Beyond Bycatch: The Species Diversity of Tonguesole (Pleuronectiformes: Cynoglossidae) in Coastal Fisheries of the Tanintharyi Region, Southern Myanmar

IRIS SEGURA-GARCIA1,*, SABAI SOE2, NYO NYO TUN1, STEPHEN BOX3
1Smithsonian Marine Station at Fort Pierce, National Museum of Natural History, Fort Pierce, Florida, United States of America
2Department of Marine Science, Myeik University, Myeik, Myanmar
3Rare - Fish Forever, Arlington, Virginia, United States of America

*E-mail: seguragarcia@si.edu | Received: 23/09/2020; Accepted: 04/03/2021

Abstract

Flatfishes in the family Cynoglossidae are an important coastal fishery in Myanmar. Due to the overlapping morphologies of multiple tonguesole species, caught both as bycatch from trawl fisheries and targeted specifically by small scale fishers, they are all marketed under a single local name, “khwayshar”. This presents a management challenge given the potential differences in the species-specific life-histories, population dynamics, fishing vulnerability and harvest rates. This study investigated the species diversity of tonguesole landings from coastal communities of the Taninthary Region of southern Myanmar. DNA barcoding was used to distinguish potentially 10 different species, of which five were identified to species level and five at the genus level. Unconfirmed genetic identifications were based on external morphology. The poor efficacy of DNA barcoding for tonguesole species identification resulted from the limited DNA barcode reference sequences available for the family Cynoglossidae in public databases. An asymmetric occurrence and relative abundance of the identified species in landing sites where samples were collected suggested that the most common species was Cynoglossus oligolepis (Bleeker, 1855), a new species record for Myanmar, followed by Cynoglossus lingua Hamilton, 1822. The results of the present study provide new information to characterise the tonguesole fishery as a first step in the development of management plans for the coastal fishery in Myanmar.

Keywords: flatfishes; coastal-fisheries; DNA-barcode, mixed-fisheries

Introduction

Tropical fisheries, operating in shallow coastal and estuarine waters, harvest large numbers and high diversity of flatfishes (Munroe, 2015a). The volume of flatfishes in these fisheries make them an important source of protein for human consumption in several countries (Cheung and Oyinlola, 2018). Despite their importance for food supply and food security, poor quantitative information on the catch volume and species composition of flatfishes exist for coastal fisheries in the tropics.

Coming both as bycatch from industrial bottom trawl fisheries and targeted by small scale fishers, flatfishes are often an essential component of local fish markets (Munroe, 2015a). Increasing fishing pressure has resulted in a historical trend of declining catches suggesting that exploitation of flatfish resources have exceeded their maximum potential (Cheung and Oyinlola, 2018). But as most countries continue to regard tropical flatfishes as bycatch of commercial fishing, fisheries departments have not prioritised their management. They have largely overlooked the importance of these species to the livelihoods and food security of coastal communities.

Flatfishes (Order Pleuronectiformes) are a highly diverse order consisting of approximately 15 families, 127 genera and 817 species found in the world’s oceans (Campbell et al., 2014, 2019). Nonetheless, the taxonomy, ecology and fisheries in tropical waters remain poorly known, especially in the Indo-Pacific, where the greatest diversity of flatfishes exists (Munroe, 2015b). Within Pleuronectoidei, the family Cynoglossidae (tonguesoles), is a diverse family of
specialised marine, estuarine and freshwater flatfishes containing about 161 species distributed in two subfamilies, Symphurinae and Cynoglossinae, and three genera: Symphorus, Cynoglossus and Paraplagusia (Campbell et al., 2014; Munroe, 2015b).

The global average annual catch of flatfishes from 2001 to 2010 indicated that Cynoglossidae is the most important family of flatfishes in contribution to total catch (78 %), followed by the Pleuronectidae with 10.4 % (Cheung and Oyinlola, 2018). In small-scale fisheries, a wide variety of species and sizes of tonguesoles are caught (Ghaffari et al., 2015; Munroe, 2015a).

In Myanmar, tonguesoles are of high socio-economic importance for many coastal communities and provide a key source of affordable, high quality protein in local markets. Tonguesoles are recorded as bycatch throughout the year in industrial trawl fishing. The inshore small-scale fisheries catch them in driftnets and as part of mixed-species assemblages caught in stationary bag nets (Lwin Lwin et al., 2014; Aung, 2018). But to date, little catch data has been collected on tonguesole fisheries in Myanmar, and the species composition had not been resolved as the identification of species has been based only on visual differentiation of external features, e.g. caudal fin rays, number of lateral lines, scale types, the shape of the body, the morphology of the snout, and colour. This may be insufficient for species determination because the genus Cynoglossus are remarkably similar with overlapping morphologies and their taxonomy at species-level remains difficult to differentiate by visual inspection (Munroe, 2015b). The difficulties with accurate identifications have resulted in all tonguesoles being marketed under a single local name “khwayshar”, and the Department of Fisheries in Myanmar recognises only this single classification for all tonguesoles captured in this country (Soe, personal communication). However, several species of tonguesoles appear in the markets of Myanmar. For example, in Mon State, the occurrence of the Bengal tonguesole, Cynoglossus cynoglossus (Hamilton, 1822), the long tonguesole Cynoglossus lingua (Hamilton, 1822), Cynoglossus macrolepidotus (Bleeker, 1851), the fourlined tonguesole, Cynoglossus bilineatus (Lacepède, 1802), the large scale tonguesole Cynoglossus arel (Bloch & Schneider, 1801), and the hooked tonguesole, Cynoglossus carpenteri Alcock, 1889 have been reported (Aung, 2018). In the Myeik archipelago, only C. lingua and C. bilineatus have been identified for the stationary bag-net fishery practised there (Lwin Lwin et al., 2014). The accurate knowledge of the species composition of a mixed-species fishery is fundamental to develop management strategies. Understanding species-specific exploitation rates connected to appropriate life histories for that species are fundamental for assessing fish stocks and wrong assumptions around both results in poor management (see Garcia-Vazquez et al., 2012).

This study is the first to disaggregate the species composition in the mixed tonguesole fisheries in the Tanintharyi Region of Myanmar using DNA barcoding. Our results provide the scientific baseline to promote the management of this important, yet unregulated, mixed-species fishery in the country.

**Materials and Methods**

This study was conducted in the Tanintharyi Region in southern Myanmar, around the two main fishing districts: Dawei and Myeik (Fig.1). This region comprises 1200 km of coast with extensive shallow marine, estuarine and mudflat areas, a common habitat of tonguesole fishes (Menon, 1977). The Dawei River estuary mudflat extends 2500 ha south from the river mouth, while around the port town of Myeik, mudflats extend approximately 4000 ha surrounded by mature mangrove (Zöckler et al., 2014).

![Fig. 1. Map of the study location of the diversity of tonguesole species in the genus Cynoglossus in the Tanintharyi Region. Red dots showing the sampling localities in Dawei and Myeik.](image)

Tissue samples and morphometric data were collected during field visits between 2016–2018 at fish landing sites and markets around the cities of Dawei (n = 109) and Myeik (n = 162) (see Fig. 1). For all individual tonguesoles, total length (TL), from the tip of the snout to the end of the longest caudal ray, was measured, keeping the fish flat on the measuring board. Tissue samples were collected from the gill cover and preserved by drying in silica gel for genetic analyses.

In addition to TL, 98 specimens were measured for the following morphometric characters: standard length (SL), head length (HL), snout length (SnL),
Genomic DNA from 271 samples was extracted and purified using the DNeasy purification kit (QIAGEN, USA). All samples were genotyped using a standard sequence of the 5' fragment of the mitochondrial gene cytochrome c oxidase subunit I (COI) amplified by polymerase chain reaction (PCR) performed in 10 µL volume consisting of 1 µL of 10× NH, reaction buffer, 2.5 mM MgCl₂, 0.125 Mm of each dNTP, 0.3 µM of each primer: Fish-BCL 5'-TCAACYAATCAYAAAGATIYGCCAC-3' and Fish-BCL 5'-ACTTCTGGGTORCRAAARAATCA-3' (Baldwin et al., 2009). 0.5 U of Biolase DNA polymerase (Bioline, USA). The cycle profile was as follows: 5 min at 95 °C, followed by 35 amplification cycles of 30 sec at 53 °C, 45 sec at 72 °C, 30 sec at 94 °C and a final elongation step of 5 min at 72 °C. For several samples, a secondary product of ~300 bp amplified consistently, hence, to eliminate the unspecific product a touchdown-PCR was performed as follows: 5 min at 95 °C, one cycle of 30 sec at 58 °C, 45 sec at 72 °C, 30 sec at 94 °C, one cycle of 30 sec at 56 °C, 45 sec at 72 °C, 30 sec at 94 °C, one cycle of 30 sec at 55 °C, 45 sec at 72 °C and 30 sec at 94 °C, followed by 25 amplification cycles of 30 sec at 53 °C, 45 sec at 72 °C and 30 sec at 94 °C and a final elongation step of 5 min at 72 °C. PCR products were purified using Exo-SAP-IT (Affymetrix, Inc., USA) and purified PCR products were sequenced using the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, USA) and ABI 3730 DNA Analyzer (Applied Biosystems, USA) at the Smithsonian Laboratory for Applied Biology, Washington DC. Sequences were checked for base call and stop codons in Geneious (http://www.geneious.com; Kearse et al., 2012), and aligned using Clustal X (Jeanmougin et al., 1998). All checked sequences were compared to homologous COI sequences using two searchable databases: BOLD to search the Barcode of Life Data Systems and BLASTn to search NCBI nucleotide repository in GenBank. We established confidence values for both BOLD (similarity >98%) and BLASTn (identity >97%) to ensure the reliable identification of species for each sample.

Unique COI haplotypes were identified using DnaSP v6 (Rozas et al., 2017). Genetic distance was estimated as COI sequence divergence percentages within different taxonomic levels using the Kimura 2-parameter (K2P) distance model (Kimura, 1980) in MEGA version 7.0 (Kumar et al., 2016). Phylogenetic relationships among COI haplotypes were examined with a K2P maximum likelihood (ML) tree tested for 1000 bootstrap replications compiled in MEGA version 7.0 (Kumar et al., 2016). In addition, phylogenetic relationships among haplotypes from Myanmar and other congeneric species were for 431 bp overlapping COI sequences available in NCBI database following the same method formerly described.

**Results**

A total of 220 tonguesole individuals were successfully sequenced for the COI gene, which recovered 42 unique COI barcodes of 543 bp (Genbank accession numbers MH235628 and MK713853–MK713893). The alignment of these COI sequences against NCBI and BOLD databases, suggested that these COI barcodes represent potentially 10 species in the genus Cynoglossus. The high percentage of identity and similarity resulting from the alignments against NCBI and BOLD, respectively, allowed us to match 35 of these COI barcodes to five species: Cynoglossus arel, C. lingua, Cynoglossus puncticeps (Richardson, 1846), C. bilineatus (C. quadrilineatus, sensu Kotteleat, 2013) and Cynoglossus oligolepis (Bleeker, 1865). The morphometric identification suggested the presence of five species in the fishery. C. arel, C. lingua, Cynoglossus, C. bilineatus and C. puncticeps, but only C. lingua, C. puncticeps and C. bilineatus were supported by genetic identification (Supplementary Table 1). The identification of C. oligolepis was not recognised based on morphometric characters, while C. cyanoglossus was not recognised based on genetic barcodes. Another seven COI barcodes (TSH31–37) returned low scores of identity to other Cynoglossus species, thus, were identified only at the genus level.

Nonetheless, the specimen holding the COI barcode TSH37 was identified as C. puncticeps based on morphological characters; this identification was supported by its close phylogenetic relationship with other barcodes matching C. puncticeps in the NJ tree (Fig. 2 and Supplementary Table 1). Other few alignments were not readily resolved; for C. bilineatus haplotypes TSH40 and TSH41 were allocated to this species BOLD Index Number (BIN: ADL6276) and confirmed by visual inspection but were partially resolved using BLASTn (Supplementary Table 1). Similarly, four ambiguous calls were found for C. lingua/C. arel when comparing the two databases. For these cases, morphometric identification supported the BLASTn matches to C. lingua (Supplementary Table 1), and were confirmed by the phylogenetic reconstruction of these haplotypes (THS23–25 and TSH30) (Fig. 2). The topology of the ML phylogenetic trees resolved monophyly for all five species identified and supported the occurrence of another six species (Figs. 2 and 3).

Estimates of COI nucleotide mean K2P divergence at intra and interspecific levels are shown in Table 1. As
Fig. 2. Phylogenetic reconstruction by maximum likelihood method of COI gene haplotypes (543 bp) of tonguesole species in the genus Cynoglossus identified in the Tanintharyi Region Myanmar. Labels at the tip of the branch correspond to COI haplotypes, the bootstrap values are shown at the nodes of the tree.

Fig. 3. Phylogenetic reconstruction by maximum likelihood method of COI gene haplotypes (543 bp) of unidentified tonguesoles, Cynoglossus spp. from the Tanintharyi Region of Myanmar using Cynoglossus arel as the outgroup. Labels at the tip of the branch correspond to COI haplotypes, the bootstrap values are shown at the nodes of the tree. Photos of sampled specimens and total length (TL) at the tip of the branch showing the difference in external morphology.
expected, mean divergence was higher at the interspecific taxonomic level (ranging 13.5–24.8 %) than intraspecific level (ranging 0.3–0.6 %). The estimated mean K2P divergence within the group of unidentified Cynoglossus spp. COI haplotypes ranged from 0.04–14.9 % and averaged 11.2 %, which is approximately as high as the interspecific level estimates (Supplementary Table 3). This suggested that these haplotypes correspond to more than one species. The visual inspection and ML phylogenetic reconstruction (Figs. 2 and 3) also support this hypothesis. The unidentified species (Cynoglossus spp.) appear to be small size species, TL registered ranged 85–113 mm (Fig. 3).

The occurrence and relative proportions of tonguesole species differ between the two studied locations; Myeik showed a higher species diversity (>5 species) compare to only three species in Dawei (Fig. 4). The breakdown by species of our sample collection suggested an asymmetrical contribution of each species to the landing’s composition (Fig. 4). Overall, the most common species was C. oligolepis which was also the dominant species in Dawei, while C. lingua was the second most common species and dominant in Myeik (Fig. 4). Whereas C. arel and C. bilineatus were found in similar proportions in both Dawei and Myeik and C. puncticeps was only found in few numbers in Myeik (Fig. 4).

Table 1. Genetic distance estimated as net evolutionary divergence, in percentage, of 543 bp COI gene haplotypes sequences from tonguesoles in the genus Cynoglossus identified at the species level in the Tanintharyi Region. Below the diagonal showing the net average evolutionary divergence between species, across the diagonal showing the average evolutionary divergence within species based on the Kimura 2-parameter model, in parenthesis the number of haplotypes identified per species.

| Species          | Cynoglossus arel | Cynoglossus lingua | Cynoglossus oligolepis | Cynoglossus puncticeps | Cynoglossus quadrilineatus |
|------------------|-----------------|-------------------|------------------------|------------------------|---------------------------|
| C. arel          | 0.3 (7)         |                   |                        |                        |                           |
| C. lingua        | 15.1            | 0.4 (8)           |                        |                        |                           |
| C. oligolepis    | 13.5            | 17.1              | 0.5 (15)               |                        |                           |
| C. puncticeps    | 14.8            | 14.7              | 14.4                   | 0.2 (2)                |                           |
| C. quadrilineatus| 20.7            | 24.8              | 22.9                   | 15.9                   | 0.6 (6)                   |

Fig. 4. Catch contribution by species. The identified tonguesole species showed differential contribution to landings between studied sites in the Tanintharyi Region, the proportion of each species based on total number of samples from each site. (“spp” includes all samples identified at genus taxonomic level).
Discussion

This is one of the first genetic studies to identify species composition of important coastal fisheries in Myanmar. Only hairtail fish and mud crab have been similarly examined (Okamoto et al., 2018; Segura-Garcia et al., 2018, respectively). DNA barcoding allowed the identification of five tonguesole species: Cynoglossus arel, C. lingua, C. puncticeps, C. bilineatus (C. quadrilineatus, sensu Kottelat, 2013) and C. oligolepis. The distribution of C. oligolepis was previously reported for the Western Pacific (Li and Wang, 1995), and was described in waters off India (Day, 1876), and the species BIN (AAZ7037) holds records of C. oligolepis from Malaysia. This study represents the first confirmed record of C. oligolepis in Myanmar, suggesting that the actual distribution of C. oligolepis is wider than previously reported.

Following taxonomic identification guides, the use of external morphometric characters allowed the identification of three of the five species C. lingua, C. puncticeps and C. bilineatus. This identification was confirmed by their genetic barcode. The diagnostic characteristics are not conspicuous for C. arel and C. lingua. (Supplementary Table 2) and there were ambiguous species calls between their barcode and morphometric diagnostics. These species were hypothesised to be closely related by Menon (1977), which was also supported by the COI gene ML phylogenetic reconstruction in this study (Fig. 2). Although, this phylogenetic relationship needs to be further tested considering a greater diversity of tonguesole species.

Other species were suggested by DNA barcoding and estimates of mean divergence between six COI haplotypes; but the taxonomy of these haplotypes was only resolved at the genus level. The alignments with NCBI and BOLD database matched Cynoglossus sp. or another congeneric species with scores of >90%. The limited efficacy of DNA barcoding for tonguesole species identification was caused by poor DNA barcode representation for the family Cynoglossidae in public databases. Accruing more COI reference sequences in public databases would improve the resolution of DNA barcoding. Alternatively, a wider genome representation or sequencing data from a fast-evolving marker, such as the mitochondrial control region, could provide additional information for species identification as was reported for species in the genus Sebastes lacking DNA barcode reference (Shum et al., 2017). The ultimate number of tonguesole species found in this study is still unclear. Based on the K2P divergence estimates (Supplementary Table 3) and the phylogenetic reconstruction (Fig. 3), we hypothesise that at least another four species could be identified in the mixed-species fisheries of south Myanmar (Fig. 3). Interestingly, other species reported to be common in the region, such as the Bengal tonguesole, C. cynoglossus, (Aung, 2018), were not confirmed using genetic barcodes. It is expected that other species contribute to the tonguesole landings in the Tanintharyi Region, as the species richness estimates are high in other neighbouring countries such as Indonesia, Malaysia and the Gulf of Thailand (Munroe, 2005), where more studies have been conducted.

This study found that misidentifications, duplicated descriptions, and mislabeling for tonguesoles have accumulated for the genus in global databases. This is indicated by the number of ambiguous species identification using both BLASTn and BOLD (Supplementary Table 1), the duplication of C. quadrilineatus (Kottelat, 2013) and the lack of monophyly of previously published haplotypes within the genus Cynoglossus (Supplementary Fig. 2). Previous studies found monophyly of the family Cynoglossidae (Chapleau, 1988); but the monophyly of the genus Cynoglossus still needs to be studied. Revisions of species names in public data bases are recommended to prevent further misidentifications.

Besides the several species of tonguesole found, we identified other flatfish species in the family Soleidae, Synaptura comemrsonii (Lacepède, 1802) and Zebrrias synapturoides (Jenkins, 1910), marketed jointly with tonguesoles as “khwayshar”. The high level of diversity of flatfishes found in mixed-species fisheries in this study is in accordance with findings throughout the Indo-West Pacific (Munroe, 2015a), in the Persian Gulf (Ghaffari et al., 2015), and at a global scale (Cheung and Oyinlola, 2018).

The differences in diversity levels and catch composition observed between the two regions in this study, Dawei and Myleik, could be explained by the different habitats at their respective fishing grounds and differences in the gear used in each of the studied communities. Fishers from the villages of San Lan and Thabuseik, in the Dawei area, catch tonguesoles in offshore waters where sandy bottoms dominate, approximately 15 km offshore near Moscos Island (Fig. 1), using a drift net of 3 m mesh that is set at ~25 m depth to target Indian mackerel (Rastrelliger kanagurta, Cuvier, 1816), croakers (Sciaenidae) and dwarf whipray (Brevitrygon walga. Müller and Henle, 1841). While across the Myleik Archipelago, the communities of Kywe Ku, Ma Eing, Min Khaung Sae and Kaw Mei (Fig. 1), catch tonguesole using bag nets around mudflat areas, a common habitat of tonguesole (Menon, 1977). Bag nets are conical nets about 12 m long with a 7 m wide mouth anchored to the substrate by a bamboo frame at about 10 m depth near mangroves. The mesh size progressively reduces from 14 cm at the mouth to 0.6 cm at the end, targeting mainly shrimp, which constitute approximately 50 % of the total catch weight, while tonguesole contributed only 1-4 % (Lwin Lwin et al., 2014).

The asymmetrical contribution of the different
species to the landings did not seem to be associated with species distribution. The most common species detected, C. oligolepis, was not previously reported for the country, while all other species show a broad distribution across the Indo-West Pacific and different depth ranges between 10 to 961 m (Munroe, 2001). Considering the broad distribution of these species, the asymmetrical contributions of these species to the landings could indicate differences at species population level.

The non-selective nature of mixed-fisheries and the fact that all tonguesole species are marketed under one label could have repercussions at population levels without being acknowledged by stakeholders. For example, one species may decline dramatically in an area changing the species-specific catch composition, but not necessarily the overall total catch levels.

**Conclusion**

The use of genetic barcode showed a high species diversity found in tonguesole landings in Tanintharyi Region of southern Myanmar. The study distinguished potentially 10 species of tonguesole marketed as one local name of “kwashar”; the most common species, Cynoglossus oligolepis, is a new record for the country. The study also found differences in species catch composition, likely due to the use of different fishing grounds and gear along the Tanintharyi.

The findings provide the baseline knowledge of the biodiversity coverage in these mixed-species fisheries. Using this information to develop fishery management and regulation for tonguesole in Myanmar is essential as tonguesoles are a source of food and income for coastal communities.

**Acknowledgements**

We are grateful to the fishers and buyers from the communities we visited across the Tanintharyi Region and the Department of Fisheries of Myanmar for support of this research project. Special thanks to Thomas Munroe for his insightful critical comments and Thu Yain Tun, whose assistance was crucial to carry out this study. This study was supported with funding from the Helmsley Charitable Trust (Grant number 504604-2017). This manuscript is contribution No. 1155 of the Smithsonian Marine Station at Fort Pierce.

**References**

Aung, T.H. 2018. Flatfishes and their catch composition in Mon State, Myanmar. International Journal of Fisheries and Aquatic Studies 6:348-355.

Baldwin, C.C., Mounts, J.H., Smith, D.G., Weigt, L. 2009. Genetic identification and color descriptions of early life-history stages of Belizean Phaeotyx and Astrapagon (Teleostei: Aponogonidae) with comments on identification of adult Phaeotyx. Zootaxa 22:1-22.

Campbell, M.A., Chanet, B., Chen, J.N., Lee, M.Y., Chen, W.J. 2019. Origins and relationships of the Pleuronectoidei: Molecular and morphological analysis of living and fossil taxa. Zoologica Scripta 48:640-656. https://doi.org/10.1111/zsc.12372

Campbell, M.A., López, J.A., Sato, T.P., Chen, W.J., Miya, M. 2014. Mitochondrial genomic investigation of flatfish monophyly. Gene 551:176-182. https://doi.org/10.1016/j.gene.2014.08.053

Carpenter, K.E., Niem, V.H. 1998. The living marine resources of the Western Central Pacific. Food and Agriculture Organization of the United Nations, Rome. 726 pp.

Chapleau, F. 1988. Comparative osteology and intergeneric relationships of the tongue soles (Pisces: Pleuronectiformes: Cynoglossidae). Canadian Journal of Zoology 66:1214-1232.

Cheung, W.W.L., Oyinlola, M.A. 2018. Vulnerability of flatfish and their fisheries to climate change. Journal of Sea Research 140:1-10. https://doi.org/10.1016/j.seares.2018.02.006

Day, F. 1876. The fishes of India: being a natural history of the fishes known to inhabit the seas and fresh waters of India, Burma, and Ceylon. Bernard Quaritch Ltd, London, pp. 321–778. https://doi.org/10.5962/bhl.title.62705

Fischer, W., Bianchi, G. 1984. Bony fishes families: Congiopodidae to Lophotidae. In: FAO species identification sheets for fishery purposes. Western Indian Ocean (Fishing Area 51). Fischer, W., Bianchi, G. (Eds.). Food and Agricultural Organization of the United Nations, Rome. Vols. 1-6: pag. var.

García-Vazquez, E., Machado-Schiaffino, G., Campo, B., Juanes, F. 2012. Species misidentification in mixed hake fisheries may lead to overexploitation and population bottlenecks. Fisheries Research 114:52-65. https://doi.org/10.1016/j.fishres.2011.05.012

Ghaffari, H., Hosseinizadeh Sahafi, H., Engelhard, G.H., Mekhanik Babaei, M. 2015. Reproductive biology of largescale tonguesole Cynoglossus arei in coastal waters of Bandar Abbas, Persian Gulf, Iran. Animal Reproduction Science 154:142–157. https://doi.org/10.1016/j.anireprosci.2014.12.004

Jeanmougin, F., Thompson, J.D., Gouy, M., Higgins, D.G., Gibson, T.J. 1998. Multiple sequence alignment with Clustal X. Trends in Biochemical Sciences 23:403–405. https://doi.org/10.1016/S0968-0004(98)01285-7

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A.A., Heled, J. 2012. Geneious. Bioinformatics, Oxford, England. https://doi.org/10.1093/bioinformatics/bts189

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111–20.

Kottelat, M. 2013. Nomenclature and identity of the tongue soles Paraplagusia bilineata, “Cynoglossus bilineatus” and Paraplagusia blochii (Teleostei: Pleuronectiformes). Raffles Bulletin of Zoology 61:763–786.

Kumar, S., Stecher, G., Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33:1780–1784. https://doi.org/10.1093/molbev/msw164

Li, S., Wang, H. 1995. Fauna Sinica, Osteichthyes. Pleuronectiformes. Science Press, Beijing. 433 pp.

Lwin Lwin, T., Tun, N.N., Thu Hein, S. 2014. Fishery of stationary bag net (bamboo-raft) in Myeik coastal waters. Myeik University Research Journal 5:123–141.

Menon, A.G.K. 1977. A systematic monograph of the tongue soles of the genus Cynoglossus Hamilton-Buchanan (Pisces: Cynoglossidae). Smithsonian Institution Press, Washington DC. 140 pp.
Munroe, T.A. 2001. Cynoglossidae. In: FAO species identification guide for fishery purposes. The Living Marine Resources of the Western Central Pacific., Carpenter, K.E., Niem, V.H. (Eds.). FAO, Rome, pp. 3890–3901.

Munroe, T.A. 2005. Tropical flatfish fisheries. In: Flatfishes biology and exploitation, Gibson, R.N. (Ed.). Oxford, UK, pp. 292–318.

Munroe, T.A. 2015a. Tropical flatfish fisheries. In: Flatfishes biology and exploitation, Gibson, R.N., Nash, R.D.M., Geffen, A.J., van der Veer, H.W. (Eds.). John Wiley & Sons, Ltd, West Sussex, UK, 576 pp.

Munroe, T.A. 2015b. Systematic diversity of the Pleuronectiformes. In: Flatfishes biology and exploitation, Gibson, R.N., Nash, R.D.M., Geffen, A.J., van der Veer, H.W. (Eds.). John Wiley & Sons, Ltd, Chichester, UK, pp. 13–51. https://doi.org/10.1002/9781119501153.ch2

Okamoto, N., Maung-Saw-Htoo-Than, Sein-Than, Koike, K., Shibata, J., Tomiyama, T. 2018. Species composition of hairtails (Trichiuridae) in Myanmar. Regional Studies in Marine Science 17:73–77. https://doi.org/10.1016/J.RSMA.2017.11.007

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34:3299–3302. https://doi.org/10.1093/molbev/msx248

Segura-García, I., Tun, T.Y., Box, S.J. 2018. Genetic characterization of the artisanal mud crab fishery in Myanmar. PLoS ONE 13:e0204905. https://doi.org/10.1371/journal.pone.0204905

Shum, P., Moore, L., Pampoulie, C., Di Muri, C., Vandamme, S., Mariani, S. 2017. Harnessing mtDNA variation to resolve ambiguity in ‘Redfish’ sold in Europe. PeerJ 5:e3746. https://doi.org/10.7717/peerj.3746

Zöckler, C., Naing, T.Z., Moses, S., Soe, Y.N., Hla, T.H. 2014. The importance of the Myanmar coast for water birds. Stilt 66:37–51.
Supplementary Fig. 1. Showing morphometric measurements recorded from each individual tonguesole. Total length (TL), standard length (SL), head length (HL), snout length (SnL), dorsal fin length (DF), anal fin length (AF), pelvic fin length (Lv), and eye diameter (O). Observations were also made of the number of caudal fin rays, types of scales, number of lateral lines and number of scales on the mid-lateral line, and a qualitative assessment of snout shape.

Supplementary Fig. 2. Maximum likelihood tree of COI gene haplotypes (431 bp) from the Tanintharyi region of Myanmar and other congeneric species occurring in the Indo-Pacific region (previously published). The labels along the branches correspond to Genbank accession numbers for published data and haplotype names for Myanmar specimens (in bold). * indicates unidentified species. Bootstrap values are shown at the nodes of the tree. Lack of monophyly and ambiguous calls marked with a star-shape. In parenthesis the country from where the haplotype was isolated.
Supplementary Table 1. Summary of DNA barcode identification showing the identity and similarity scores as well as the diagnose based on morphometric examination. * indicate low score or mismatches.

| COI gene haplotype | Voucher ID | NCBI match | NCBI accession number | % identity | BOLD match | % similarity | Morphometric |
|-------------------|-----------|------------|-----------------------|------------|------------|--------------|--------------|
| TSH01             | Bs29      | arel       | 99.6                  | arel       | 100        | MK713853     | -            |
| TSH04             | Bs64      | arel       | 99.8                  | arel       | 99.82      | MK713854     | -            |
| TSH03             | Bs102     | arel       | 99.2                  | arel       | 99.8       | MK713855     | -            |
| TSH04             | Bs45      | arel       | 99.8                  | arel       | 99.46      | MK713856     | -            |
| TSH05             | Bs61      | arel       | 99.8                  | arel       | 99.82      | MK713857     | -            |
| TSH06             | Bs11      | arel       | 99.8                  | arel       | 99.82      | MK713858     | -            |
| TSH07             | Bs106     | arel       | 99.6                  | arel       | 99.82      | MK713859     | -            |
| TSH08             | Bs46      | oligolepis | 97.3                  | oligolepis| 99.46      | MK713860     | arel         |
| TSH09             | Bs42      | oligolepis | 97.9                  | oligolepis| 100        | MK713861     | -            |
| TSH10             | Bs239     | oligolepis | 99.5                  | oligolepis| 99.64      | MK713862     | -            |
| TSH11             | Bs252     | oligolepis | 99.5                  | oligolepis| 99.54      | MK713863     | -            |
| TSH12             | Bs48      | oligolepis | 97.7                  | oligolepis| 99.28      | MK713864     | -            |
| TSH13             | Bs54      | oligolepis | 97.7                  | oligolepis| 99.1       | MK713865     | -            |
| TSH14             | Bs243     | oligolepis | 97.7                  | oligolepis| 99.1       | MK713866     | -            |
| TSH15             | Bs257     | oligolepis | 99.5                  | oligolepis| 99.64      | MK713867     | -            |
| TSH16             | Bs32      | oligolepis | 97.5                  | oligolepis| 99.28      | MK713868     | -            |
| TSH17             | Bs41      | oligolepis | 97.7                  | oligolepis| 99.1       | MK713869     | -            |
| TSH18             | Bs135     | oligolepis | 97.5                  | oligolepis| 99.37      | MK713870     | arel         |
| TSH19             | Bs33      | oligolepis | 97.5                  | oligolepis| 99.28      | MK713871     | -            |
| TSH20             | Bs44      | oligolepis | 97.5                  | oligolepis| 99.28      | MK713872     | -            |
| TSH21             | Bs259     | oligolepis | 99.2                  | oligolepis| 99.28      | MK713873     | -            |
| TSH22             | Bs40      | oligolepis | 97.7                  | oligolepis| 99.46      | MK713874     | -            |
| TSH23             | Bs80      | lingua     | 99.8                  | arel       | 99.81      | MK713875     | lingua       |
| TSH24             | Bs197     | lingua     | 99.7                  | arel       | 99.81      | MK713876     | lingua       |
| TSH25             | Bs223     | lingua     | 100                   | arel/lingua| 99.81      | MK713877     | lingua       |
| TSH26             | Bs180     | lingua     | 99.1                  | lingua     | 99.82      | MK713878     | lingua       |
| TSH27             | Bs229     | lingua     | 99.6                  | lingua     | 99.82      | MK713879     | lingua       |
| TSH28             | Bs190     | lingua     | 99.6                  | lingua     | 99.82      | MK713880     | lingua       |
| TSH29             | Bs212     | lingua     | 97.8                  | lingua     | 100        | MK713881     | lingua       |
| TSH30             | Bs160     | lingua     | 98.3                  | arel       | 99.81      | MK713882     | lingua       |
| MM7               | MM7       | lingua     | 99.8                  | lingua     | 100        | MH235628     | -            |
| TSH31             | Bs139     | sp         | 87.4*                 | no match   | MK713883   | arel         |              |
| TSH32             | Bs219     | attenuatus | 82.9*                 | no match   | MK713884   | cynoglossus  |              |
| TSH33             | Bs217     | attenuatus | 82.5*                 | no match   | MK713885   | cynoglossus  |              |
| TSH34             | Bs214     | sp         | 88*                   | no match   | MK713886   | unknown      |              |
| TSH35             | Bs215     | sp         | 96.6                  | cynoglossus| 98.57      | MK713887     | unknown      |
| TSH36             | Bs216     | sp         | 85.6*                 | no match   | MK713888   | unknown      |              |
| TSH37             | Bs147     | sp         | 92.2*                 | no match   | MK713889   | puncticeps   |              |
| TSH38             | Bs220     | puncticeps | 99.7                  | puncticeps| 100        | MK713890v    | puncticeps   |
| TSH39             | Bs221     | puncticeps | 99.7                  | puncticeps| 99.82      | MK713891     | puncticeps   |
| TSH40             | Bs270     | bilineatus | 95.95                 | bilineatus | 100        | MK713892     | bilineatus   |
| TSH41             | Bs89      | sp         | 99.6                  | bilineatus | 99.46      | MK713893     | -            |
Supplementary Table 2. Diagnostic characters of tonguesole species distinguished using external morphometric characters.

| Species               | Diagnostic features                                                                 | Photo |
|-----------------------|--------------------------------------------------------------------------------------|-------|
| Cynoglossus lingua    | Snout obtusely pointed, body elongated, ctenoid scale on eyed side, caudal fin with 10 rays, irregular brown–black patches on eyed side. |       |
| Cynoglossus puncticeps| Snout rounded, rostral hook short, eye nearly continuous, ctenoid scale on both side of body, caudal fin with 10 rays, very distinct irregular dark brown blotches, often forming irregular cross bands on eyed side; some rays of dorsal and anal fins dashed with dark brown. |       |
| Cynoglossus bilineatus| Snout rounded, ctenoid on eyed side, cycloid on blind side of body, caudal fin with 12 rays, brown in eyed side of body. |       |

Supplementary Table 3. Estimates of net evolutionary divergence, in percentage, of 543 bp COI gene haplotypes sequences from tonguesoles in the genus Cynoglossus identified at the genus level in the Tanintharyi region. Below the diagonal showing the net average evolutionary divergence based on the Kimura 2-parameter model between suspected species.

| Cynoglossus sp. haplotype | TSH31 | TSH32 | TSH33 | TSH34 | TSH35 |
|---------------------------|-------|-------|-------|-------|-------|
| TSH31                     | -     |       |       |       |       |
| TSH32                     | 0.4   | -     |       |       |       |
| TSH33                     | 0.7   | 0.7   | -     |       |       |
| TSH34                     | 1.1   | 1.1   | 1.1   | -     |       |
| TSH35                     | 11.3  | 11.8  | 11.7  | 10.8  | -     |
| TSH36                     | 14.4  | 14.4  | 14.9  | 13.7  | 14    |