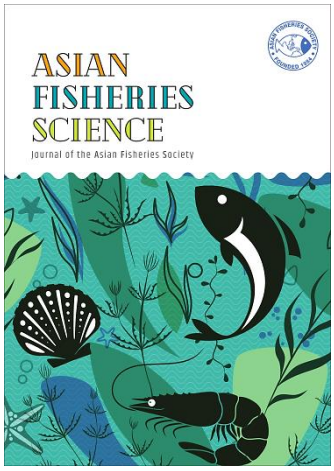

Beyond Bycatch: The Species Diversity of Tonguesole (Pleuronectiformes: Cynoglossidae) in Coastal Fisheries of the Tanintharyi Region, Southern Myanmar

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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 Tanintharyi 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: *Symphurus*, *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).

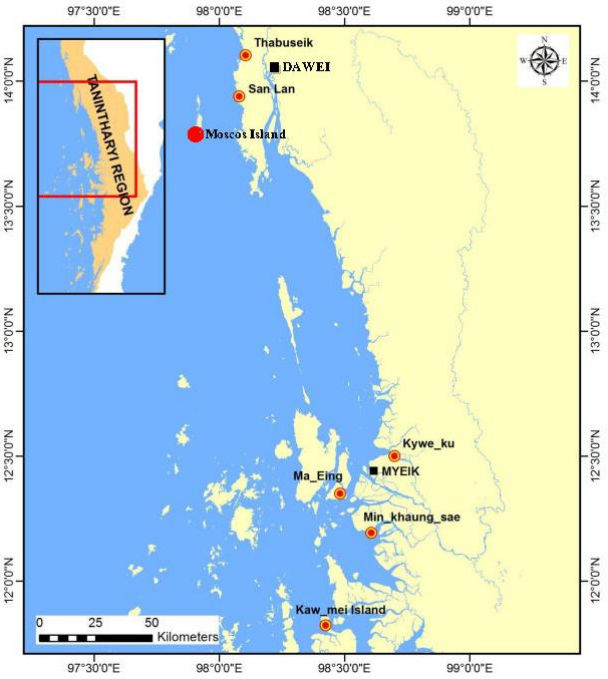

Fig. 1. Map of the study location of the diversity of tonguesole species in the genus *Cynoglossus* in the Tanintharyi Region. Red dots showing the sampling localities in Dawei and Myeik.

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),

dorsal fin length (DF), anal fin length (AF), pelvic fin length (Lv), and eye diameter (O) (Supplementary Fig.1). 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. These external morphological characters were then used for species identifications using the following available identification guides for the region (Day, 1876; Fischer and Bianchi, 1984; Carpenter and Niem, 1998).

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_4 reaction buffer, 2.5 mM $MgCl_2$, 0.125 Mm of each dNTP, 0.3 µM of each primer: Fish-BCL 5′-TCAACYAATCAYAAAGATATYGGCAC-3′ and Fish-BCL 5′-ACTTCYGGGTGRCCRAARAATCA-3′ (Baldwin et al., 2009), 0.5 U of Biolase DNA polymerase (Bioline, USA). The cycle profile was as follow: 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 NBCI 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* Kottelat, 2013) and *Cynoglossus oligolepis* (Bleeker, 1855). The morphometric identification suggested the presence of five species in the fishery (*C. arel, C. lingua, C. cynoglossus, C. bilineatus* and *C. puncticeps*), but only *C. lingua*, *C. puncticep* 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. cynoglossus* 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

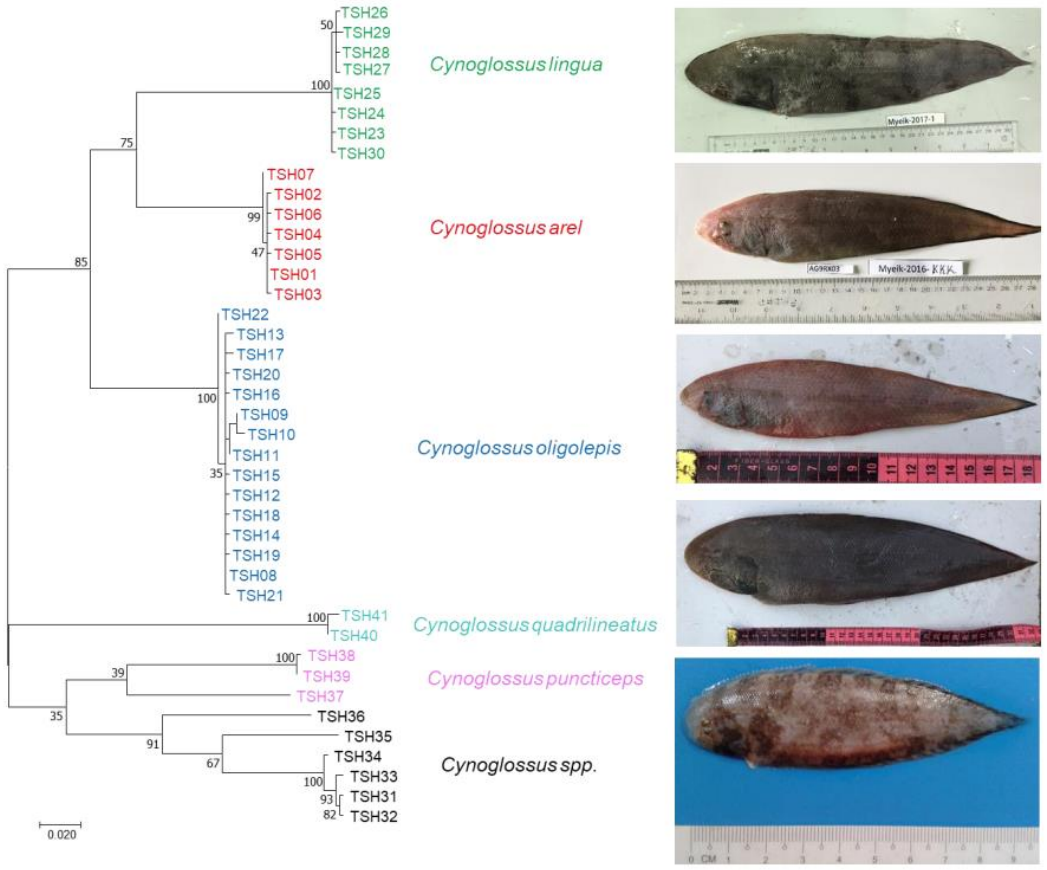

Fig. 2. Phylogenetic reconstruction by maximum likelihood method of *COI gene* haplotypes (543 bp) of tonguesole species in the genus *Cynoglossus* identified in the Tanintharyi Region Myanmar. Labels at the tip of the branch correspond to *COI* haplotypes, the bootstrap values are shown at the nodes of the tree.

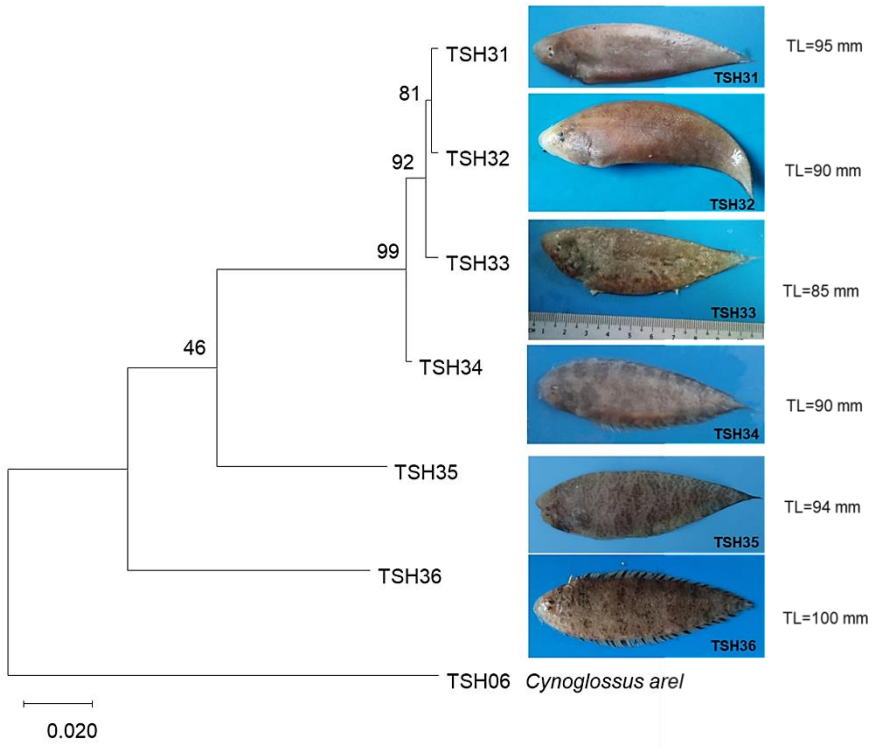

Fig. 3. Phylogenetic reconstruction by maximum likelihood method of *COI gene* haplotypes (543 bp) of unidentified tonguesoles, *Cynoglossus* spp. from the Tanintharyi Region of Myanmar using *Cynoglossus arel* as the outgroup. Labels at the tip of the branch correspond to *COI* haplotypes, the bootstrap 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)

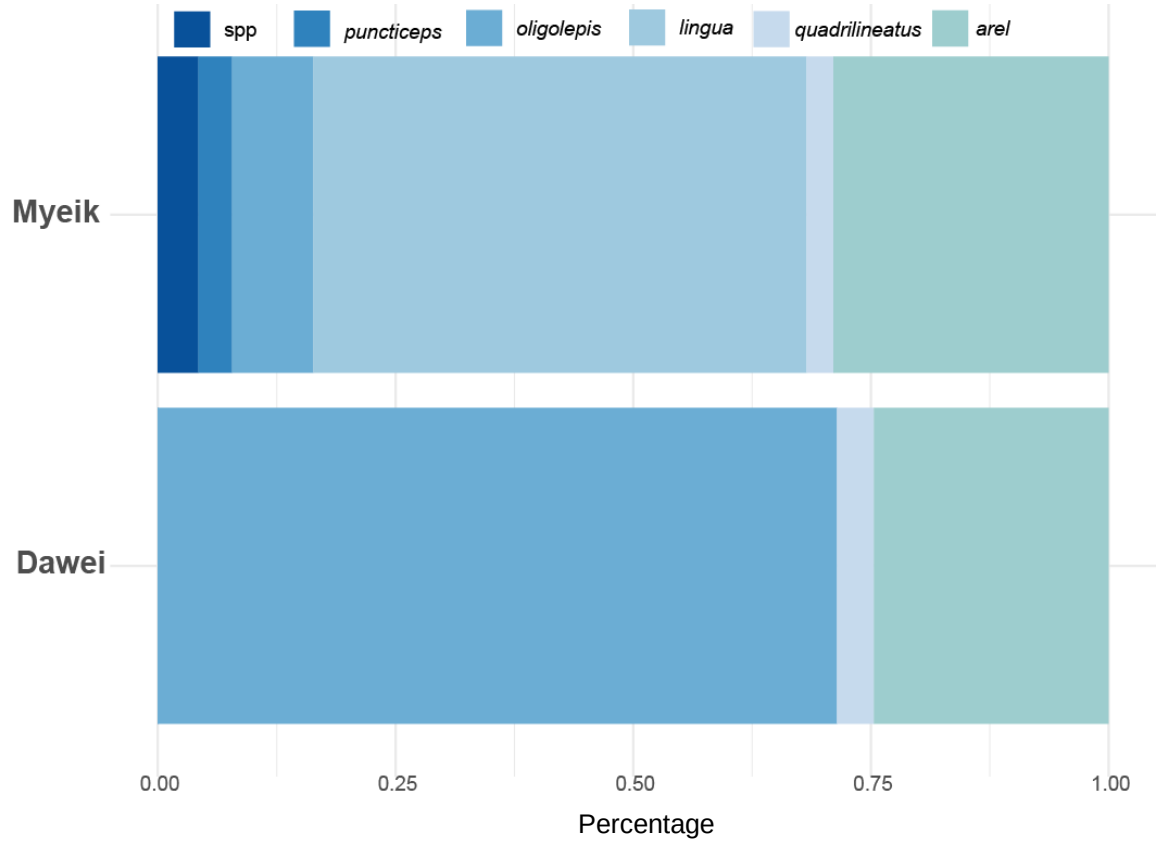

Fig. 4. Catch contribution by species. The identified tonguesole species showed differential contribution to landings between studied sites in the Tanintharyi Region, the proportion of each species based on total number of samples from each site. ("spp" includes all samples identified at genus taxonomic 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-García et al., 2018, respectively). DNA barcoding allowed the identification of five tonguesole species: *Cynoglossus arel, C. lingua, C. puncticeps, C. bilinieatus* (*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 *Zebrias 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 Myeik, 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" 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 Myeik 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

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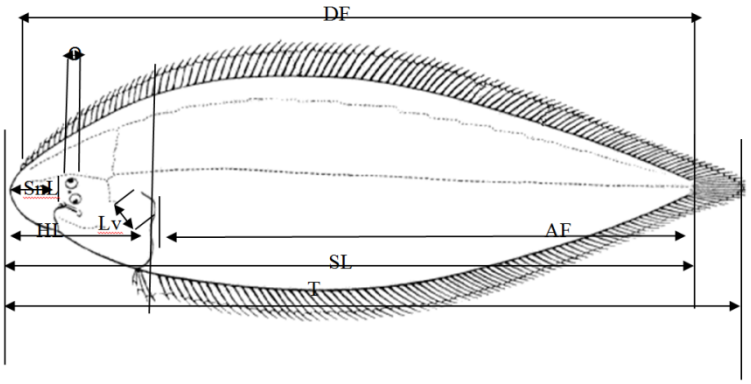
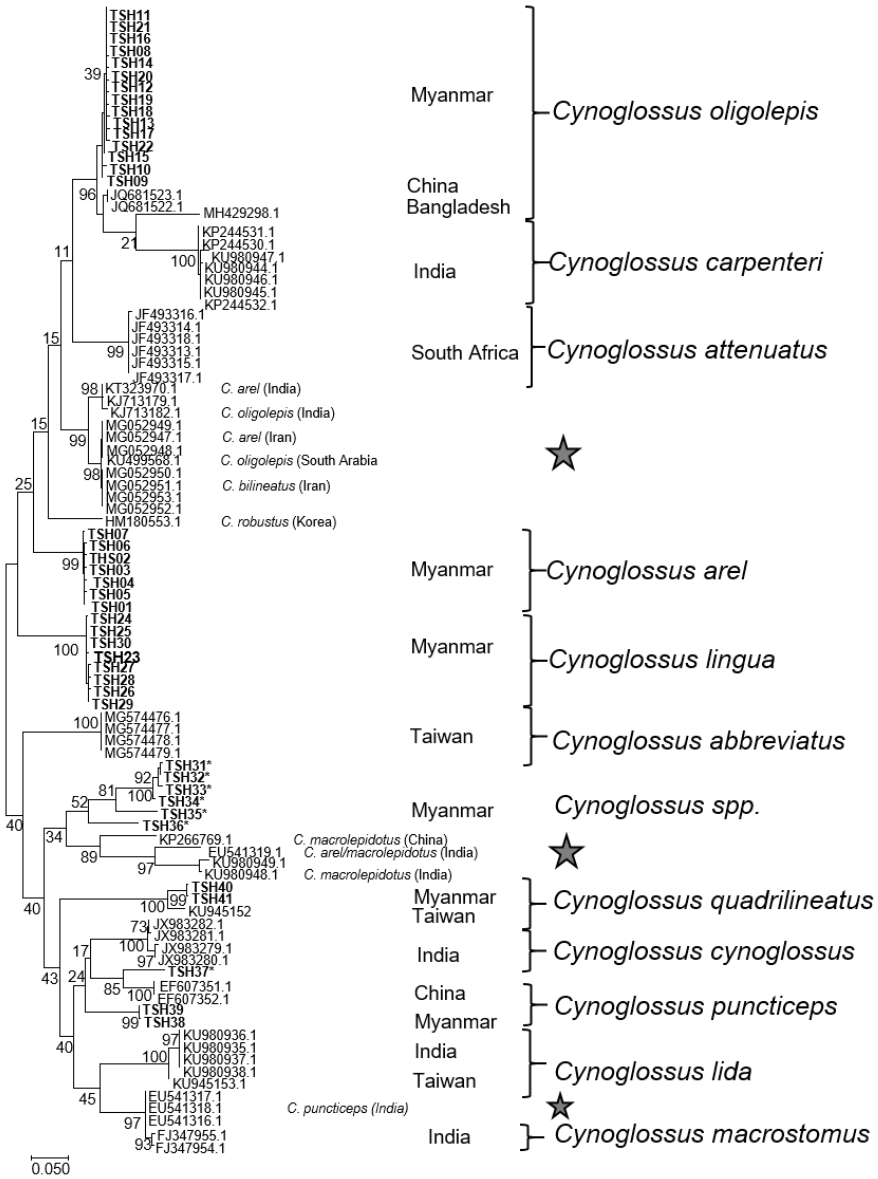


Supplementary Fig. 1. Showing morphometric measurements recorded from each individual tonguesole. Total length (TL), standard length (SL), head length (HL), snout length (SnL), dorsal fin length (DF), anal fin length (AF), pelvic fin length (Lv), and eye diameter (O). Observations were also made of the 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.

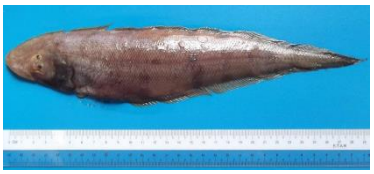
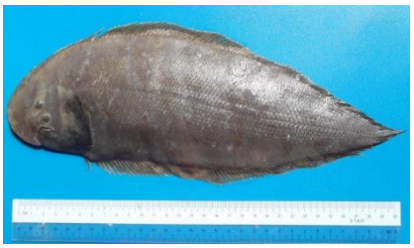


Supplementary Fig. 2. Maximum likelihood tree of *COI gene* haplotypes (431 bp) from the Tanintharyi region of Myanmar and other congeneric species occurring in the Indo-Pacific region (previously published). The labels along the branches correspond to Genbank accession numbers for published data and 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	% identity	BOLD match	% similarity	NCBI accession number	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	Bs69	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