animal species by using cytochrome oxidase I (COI) gene. This approach is highly dependent on readily availabe COI sequences in public databases. However, the accuracy of species identification and the quality of COI sequences available in public databases such as GenBank and BOLD Systems are still unknown. Here, a total of 232 sequences of 24 species from Family Leiognathidae has been downloaded from these public databases. A total of 14 COI sequences showed ambiguous sites and therefore were excluded for further analysis. From all the sequences that has been downloaded, a total of 88 sequences has been detected as potential misidentification as these sequences did not group with their own taxa. The mean intraspecific K2P divergences were 1.44% among individuals within species and 5.63% within the genera. There are four species (*Equulites elongatus, Equulites leuciscus, Gazza minuta* and *Secutor indicius*) that had shown deep divergences among individuals. These had been assigned to a single taxon with maximum intraspecific divergence of 8.1%, 7.2%, 4.0% and 3.1% respectively. Although GenBank and BOLD Systems have been established as public sequence libraries, the accuracy of deposited sequences should be monitored to ensure the success of species identification.
Key Words: COI, Cytochrome oxidase I, DNA barcoding, Leiognathidae.

Introduction. Malaysia is a mega-diverse country with approximately 640 species of freshwater fish and 1407 species of marine fishes according to Froese & Pauly (2015). In the recent decade, the loss of biodiversity has been recognized as a major global environmental problem and much effort has been made focusing on biodiversity conservation (Blaxter 2003; Wilson 2003). So, it is in need of taxonomic experts to identify specimens and record the present biodiversity (Motomura & Ishikawa 2013). The identification work is mostly dependent on the knowledge held by family-specialized taxonomist whose work sometime cannot cover all fish taxa and commonly require other fish experts. Therefore, the problematic taxonomic issues need to be solved in details to provide comprehensive knowledge to learner. There are various methods that have been used in classifying and determining the organisms, for example using morphological identification, physiological, biochemical observation and genetic investigation (Strauss & Bond 1990). However, through morphology identification, there are disadvantages when two species are morphologically very similar, but genetically distinct (cryptic species). To overcome this problem, genetic information, specifically DNA sequences, has been suggested to serve as a criterion, or at least a complement, in taxonomic identification (Blaxter 2003; Savolainen et al 2005).

DNA barcoding is an identification technique increasingly used by ecologist and it relies on the use of a short section of standardized DNA region as tag for accurate species identification (Hebert et al 2003a; Hebert & Gregory 2005). This approach is not only able to identify a single species from a specimen but also determine the species composition of environment sample called the environmental DNA (eDNA) (Hebert et al 2003a). The use of DNA sequence in identifying different species is the same way as supermarket scanner uses in scanning the black stripes barcode to identify the product. The gene region that is being used for almost all animal groups is a 648 base-pair region in the mitochondrial cytochrome c oxidase I gene or COI (Hebert et al 2003b). This region has been highly proving to be effective in identifying fish, birds and many other animal groups (Hogg & Hebert 2004; Hebert et al 2004a,b; Ward et al 2005; Costa et al 2007). The advantages in using COI is that it is short enough to be sequenced quickly and cheaply yet effective enough to identify variations among species (Hebert et al 2003b).

Species identification through DNA barcoding can be achieved by the retrieval of COI region, the 'barcode' from the unknown specimen. The barcode sequence will then be compared with COI data derived from individuals of known identity available in public databases such as GenBank and BOLD Systems (Hajibabaei et al 2007). A specimen is identified if its sequence closely matches the one in the public databases. Species identification using DNA barcoding approach is highly dependent on the readily availabe COI sequences in public databases. However, the accuracy and quality of COI sequences available in public databases are still unknown. Low quality of sequences and misidentification of commercially important species in public databases can lead to inaccurate data interpretation for management and conservation purposes.

Ponyfishes (Perciformes: Leiognathidae) are commonly known as "Kekek" in Malaysia (Seah et al 2008). This family is most widely known as slimys or slipmouths, which consists of nine genera and 50 species (Seah et al 2012; Eschmeyer et al 2016). Almost half of all ponyfish species can be found in Malaysian water (Seah et al 2011b). Ponyfishes are commercially important 'by-catch' fishes in Malaysian fisheries industry. Though small in size, these species have a great potential for commercial exploitation, being an important source of food and fish meal (Seah et al 2011a).

Until now, the identification and classification of the fish from family Leiognathidae are relying on phenotypic characters and required detail inspection of the specimen (Mazlan & Seah 2006). These traditional methods are largely based on external characteristics like shape, size and colours of body parts; sometimes, the identification needs an experienced professional taxonomist to discriminate among the species. Misidentification of economically important fish can affect the accuracy of data collection. Therefore, accurate identification at the species-level is important to ensure the successful management of the fisheries stocks, and here, the accuracy of COI sequences available in public databases were evaluated and analysed.

Materials and Methods

Data collection and validation

A total of 232 COI sequences of 24 species from Family Leiognathidae were downloaded from public databases GenBank (www.ncbi.nlm.nih.gov) and BOLD Systems (www.boldsystems.org). However, 14 COI sequences showed ambiguous sites and therefore were excluded from further analysis. After exclusion of these 14 sequences, the data set consisted of a total of 218 sequences. Incorrect taxonomic identification can affect the divergence assessment of the data set. Therefore all COI sequences were aligned and a Neighbour-Joining (NJ) tree was constructed using MEGA6 (Tamura et al 2011). The samples which did not cluster with their own taxa had their photograph reviewed and their taxonomy checked. A total of 75 specimens did not cluster with their own taxa and this revealed potential misidentification. Subsequently a total of 218 sequences were analysed from 24 species and seven genera from Family Leiognathidae.

COI divergence assessment

Multiple alignments were performed for the 218 sequences in MEGA6 (Tamura et al 2011) software, and the amino acid translation was examined to ensure that no gaps or stop codons were present in the alignment. Kimura 2-Parameter (K2P) distance measure was employed to infer the genetic distances within and between species. Kimura-2-parameter and gamma distribution for the rate variation among site was used as the best-fit DNA substitution model in NJ analyses. Phylogenetic tree through Neighbor-joining (NJ) method was then constructed using 10000 bootstrap replications.

Results and Discussion

General finding

According to the FishBase, there are 48 species and nine genera that were recorded in Family Leiognathidae worldwide. However, only 24 species from seven genera have their COI barcode records in GenBank and BOLD Systems. The number of sequences per species varied between two (*Equulites lineolatus*, *Equulites stercorarius*, *Gazza achlamys*, *Leiognathus robustus*, *Leiognathus cf. striatus*) to 25 (*Leiognathus sp* 3). There are approximately 6% (14 sequences) of the sequences that showed ambiguous sites and were considered as low quality sequence. These sequences were not suitable for further analysis, while 34.4% (75 sequences) was found to be misidentified. These findings showed that taxonomic confusion may occur between species in family Leiognathidae and this may lead to misidentification of the specimens. No ambiguities were observed among all the sequences and there were no insertions, deletion or stop codons, supporting the view that all the public sequences downloaded constitute functional mitochondrial COI sequences.

COI divergence assessment

COI nucleotide divergences were calculated for the data set of 218 sequences of 24 species and seven genera. The average within species K2P distance was 1.44% with far less 0.00% for *Equulites stercorarius, Leiognathus fasciatus, Leiognathus cf. striatus* and *Secutor sp* 2. The highest was 5.8% for *Equulites elongates* (Table 1). There were four species (*Equulites elongatus*, *Equulites leuciscus*, *Gazza minuta* and *Secutor indicius*) that showed slightly higher within species divergence (Table 1). The average congeneric distance was 5.63% which is four times higher than conspecific distance. The congeneric distances were lowest among genera, *Nuchequula* (0.1% - 1 species), followed by *Photopectoralis* (0.4% - 1 species); *Eubleekeria* (0.9% - 1); *Gazza* (4.2% - 2 species); *Equulites* (7.7% - 5 species); *Secutor* (12.4% - 6 species) and the highest variation observed in the genus *Leiognathus* (13.7% - 8 species) (Table 2).

Potential misidentification

There are 75 sequences have been detected as potential misidentification. Sixty nine specimens formed their own cluster, while the other six specimens grouped with the other taxa (Table 3).

Through detail examination, the findings showed that *L. equulus, S. indicius* and *E. leuciscus* were misidentified as *S. insidiator*. In addition, the later also misidentified as *E. elongatus* FJ607429. The first and last species have distinct morphological characteristic differences compare to *S. insidiator* whereas the *S. indicius* was frequently misidentified as *S. insidiator* (Woodland et al 2001). Generally, *S. indicius* can be distinguished from *S. insidiator* by having a greater number of columns consisting bars and spots on dorsolateral (17-22 vs. 11-15), dorsal fin origin is behind a vertical from pelvic fin insertion (vs. located along the same vertical in *S. insidiator*) and presence of a black dot-line from pectoral axil to pelvic fin (vs. indistinct pattern in *S. insidiator*) (Woodland et al 2001; Seah et al 2012). However, both species were always easily mixed up due to the condition of specimen (faint color, deformation caused by trawl pressure) and lack of detail observation. No nomenclatural problems have been reported between *E. leuciscus* and *E. elongatus* because both species are efficiently observable either in fresh or preserved conditions. *Equulites leuciscus* readily identified from *E. elongatus* by having moderately deep body, 2-3 times in standard length (SL) (vs. elongated body,

>3.3 times in SL), distinctly elongated 2nd dorsal fin spine (vs. not elongate), speckled pigmentation on dorsolateral (vs. blotched marking) and male having translucent triangular patch on belly (vs. translucent bullet-shaped patch) (Woodland et al 2001; Chakrabarty et al 2011). On the contrary, *S. insidiator* was misidentified as *P. bindus*. Moreover, *P. bindus* was misidentified as *E. splendens* DADB009-12. These three species have no complicated morphological diagnostics and original descriptions were sufficient to differentiate them by referring Woodland et al (2001).

Table 1

Intraspecific nucleotide K2P distances for 24 species of Family Leiognathidae

Species	No. of sequences (n)	Mean K2P distance (%)	Min (%)	Max (%)
Equulites elongates	3	5.8	1.6	8.1
Equulites leuciscus	23	0.9	0	7.2
Equulites lineolatus	2	0.3	0.3	0.3
Equulites rivulatus	4	0.1	0	0.2
Equulites stercorarius	2	0	0	0
Eubleekeria splendens	9	0.9	0	1.9
Gazza achlamys	2	0.2	0.2	0.2
Gazza minuta	24	2.1	0	4.0
Leiognathus aureus	3	0.4	0	1.0
Leiognathus daura	11	0.3	0	0.8
Leiognathus fasciatus	11	0	0	0
Leiognathus robustus	2	0.3	0.3	0.3
Leiognathus cf. striatus	2	0	0	0
Leiognathus sp 1	10	0.2	0	0.5
Leiognathus sp 2	12	0.4	0	0.6
Leiognathus sp 3	25	0.4	0	1.4
Nuchequula nuchalis	12	0.1	0	0.3
Photopectoralis sp 1	5	0.4	0	1.0
Secutor indicius	4	2.1	0	3.1
Secutor insidiator	19	0.1	0	0.5
Secutor megalolepis	3	1.4	0.3	2.1
Secutor sp 1	4	0.2	0	0
Secutor sp 2	4	0	0	0
Secutor sp 3	22	0.1	0	0.5

Table 2

Congeneric nucleotide K2P distances for seven genera in Family Leiognathidae

Genus	No. of sequences (n)	Mean K2P distance (%)
Equulites	34	7.7
Eubleekeria	9	0.9
Gazza	26	4.2
Leiognathus	76	13.7
Nuchequula	12	0.1
Photopectoralis	5	0.4
Secutor	56	12.4

Table 3

Six specimens of potential misidentification which grouped with other taxa in NJ tree

Sequence ID/GenBank no	Species	Group with taxa in NJ tree
DSLAG933-10	*Secutor insidiator*	*Leiognathus equulus*
HQ993155	*Photopectoralis bindus*	*Secutor insidiator*
HQ993161	*Secutor insidiator*	*Secutor indicius*
FJ265837	*Secutor insidiator*	*Equulites leuciscus*
FJ607429	*Equulites elongatus*	*Equulites leuciscus*
DADB009-12	*Eubleekeria splendens*	*Photopectoralis bindus*

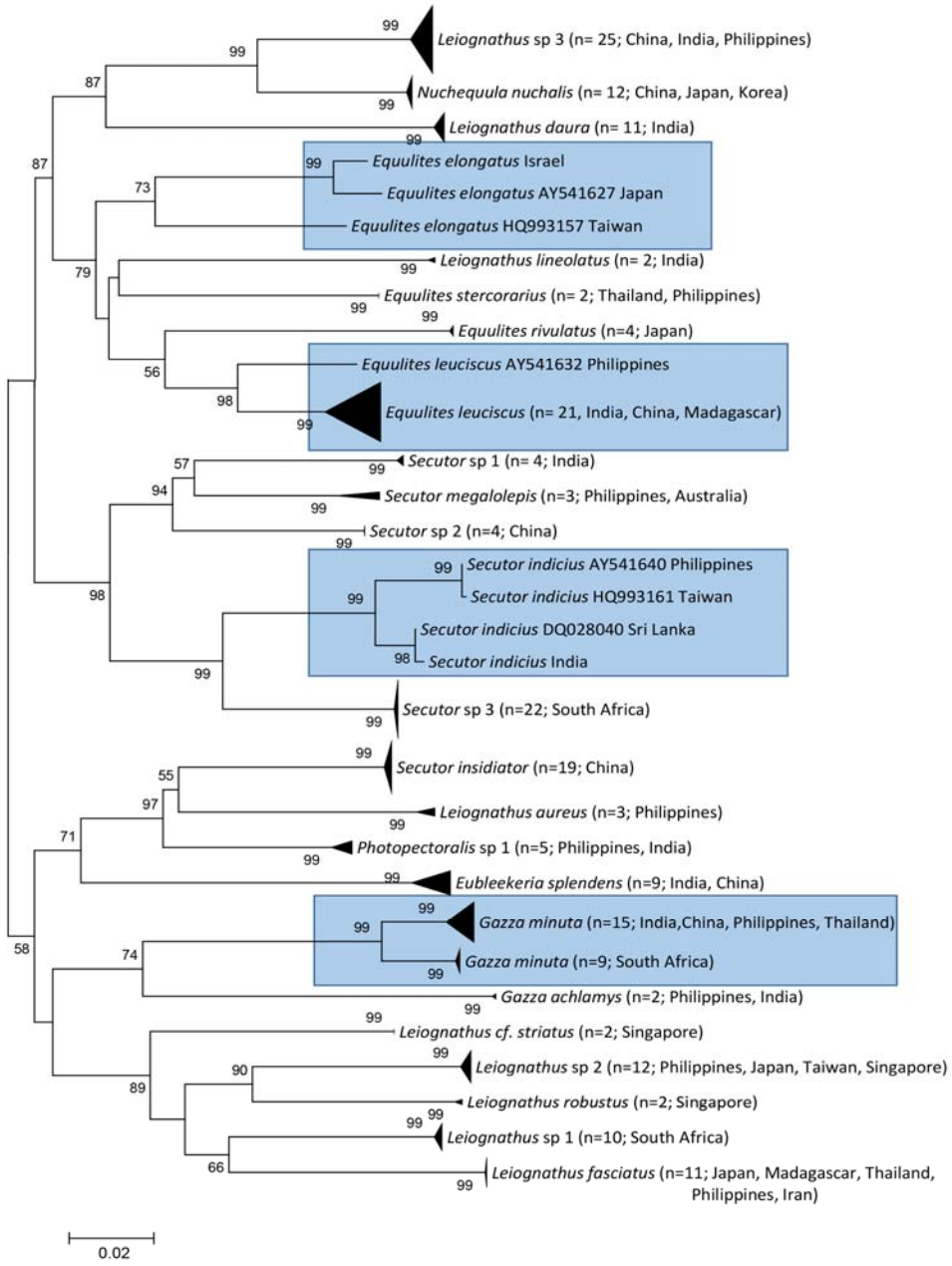

Figure 1. Neighbour-Joining tree (K2P distance) of 24 species of Family Leiognathidae found in GenBank and BOLD Systems. Only bootstrap values greater than 50 are shown. Blue box showed deep divergence within species.

Deep intraspecific divergences

In four species, we detected deep divergences among individuals that have been assigned in a single taxon. The divergence is too high for the same species. According to Hebert et al (2003b), the nucleotide distance should be lower than 3% for individuals in the same species. Closer observation of the data associated with *E. elongatus*, *E. leuciscus, G. minuta* and *S. indicius* showed maximum intraspecific divergence of 8.1%, 7.2%, 4.0% and 3.1% respectively (Table 1), revealing that the individuals formed two clusters in NJ analysis (Figure 1). Divergent as they were, members of the two clusters nonetheless were more similar to each other than to members of any other species in the data set. These high sympatric divergences suggest that each might result from inadequate taxonomy resolution (the non-recognition of cryptic species complexes) (Ward 2009; Mat Jaafar et al 2012).

Equulites elongatus

Maximum K2P distance within species of *E. elongatus* was 8.1% nucleotide divergence. There were only three COI sequences available for this species in GenBank and BOLD Systems. All of the specimens are from different localities (Israel, Japan and Taiwan). In the NJ tree, specimens from Israel and Japan were grouped in the same cluster, while the other cluster consisted only specimen from Taiwan (Figure 2). However, no geographic structure was apparent.

Secutor indicius

Phylogenetic analyses revealed two clusters generated from three specimens of *S. indicius* (Figure 3). Mean K2P distance within species was 2.1% with a maximum of 3.1% nucleotide divergence. Cluster I consisted one individual of *S. indicius* from Philippines and one individual of *S. insidiator* (potential misidentification) from Taiwan supported by a bootstrap value of 100%. Meanwhile, Cluster II comprised two individuals from India. The deep divergence among individuals may be due to the different geographical region. Cluster I comprising individuals from Pacific Ocean while Cluster II comprised individual from Indian Ocean.

Equulites leuciscus

Phylogenetic analysis also revealed two clusters generated from 22 *E. leuciscus* samples (Figure 4). Mean K2P distance within species was 0.9% with a maximum of 7.2% nucleotide divergence. Cluster I, the major lineage containing specimens of *E. leuciscus* from China, India, Japan and Madagascar and one specimen of *S. insidiator* from India (potential misidentification). This cluster was strongly supported with a bootstrap value of 100%. In contrast, Cluster II, containing only a single specimen from Philippines.

Gazza minuta

Twenty four COI sequences of *G. minuta* which were downloaded from GenBank and BOLD Systems formed two clusters (Figure 5) with Cluster I comprised 15 individuals from China, Philippines and India. While Cluster II comprised nine individuals from South Africa with a high bootstrap value of 98 - 100%. These clusters were separated by 4% nucleotide divergence. This deep divergence is probably due to the geographical factor as the South Africa is located western of the Indian Ocean while the other localities are located at the eastern of Indian Ocean.

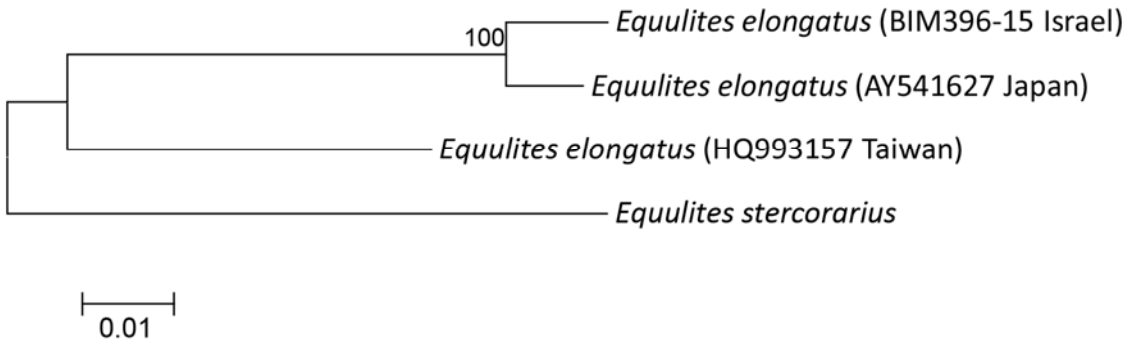

Figure 2. Neighbour-Joining tree (K2P distance) of 3 *CO*I sequences of *Equulites elongatus* found in GenBank and BOLD Systems. Only bootstrap values greater than 50 are shown.

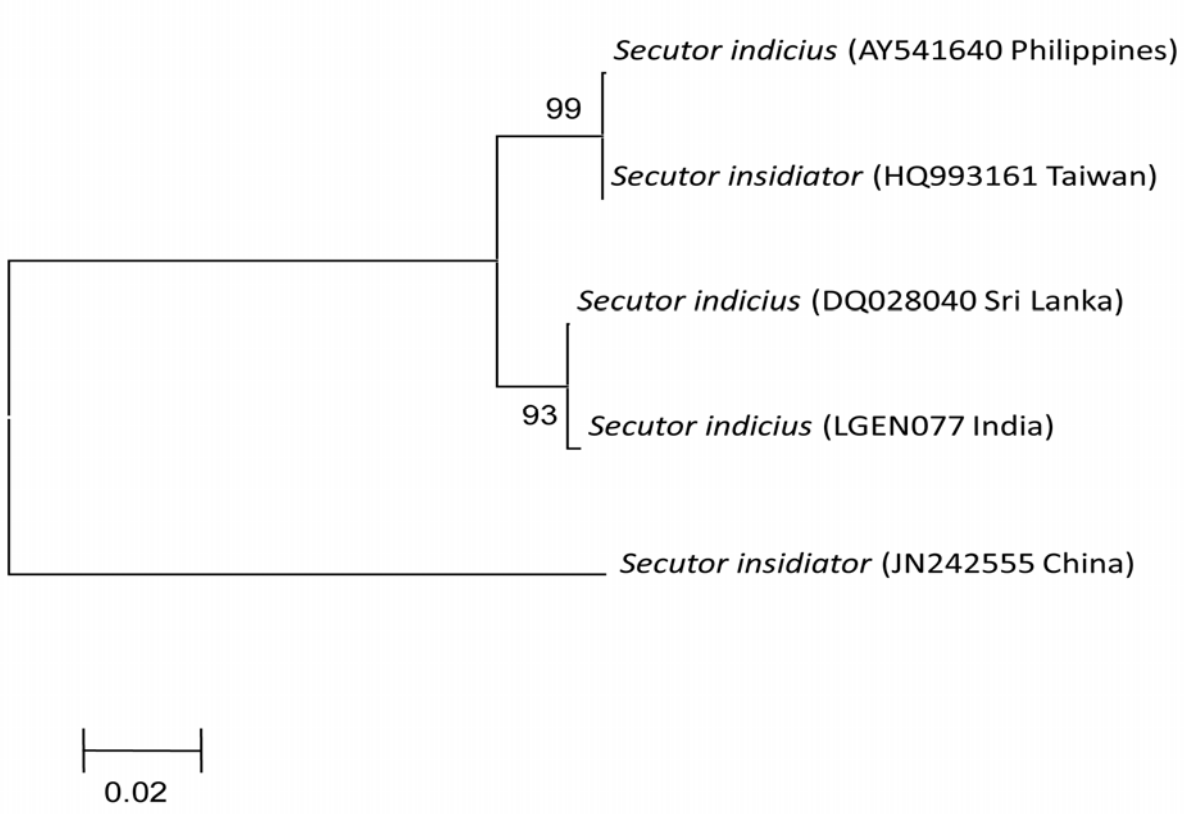

Figure 3. Neighbour-Joining tree (K2P distance) of three *CO*I sequences of *Secutor indicius* found in GenBank and BOLD Systems. Only bootstrap values greater than 50 are shown.

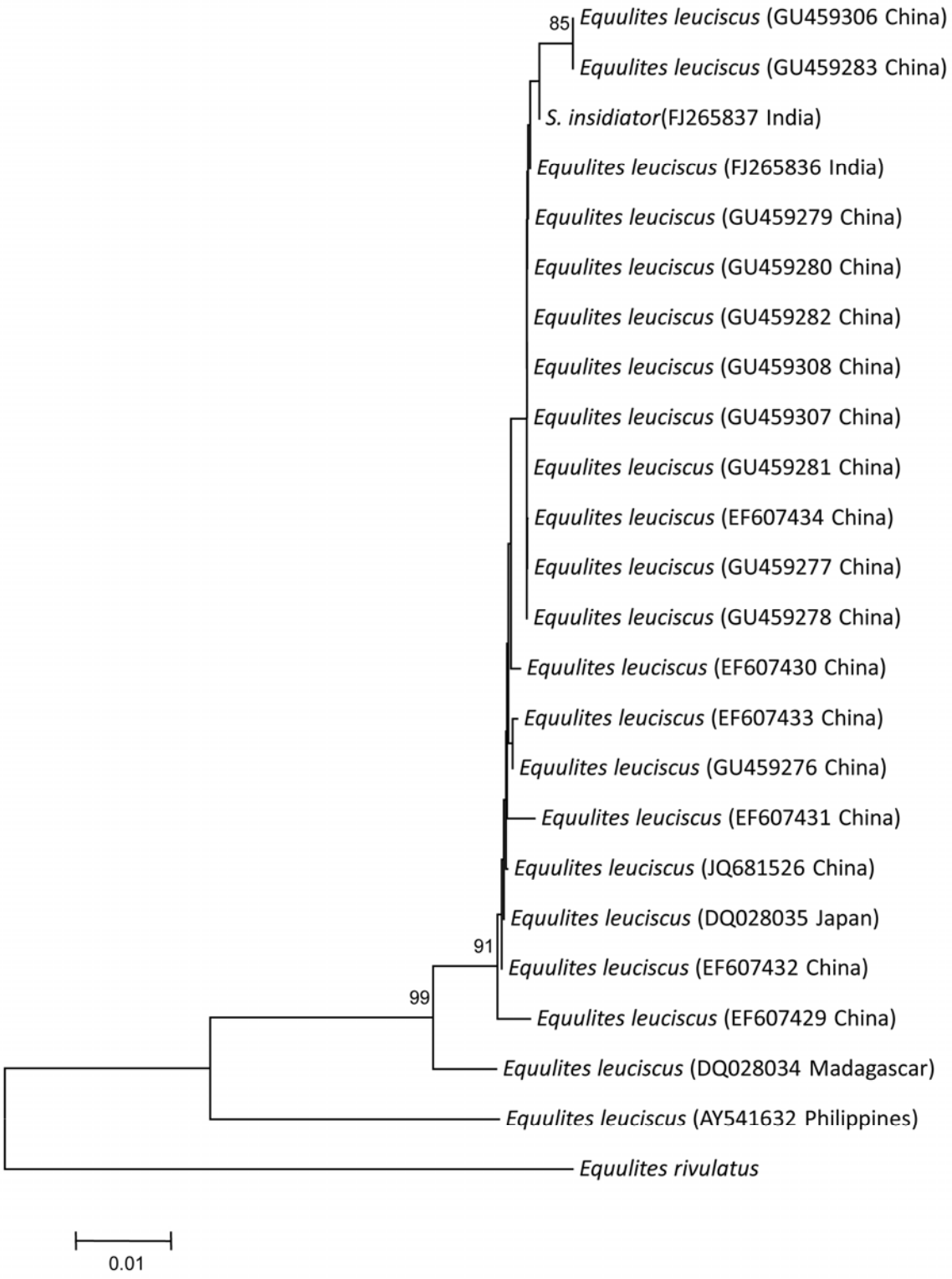

Figure 4. Neighbour-Joining tree (K2P distance) of 23 *CO*I sequences of *Gazza minuta* found in GenBank and BOLD Systems. Only bootstrap values greater than 50 are shown.

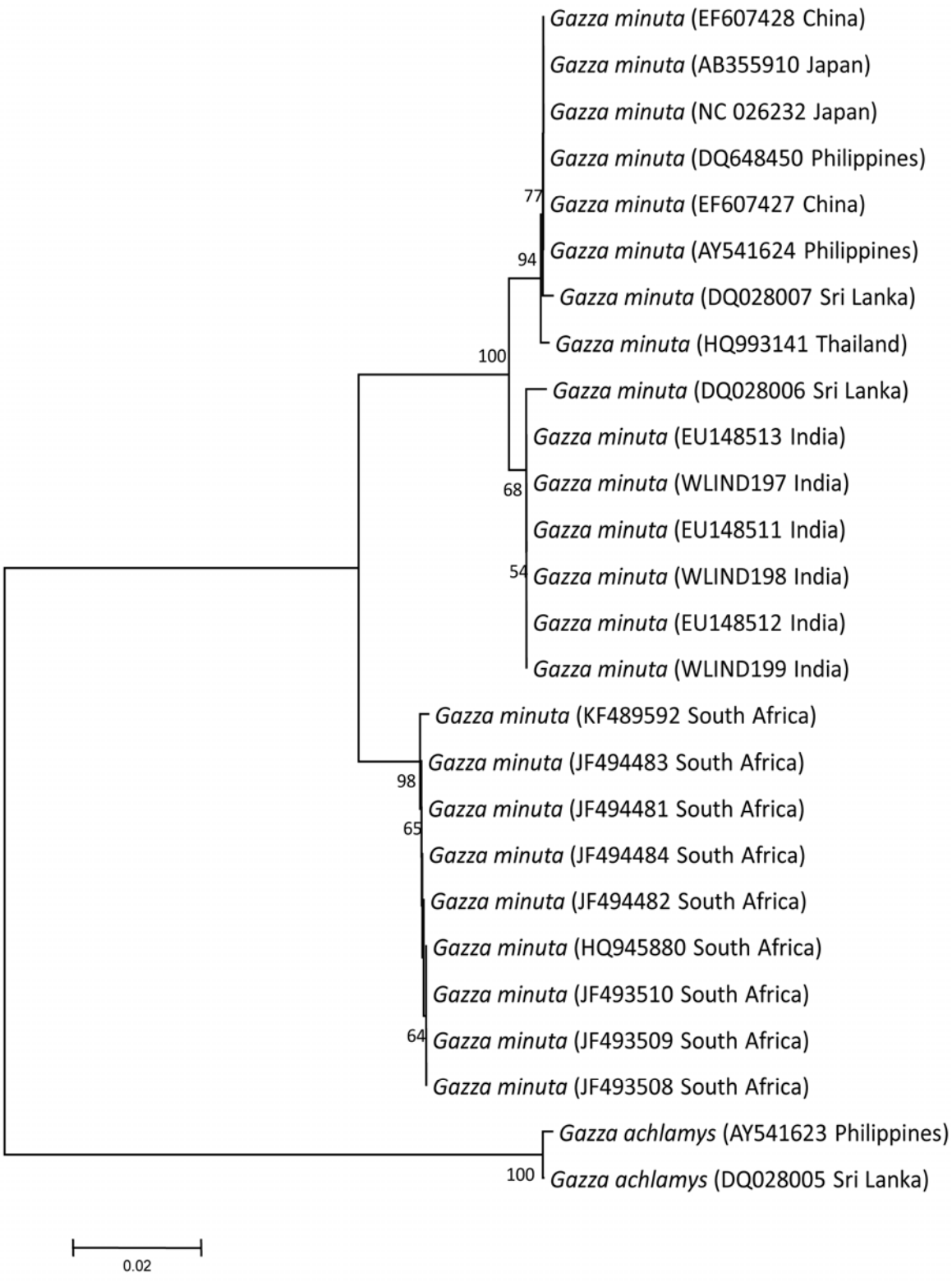

Figure 5. Neighbour-Joining tree (K2P distance) of 24 *COI* sequences of *Gazza minuta* found in GenBank and BOLD Systems. Only bootstrap values greater than 50 are shown.

Conclusion. The establishment of public databases for COI sequences contributed to the global DNA barcoding effort to document and catalogue the diversity of life, particularly with regard to conservation and management applications. However, the success of DNA Barcoding in discrimination and identification of species is highly dependent on the accuracy and quality of sequences available in public databases. Although GenBank and BOLD Systems have been established as public sequence libraries, the accuracy of deposited sequences should be monitored to ensure the success of species identification.

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References

Blaxter M., 2003 Molecular systematics - Counting angels with DNA. Nature 421: 122-124. doi:10.1038/421122a

Chakrabarty P., Davis M.P., Smith W.L., Berquist R, Gledhill K.M., Frank L.R., Sparks J.S., 2011 Evolution of the light organ system in ponyfishes (Teleostei: Leiognathidae). Journal of Morphology 272: 704-721. doi: 10.1002/jmor.10941

Costa F.O., deWaard J.R., Boutillier J., Ratnasingham S., Dooh R., Hajibabaei M., Hebert P.D.N., 2007 Biological identifications through DNA barcodes: the case of the Crustacea. Canadian Journal of Fisheries and Aquatic Sciences 64: 272-295. doi: 10.1139/f07-008

Eschmeyer W.N., Fricke R., van der Laan R. (eds). Catalog of fishes: genera, species, references.(http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp). Electronic version accessed 22 February 2016.

Froese R., Pauly D. (eds.) 2015 FishBase. World Wide Web electronic publication. www.fishbase.org, version (10/2015).

Hajibabaei M., Singer G.A.C., Hebert P.D.N., Hickey D.A., 2007 DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. Trends in Genetic 4: 167-172. doi:10.1016/j.tig.2007.02.001

Hebert P.D.N., Cywinska A., Ball S.L., de Waard J.R., 2003a Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270: 313–322. doi: 10.1098/rspb.2002.2218

Hebert P.D.N., Gregory T.R., 2005 The promise of DNA barcoding for taxonomy. Systematic Biology 54: 852–859. doi: 10.1080/10635150500354886

Hebert P.D.N., Penton E.H., Burns J.M., Janzen D.H., Hallwachs W., 2004a Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proceedings of the National Academy of Sciences USA 101: 14812-14817. doi: 10.1073/pnas.0406166101

Hebert P.D.N., Ratnasingham S., de Waard J.R., 2003b Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of Royal Society London B 270: S96–S99. doi: 10.1098/rsbl.2003.0025

Hebert P.D.N., Stoeckle M.Y., Zemlak T.S., Francis C.M., 2004b Identification of birds through DNA barcodes. PLoS Biology 2: 1657-1663. doi: 10.1371/journal.pbio.0020312.sd001

Hogg I.D., Hebert P.D.N., 2004 Biological identification of springtails (Collembola: Hexapoda) from the Canadian Arctic, using mitochondrial DNA barcodes. Canadian Journal of Zoology 82: 749-754. doi: 10.1139/z04-041

Mat Jaafar T.N.A., Taylor M.I., Mohd Nor S.A., de Bruyn M., Carvalho G.R., 2012 DNA Barcoding Reveals Cryptic Diversity within Commercially Exploited Indo-Malay Carangidae (Teleosteii: Perciformes). PLoS ONE 7: e49623. doi:10.1371/journal.pone.0049623

Mazlan A.G., Seah Y.G., 2006 Meristic and length-weight relationship of ponyfishes (Leiognathidae) in the coastal water of Pulau Sibu-Tinggi, Johor, Malaysia. Malaysian Applied Biology 35: 27–35.

Motomura H., Ishikawa S. (eds.), 2013 Fish collection building and procedures manual. English edition. The Kagoshima University Museum, Kagoshima and the Research Institute for Humanity and Nature, Kyoto. 70p.

Savolainen V., Cowan R.S., Vogler A.P., Roderick G.K., Lane R., 2005 Towards writing the encyclopaedia of life: an introduction to DNA barcoding. Philosophical Transactions of the Royal Society B-Biological Sciences 360: 1805-1811. doi : 10.1098/rstb.2005.1730

Seah Y.G., Mazlan A.G., Usup G., 2008 Phylogeny of ponyfishes from coastal waters of the South China Sea. Journal of Applied Biological Sciences 2: 125-132.

Seah Y.G, Mazlan A.G., Abdullah S., Zaidi C.C., Usup G., Mohamed C.A.R., 2011a Feeding guild of the dominant trawl species in the southeastern waters of Peninsular Malaysia. Journal of Biological Sciences 11: 221-225. doi: 10.3923/jbs.2011.221.225

Seah Y.G., Mazlan A.G., Simon K.D., Arshad A., Mohamed C.A.R., Usup G., 2011b Overview of the leiognathid species in Malaysia. AACL Bioflux 4: 505-510.

Seah Y.G., Usup G., Mohamed C.A.R., Arshad A., Mazlan A.G., 2012 Phylogeny and morphological delineation of leiognathids in the waters of Peninsular Malaysia. Coastal Marine Science 35: 91-95.

Strauss R.E., Bond C.E., 1990 Taxonomic methods: morphology. In: Schreck C.B., Moyle P.B. Methods for fish biology. American Fisheries Society, Maryland, pp 109–140.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S., 2011 MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biological Evolution 521:2731-2739. doi: 10.1093/molbev/msr121

Ward R. D., 2009 DNA barcode divergence among species and genera of birds and fishes. Molecular Ecology Resources 9: 1077–85. doi:10.1111/j.1755-0998.2009.02541.x

Ward R.D., Zemlak T.S., Innes B.H., Last P.R., Hebert P.D.N., 2005 DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B 360: 1847-1857. doi: 10.1098/rstb.2005.1716

Wilson E.O., 2003 Biodiversity in the information age. Issues in Science and Technology 19: 45-46.

Woodland D.J., Premcharoen S., Cabanban A.S., 2001 Leiognathidae. Slipmouths (ponyfishes). In: [FAO species identification guide for fishery purposes. The living marine resources of Western Central Pacific. Volume 5. Bony fishes part 3 (Menidae to Pomacentridae)]. Carpenter, K.E. and Niem, V.H. (eds.), pp. 2792-2823, FAO, Rome.

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