Molecular and Morphological Identification of *Mola* Sunfish Specimens (Actinopterygii: Tetraodontiformes: Molidae) from the Indian Ocean

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We employed molecular systematic analysis based on mitochondrial D-loop sequences to identify two specimens of ocean sunfish from Omani coastal waters that had previously been identified morphologically as *Mola mola* (Linnaeus, 1758) and *M. ramsayi* (Giglioli, 1883). Of the three molecular species that are recognized globally, *Mola sp. A*, *Mola sp. B*, and *Mola sp. C*, we confirmed that both specimens from Oman are *Mola sp. A*. This result suggested a broader distribution of this species than had ever been thought, but it also revealed a discrepancy between morphological and genetic characters in *Mola* taxonomy. We reexamined the morphology of the two specimens from Oman to clarify the extent to which the key morphological features correspond with molecular genetic identifications, and discovered errors in the original reports on both specimens.

**Key Words:** Molidae, *Mola*, morphological discrepancy, DNA analysis, Indian Ocean, morphological change.

**Introduction**

Individuals of ocean sunfish genus *Mola* (family Molidae), which is distributed widely in tropical and temperate waters of the world, grow to over 300 cm in total length (TL) and may weigh over 2000 kg (Pope et al. 2009; Sawai 2012). *Mola* species have a unique body shape and completely lack caudal elements, which are replaced with a rear fin (called the clavus) comprising highly modified elements of the dorsal and anal fins (Fraser-Brunner 1951; Johnson and Britz 2005; Nakae and Sasaki 2006). The genus has been recognized to contain two species, *M. mola* (Linnaeus, 1758) and *M. ramsayi* (Giglioli, 1883), according to morphological descriptions by Fraser-Brunner (1951). The numbers of fin rays in, and ossicles on, the clavus have been suggested as major key morphological characteristics to distinguish the two species of *Mola* (Fraser-Brunner 1951).

Recent molecular phylogenetic studies of *Mola* have resulted in different taxonomies (Bass et al. 2005; Sagara et al. 2005; Yoshita et al. 2009; Yamanoue et al. 2010; Thys et al. 2013). Bass et al. (2005), based on a mitochondrial DNA analysis, proposed that *M. mola* and *M. ramsayi* can each be subdivided into Atlantic and Pacific groups (Fig. 1). Subsequently, mitochondrial DNA analysis by Yoshita et al. (2009) revealed that the genus *Mola* contains at least three groups around the world, which were later recognized as independent species. They have been designated provisionally as *Mola* species A, B, and C await definitive assignment of valid scientific names (Yamanoue et al. 2010; Yamanoue and Sawai 2012). In the phylogenetic tree generated by Yoshita et al. (2009), these three species consistently correspond to the previously proposed groups of Bass et al. (2005): *Mola sp. A*, *Mola sp. B*, and *Mola sp. C* were identical to the Pacific *M. ramsayi* group, group, and Atlantic *M. ramsayi* group, respectively (Fig. 1). It is still unclear, however, how these genetically identified species correspond to the morphology-based taxonomy of Fraser-Brunner (1951), so here we follow Yoshita et al. (2009).

Bass et al. (2005) did not conduct morphological research on *Mola*; however, for *Mola sp. A* and *Mola sp. C*, differences in morphology and ecological characteristics have been extensively studied in specimens from Japanese waters (Yoshita et al. 2009; Sawai et al. 2011). Additional papers (e.g., Hatooka and Hagiwara 2013) accepted these as different species. However, confirmation of a correspondence between morphological and molecular-phylogenetic taxa has never been done elsewhere, including the Indian Ocean.

The first and only Indian Ocean specimen of *Mola* used for molecular analysis was from Algoa Bay, South Africa (Bass et al. 2005). Probably due to the location of the sampling site close to the border between the Atlantic and Indian ocean basins, this sample was treated as an Atlantic rep-
representative in the phylogenetic analysis of Bass et al. (2005) (EMBL/GenBank/DDBJ accession number AY940827). The specimen was subsequently confirmed to belong to *Mola* sp. C (Yoshita et al. 2009). Here we obtained two further specimens (OMMSFC 1085 and OMMSTC 1097) from the Indian Ocean for DNA and morphological analyses. Using the morphological descriptions of Fraser-Brunner (1951), these two specimens had been identified as *M. ramsayi* and *M. mola*, and they were published as the first records of each species from Omani waters (Jawad et al. 2012; Jawad 2013).

No other records of *Mola* are known from this region so far. In this study, we conducted a phylogenetic analysis to determine which genetically recognized species, i.e., *Mola* sp. A, *Mola* sp. B, or *Mola* sp. C, can accommodate the two specimens. We also briefly discuss the correspondence between genetic identity and morphological taxonomy in *Mola* sunfishes and their respective distributional ranges in the Indian Ocean.

**Materials and Methods**

**Material examined.** OMMSFC 1085, 91.6 cm TL, coast of Sur, Sultanate of Oman, 10 September 2011, stranded (Jawad et al. 2012); OMMSTC 1097, 135.0 cm TL, coast of Quriat, Sultanate of Oman, 1 April 2012, stranded (Jawad 2013). The whole bodies of both specimens are deposited in the fish collection of the Marine Science and Fisheries Centre, Ministry of Agriculture and Fisheries Wealth, Muscat, Sultanate of Oman. Tissue samples from both specimens were taken as described below before the specimens were preserved and deposited in the fish collection.

**DNA analysis.** Fin tissues were excised from the two specimens when they were fresh, and stored in absolute ethanol. Total genomic DNA was extracted using a Gentra Puregene Tissue Kit (QIAGEN) following the manufacturer’s protocol. The major non-coding region of the mitochondrial genome, located between the tRNAPro and tRNAPhe genes, commonly called the D-loop or control region, was analyzed in this study. The entire D-loop region of each specimen was amplified by PCR technique with the PCR primers L15927-Thr (5′-AGA GCG TCG GTC TTG TAAKCCG-3′; Miya and Nishida 1999) and H599-Phe (5′-AGC ATC TTC AGT GTT ATG CTT-3′). The amplification reaction mixture (total 10 µl) included 0.5 µl of DNA template, 0.04 µl of TaKaRa Ex Taq (2.5 U), 0.8 µl of dNTP mixture, 1.0 µl of 10× Ex Taq buffer, and 7.66 µl of sterile distilled water. The thermal cycle profile was as follows: denaturation at 94°C for 15 s, annealing at 52°C for 15 s, and extension at 72°C for 1 min. A double-stranded PCR product, purified using an ExoSAP-IT (USB), was subsequently used for direct cycle sequencing using a Big-dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster, CA, USA) and the primer L16504-CR (5′-TGAWYTAT TCC TGG CAT TTG GYTC-3′; Miya and Nishida 1999) in addition to the same primers used for the PCR reactions. Sequencing reactions were performed according to the manufacturer’s instructions, and dye-labeled fragments were analyzed using Model 3130 DNA sequencers (Applied Biosystems). The DNA sequence obtained was edited and analyzed with EditView ver.1.01, AutoAssembler ver.2.1 (Applied Biosystems) and DNASIS ver.3.2 (Hitachi Software Engineering Co. Ltd.). The complete D-loop sequences of the two Omani *Mola* specimens were added to the 146 *Mola* sequences available in the database (DDBJ/EMBL/GenBank), together with those of *Ranzania laevis* (Pennant, 1776) and *Masturus lanceolatus* (Liénard, 1840) as outgroups, to construct a dataset for analysis (Table 1). The dataset was aligned using MAFFT v7 (Katoh and Standley 2013) and the aligned sequences were automatically trimmed using trimAL (“-automated1” option; Capella-Gutiérrez et al. 2009). Phylogenetic relationships were inferred for species identification with the neighbor joining (NJ), maximum likelihood (ML), and maximum parsimony (MP) methods, which were conducted with MEGA v6 (Tamura et al. 2013). A bootstrap analysis was performed to calculate the robustness of each branch of the resultant NJ, ML, and MP trees. The ML and MP trees are not reported.

Fig. 1. Correspondence between species names of *Mola* sunfishes in previous studies. The dotted arrows indicate unclear relationships, while the solid arrows indicate clear relationships.
in detail here, because these trees displayed almost the same form as the NJ tree. The complete D-loop sequences of OMMSFC 1085 (sample code QuOma-Mo1) and OMMSTC 1097 (QuOma-Mo2) have been registered in DDBJ/EMBL/GenBank under the accession numbers LC010276 and LC010277, respectively.

**Morphological analysis.** The two specimens were re-

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample code (Accession number)</th>
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<tbody>
<tr>
<td><em>Ranzania laevis</em></td>
<td>IK1^1</td>
</tr>
<tr>
<td><em>Masturus lanceolatus</em></td>
<td>MTNP1^1</td>
</tr>
<tr>
<td><em>Mola</em> sp. A</td>
<td>AM-13^1, AM-39^1, OL-1^1, AM-54^1, IO-1^1, IS-1^1, JNSW-2^1, KiM-58^1, OT-1^1, OT-3^1, OT-4^1, OT-6^1, SNSW-3^1, TW-3^1, YI-6^1, YI-7^1, YI-9^1, YI-26^1, YI-27^1, FI-19^1, FI-97b^1, QuOma-Mo1^1, QuOma-Mo2^1</td>
</tr>
<tr>
<td><em>Mola</em> sp. B</td>
<td>AM-12^1, AM-25^1, AM-35^1, AM-36^1, IN-1^1, IN-2^1, IN-3^1, KAIK-4^1, KASK-3^1, KM-1^1, KM-2^1, KO-13^1, KO-17^1, KY-2^1, MA-1^1, MA-2^1, MA-4^1, MA-5^1, (AY940814)^2, (AY940815)^2, (AY940815)^2, (AY940816)^2, (AY940817)^2, (AY940818)^2, (AY940819)^2, (AY940820)^2, (AY940821)^2, (AY940822)^2, (AY940823)^2, AM-15^3, AM-16^3, AM-17^3, AM-26^3, AM-27^3, AM-28^3, AM-29^3, AM-51^3, AM-52^3, AM-53^3, AM-55^3, BMNH-1^1, IK-3^1, IK-5^1, IK-7^1, IN-4^1, IS-2^1, IS-3^1, KASK-1^1, KC-1^1, KI-5^1, KI-8^1, KIM-59^1, KO-4^1, KO-5^1, KO-50^1, KO-51^1, KT-1^1, KT-2^1, KW-1^1, KY-1^1, NDI-1^1, NDI-21, NDO-1^1, NP-1^1, NP-2^1, NP-3^1, NP-4^1, NP-5^1, NP-6^1, NP-7^1, NP-8^1, NP-9^1, NP-10^1, NP-11^1, NP-12^1, NP-13^1, NS-1^1, OS-1^1, Oti-8^1, Oti-9^1, Oti-10^1, Oti-12^1, Oti-13^1, SaK-1^1, SaK-4^1, SaK-5^1, SaK-6^1, SaK-9^1, TW-4^1, TW-7^1, YI-1^1, YI-5^1, YI-8^1, YI-11^1, YI-12^1, YI-13^1, YI-14^1, YI-15^1, YI-16^1, YI-17^1, YI-18^1, YI-19^1, YI-20^1, YI-21^1, YI-22^1, YI-23^1, YI-24^1, YI-25^1, YS-2^1, AT-1^1, FI-8^1, IBUK-1^1, IBUK-13^1, MS-1^1, NH-2^1, OL-91^1, SH-3^1, MOL-1^1, MOL-2^1</td>
</tr>
<tr>
<td><em>Mola</em> sp. C</td>
<td>(AY940826)^2, (AY940827)^2, NNSW-1^1</td>
</tr>
</tbody>
</table>

References for each sample code: ^1 Sagara et al. (2005), ^2 Bass et al. (2005), ^3 Yoshita et al. (2009), ^4 Yamanoue et al. (2010), ^5 unpublished, ^6 this study.

Fig. 2. The phylogenetic placements of the two specimens from Oman in the neighbor-joining tree of *Mola* species inferred from D-loop sequences available in the DDBJ/EMBL/GenBank databases. The Omani specimens are shown in bold. The numbers beside branches indicate bootstrap values (values of less than 50% and those within the *Mola* sp. B and *Mola* sp. C clades are not shown). The scale indicates expected nucleotide substitutions per site.
examined based on photographs in order to reconfirm the expression in each of them of the four diagnostic characters proposed by Fraser-Brunner (1951), which were also used by Jawad et al. (2012) and Jawad (2013): number of ossicles; number of clavus fin rays; presence or absence of a smooth band of reduced denticles along the base of the dorsal fin, clavus, and anal fin; and comparison of ossicle size and width of spaces between ossicles. Moreover, two diagnostic characters proposed by Yoshita et al. (2009) for distinguishing *Mola* sp. A and *Mola* sp. B, the presence or absence of a head bump and the shape of the clavus, were also checked in these two specimens.

**Results**

The total lengths of the complete D-loop sequences of OMMSFC 1085 (sample code QuOma-Mo1) and OMMSTC 1097 (QuOma-Mo2) were 817 bp and 821 bp, respectively, which are typical among those of *Mola* species. Their difference in aligned sequences excluding gaps was only 10 bp, i.e., 1.22% of the total length. Phylogenetic analysis of the whole dataset showed that each species of *Mola* formed a clade with high bootstrap values (91–100%), and the two Omani specimens were both nested within the *Mola* sp. A clade (Fig. 2).

As for morphology, OMMSFC 1085 had 12–13 ossicles and OMMSTC 1097 had 11–13 (Fig. 3). OMMSFC 1085 had 19 clavus fin rays (Fig. 3), but those of OMMSTC 1097 could not be counted from the photograph owing to its thick skin. Both OMMSFC 1085 and OMMSTC 1097 had a smooth band (Fig. 3). The ossicles of OMMSFC 1085 and OMMSTC 1097 were larger than the width of spaces between them (Fig. 3).

**Discussion**

*Mola* sp. A has been found only in the Pacific Ocean, around Japan, Taiwan, Australia, and the Galápagos Islands (Bass et al. 2005; Yoshita et al. 2009; Thys et al. 2013), where Bass et al. (2005) and Thys et al. (2013) called it *M. ramsayi*. The present study confirmed that both of the specimens from Oman, which were first published as *Mola ramsayi* and *Mola mola* (Jawad et al. 2012; Jawad 2013), are genetically and morphologically *Mola* sp. A. This shows that, similarly to the wide distributional range of *Mola* sp. B (Yoshita et al. 2009), *Mola* sp. A also has a wide geographic distribution in both the Pacific and Atlantic ocean basins.

In the present analysis, the Algoa Bay specimen of Bass et al. (2005) was nested within the *Mola* sp. C clade, as it also was in the analysis of Yoshita et al. (2009) (Fig. 2). Thus, both *Mola* sp. A and *Mola* sp. C are found in the Indian Ocean. Owing to a dearth of recorded sightings and catch data of *Mola* sunfishes in the Indian Ocean (Pope et

![Fig. 3. Photographs of the clavus of the two specimens of *Mola* sp. A from Oman (A: OMMSFC 1085; B: OMMSTC 1097). Solid arrows indicate clavus fin rays. Open arrows indicate ossicles. The double-headed arrow indicates the width of the smooth band. Question marks indicate unclear ossicles.](image)

### Table 2. Comparison of morphological characters in two specimens of *Mola* from Oman as reported in previous and present studies.

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<tbody>
<tr>
<td></td>
<td>OMMSFC 1085</td>
<td></td>
<td>OMMSTC 1097</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of specimens</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Total length (cm)</td>
<td>91.6</td>
<td>135.0</td>
<td>181–269</td>
<td>194–277</td>
<td></td>
</tr>
<tr>
<td>Ossicles</td>
<td>12–13</td>
<td>12</td>
<td>11–13</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Clavus fin rays</td>
<td>19</td>
<td>16</td>
<td>No data</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Smooth band</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>Ossicle size (OS) versus width of spaces between ossicles (SS)</td>
<td>OS&gt;SS</td>
<td>OS&gt;SS</td>
<td>OS&gt;SS</td>
<td>OS&lt;SS</td>
<td></td>
</tr>
<tr>
<td>Head bump</td>
<td>Absent</td>
<td>No data</td>
<td>Absent</td>
<td>No data</td>
<td>Present</td>
</tr>
<tr>
<td>Shape of clavus</td>
<td>Round</td>
<td>No data</td>
<td>Round</td>
<td>No data</td>
<td>Wavy</td>
</tr>
</tbody>
</table>
More extensive research is needed to contribute to a more accurate understanding of the distributions of *Mola* species there.

The two specimens from Oman were originally regarded as belonging to species because of apparent differences in their morphology (Jawad et al. 2012; Jawad 2013), but the present study, based on both genetics and morphology, found them to be the same species (Table 2). The counts of the clavus fin rays and ossicles of these specimens in the previous studies (Jawad et al. 2012; Jawad 2013) were lower than those in this study (Table 2). OMMSFC 1085 was identified as *M. ramsayi* by Jawad et al. (2012) because of its supposed lack of a smooth band in the clavus, but we found such a smooth band in this specimen (Fig. 3; Table 2). Smooth bands are present in both *Mola* sp. A and *Mola* sp. B, but they are more difficult to observe in the former probably due to a difference in body scales (Sawai et al. 2015).

This no doubt led the earlier researchers to the wrong conclusion. OMMSTC 1097 was identified as *M. mola* by Jawad (2013) because he considered that the ossicles were smaller than the width of the spaces between them, while we found the opposite (Fig. 3; Table 2). The different results in species identification obtained by two groups of researchers highlight fundamental problems in the definition of key characters for *Mola* species. Furthermore, the studies of Fraser-Brunner (1951) were based on cursory examinations of relatively few specimens (Matsuura 2015).

The morphological features of *Mola* sp. A seem to be well consistent with the key characters (16 clavus fin rays; 12 ossicles; ossicles larger than the width of the space between them) of *M. ramsayi* proposed by Fraser-Brunner (1951), except for the presence or absence of the smooth band. Thys et al. (2013) and Matsuura (2015) synonymized *M. ramsayi* with *Mola* sp. A, but we think this was premature and that it is necessary to conduct further investigations, including a morphological reappraisal of the type specimen of *M. ramsayi*.

Owing to a lack of small-sized specimens of *Mola* sp. A (181–332 cm TL; Yoshita et al. 2009; Yamanoue et al. 2010), the key characters for morphological discrimination between *Mola* sp. A and *Mola* sp. B can only be evaluated for specimens of TL over 180 cm (Yoshita et al. 2009; Hatooka and Hagiwara 2013). The specimens from Oman used in the present study are far smaller than other recorded specimens from Japan (Yoshita et al. 2009), and at 91.6 cm and 135.0 cm TL. They are the smallest confirmed specimens of *Mola* sp. A. In comparison with larger specimens of *Mola* sp. A (Fig. 4; cf. Yoshita et al. 2009; Hatooka and Hagiwara 2013; Sawai et al. 2015), the specimens from Oman have a similar rounded clavus (Fig. 3), the specimens from Oman lack a bump on the head (Fig. 4; cf. Jawad et al. 2012; Jawad 2013). The shapes of the head parts seem to be similar to those of *Mola* sp. B (cf. Yoshita et al. 2009; Sawai et al. 2015). Additionally, the bump of the chin under the lower jaw of these small Omani specimens is less prominent than that of larger specimens of *Mola* sp. A (Fig. 4). These are possible indications that the bumps of the head and chin in *Mola* sp. A develop with age and that their form may be an ontogenetic feature.

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