Research Article

Otolith shape analysis of three mudskipper species of Persian Gulf

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Abstract

The variation in otolith shape of three syntopic species of mudskippers *Boleophthalmus dussumieri*, *Periophthalmus waltoni*, and *Scartelaos tenuis* from the tidal flats of Qeshm Island in Persian Gulf was studied based on Elliptic Fourier Analysis. Principal component analysis and Discriminant analysis could separate the specimens of every species with 100% classification success, which proves the variability of otolith among the three fish species. Cluster analysis produced two main clusters, one cluster encompasses only specimens from *P. waltoni* and the other contains *S. tenuis* and *B. dussumieri.* The result of the present study revealed the power of geometric morphometrics in discriminating the three mudskippers. It is also concluded that otolith traits especially its geometric morphometric could be regarded as an invaluable source of information for paleontology, phylogeny, taxonomy and ecology of fishes.

Keywords: Mudskippers, Persian Gulf, Otolith shape, Elliptic Fourier Analysis

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Introduction

Fish otoliths are located in the inner ear of bony fishes, where they operate as integral pieces of the hearing and equilibrium organs (Popper and Fay, 2011). They consist of aragonite and develop independently from the skeletal system (Maisey, 1987). Three pairs of otoliths exist - the saccular otoliths or sagittae, the utricular otoliths or lapilli, and the lagenar otoliths or asterisci (Nolf, 1985). In most teleosts, the morphology of sagitta represents a meaningful taxonomic tool for species identification (Tuset *et al.,* 2008; Nolf, 2013), although considerable intraspecific variation can occur, especially when connectivity between populations is restricted (Teimori *et al.*, 2012a; Gholami *et al.*, 2015; Teimori *et al.*, 2018). Furthermore, environmental factors and ecological or behavioral characteristics can influence otolith morphology (Torres *et al.*, 2000; Volpedo and Echeverria, 2003; Lombarte *et al.*, 2010; Gierl *et al.*, 2018; Doustdar *et al.*, 2019).

Elliptical Fourier-shape descriptors have been used to discriminate between species of species-rich genera such as *Sebastes* (Stransky and MacLellan, 2005). Otolith shape analysis of fishes has also been found effective to discriminate stocks of some species such as *Gadus morhua* (Campana and Casselman, 1993), two anchovy species, silver and blue anchovies (Karahan *et al.*, 2014), three populations of *Lutjanus kasmira* (Vignon and Morat, 2010), and 42

rockfish species (*Sebastes* spp.) (Tuset *et al.*, 2016).

Moreover, using otolith morphometric traits has shown geographical variation between populations of *Aphaniops stoliczkanus* from Southeastern Arabian Peninsula (Reichenbacher *et al.*, 2009) and Southern Iran (Teimori *et al.*, 2012a). These characteristics have been used on genus *Aphanius* to differentiate between species and identify new species (Teimori *et al.*, 2012b; Gholami *et al.*, 2014) which revealed its importance in taxonomic and phylogenetic studies.

Some studies investigated otolith shape analyses in the family Gobiidae (Lord *et al.*, 2012; Yu *et al.*, 2014; Bănaru *et al.*, 2017; Lombarte *et al.*, 2018; Gut *et al.*, 2020). Wang *et al.* (2011) used this method for stock discrimination of spotted tail goby *Synechogobius ommaturus* from the Yellow Sea. Five lineages of the gobionelline-like Gobiidae (sensu Agorreta *et al.*, 2013) were recognized using otolith shape analysis; the outcome revealed that *Pomatoschistus* and *Periophthalmus* lineages were discriminated better than other lineages (Gierl *et al.*, 2018).

Mudskippers (Gobioidei, Gobiidae, Oxudercinae) reside in intertidal and supratidal mudflats and mangroves of Africa, Indian Ocean, and whole Indo-West Pacific region (Murdy,1989; Jaafar and Murdy, 2017). These fishes encompass ten genera, but only four, namely *Boleophthalmus*, *Periophthalmodon*, *Periophthalmus* and

Scartelaos, spend time on land as part of their daily life cycle (Murdy 1989, 2011). In Persian Gulf and Gulf of Oman, three species of mudskippers live in mangroves and tidal flats: *Boleophthalmus dussumieri* Valenciennes, 1837, *Periophthalmus waltoni* Koumans, 1955, and *Scartelaos tenuis* (Day, 1876) (Polgar *et al.*, 2017). The aims of this study were A) to present and compare otolith shape characteristics of the three mudskippers from Persian Gulf based on Elliptic Fourier analysis, and B) to compare their otolith shape relationships with their phylogenetic relations as revealed by molecular trees of the past studies.

Materials and methods

A total of 34 mudskipper specimens (*B. dussumieri*, 10 specimens; *P. waltoni*, 16; *S. tenuis*, 8) were captured from a station located in Qeshm Island (Dokuhak, $26°59'58''$N, $56°09'23''$E) during June 2017, Qeshm is the largest Iranian Persian Gulf Island (Fig. 1).
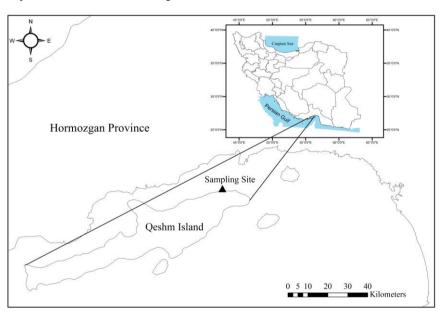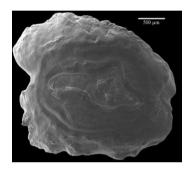

Figure 1: Sampling site for the three mudskipper species in Qeshm Island (Dokuhak, $26°59'58''$N, $56°09'23''$E).
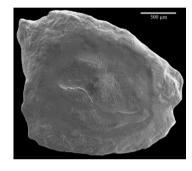
Fish samples were collected using a hand-net during low-tide and transported to the laboratory for further examination. Specimens were identified using morphological characters according to Murdy (1989). Otoliths were removed by dissecting the fish skulls. Otoliths were immersed in 1% KOH solution for 3-4 hours t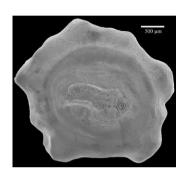o remove organic debris, which were subsequently put in distilled water for 4-5 hours and then rinsed several times. Left otoliths were photographed using a stereomicroscope and scanning electron microscopy (SEM LEO 1430 VP at the Zoological State Collection Munich, Germany and VEGA3 TESCAN at Alzahra University, Tehran, Iran). A representative image of sagitta (termed otolith in the following) of each

mudskipper species is shown in Figure 2. The outline of the otolith was traced using the soft-wares tpsUtil v. 1.38, tpsDig v. 2.16 and GMTP (Rohlf, 2006, 2013; Taravati and Darvish, 2010). The x- and y- coordinates of 300 harmonics were obtained from points equally spaced along the otolith's outline using tpsDig v. 2.16, and the TPS files opened in PAST 3.20 (Hammer *et al.*, 2001). The x- and y- coordinates of

Fourier harmonics were made invariant to otolith size, rotation and starting position of the tracing of the outline (Ferson *et al.*, 1985; Rohlf and Slice, 1990). Variation in otolith shape was assessed using Elliptical Fourier analysis (Ferson *et al.*, 1985). Principal component analysis, Discriminant analysis and Cluster analysis were run using EFA PC scores with PAST 3.20.

Boleophthalmus dussumieri   *Scartelaos tenuis*   *Periophthalmus waltoni*

Figure 2: SEM photographs of otoliths from the three mudskipper species.

Results

Thirty-four SEM pictures of sagittae otoliths of three mudskippers from Qeshm Island were used to conduct the analyses. The outcome of the principal component analysis (PCA) based on EFA PC scores assigned the specimens of each species to a separate group (Fig. 3). The first four principal components (PCs) accounted for 40.9%, 18.8%, 9.6%, and 5.7% of total variance, respectively. PC1 separated *P. waltoni* from *B. dussumieri*, but with some overlap. PC2 clearly isolated *S. tenuis* from the other two species. Discriminant analysis based on PC

scores could recognize every species as a separate group, with 100% classification success (Fig. 4). The first two canonical variates (CVs) accounted for 87.25% and 12.75% of total variance, respectively. CV1 separates *P. waltoni* from *B. dussumieri* and *S. tenuis*. Cluster analysis based on PC scores showed that there were two main clusters, one cluster included of only specimens from *P. waltoni* and the other cluster contained *S. tenuis* and *B. dussumieri* (Figure 5). Cluster analysis recognized *B. dussumieri* and *S. tenuis* as more similar taxa based on otolith shape analysis (yellow line in Fig. 5).
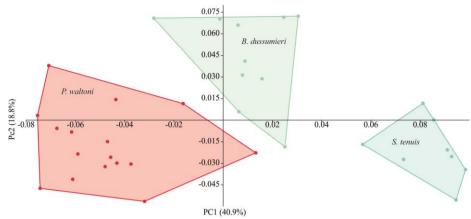
Figure 3: Principal component scores for the otolith shapes of the three mudskipper species, *B. dussumieri*, *P. waltoni* and *S. tenuis*.
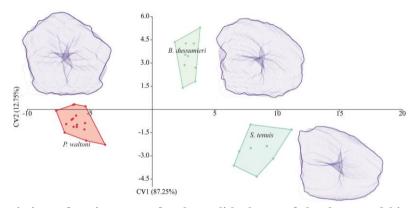

Figure 4: Discriminant function scores for the otolith shapes of the three mudskipper species, *B. dussumieri*, *P. waltoni* and *S. tenuis*.
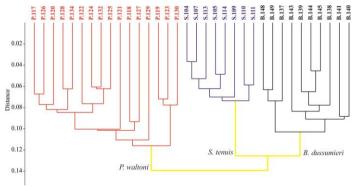

Figure 5: Cluster analysis for the otolith shapes of the three mudskipper species, *B. dussumieri*, *P. waltoni* and *S. tenuis*.

Discussion

In the present study, three mudskipper species from three genera are compared using otolith shape analysis (outline method). *Boleophthalmus dussumieri* and *Scartelaos tenuis* are identified as more similar taxa while *Periophthalmus waltoni* is recognized as a separate clade (Figs. 3 to 5). The body shape of the examined species has been

evaluated by geometric morphometric approach (14 landmarks) in a previous study (Polgar *et al.*, 2017); the resulting PCA scatter plot is similar to the classification of the otoliths of our study; *Periophthalmus waltoni* was separated from *Boleophthalmus dussumieri* and *Scartelaos tenuis* with PC1 (89% of variance).

Molecular studies define *Periophthalmus* lineage within Gobiidae family, which constitutes two traditional subfamilies *Amblyopinae* and *Oxudercinae*, including *Periophthalmus*, *Boleophthalmus*, *Scartelaos* and some other genera (Agorreta *et al.*, 2013; Ghanbarifardi *et al.*, 2016; Polgar *et al.*, 2017). The mentioned studies are in consistent with our result and recognize *Scartelaos* and *Boleophthalmus* as sister taxa and *Periophthalmus* as an isolated genus for all other genera within *Periophthalmus* lineage. Otolith morphometry of mudskippers from Persian Gulf were compared and it is documented that Otolith morphometry were more clearly separated *P. waltoni* from *B. dussumieri*, than *S. tenuis* from either *P. waltoni* or *B. dussumieri* (Ghanbarifardi *et al.*, 2020b). Murdy (1989) used 39 morphological (mostly osteological) apomorphies to conduct a cladistic analysis on Oxudercines. That study puts *Periophthalmus*, *Periophthalmodon, Boleophthalmus*, *Scartelaos* in one clade, isolated from other genera of Oxudercinae, which is in contradiction with the result of the present study and molecular

examinations (Agorreta *et al.*, 2013; Ghanbarifardi *et al.*, 2016; Polgar *et al.*, 2017). Therefore, the otolith characters differentiate mudskippers phylogenetically better than the other osteological characters. The posterior vertebral column and the caudal skeleton of ten mudskipper species were examined and showed isolation of *Periophthalmus* and *Periophthalmodon* from other mudskipper genera to some extent (Ghanbarifardi *et al.*, 2020a). Teimori *et al.* (2012b) have concluded that otolith morphology of *Aphanius* species probably has a higher rate of divergence compared to other morphological traits. Similarly, it seems mudskippers' otoliths could discriminate mudskippers better than other morphological characters (Murdy, 1989; Ghanbarifardi *et al.*, 2020a). Otolith characters (the present study), skull bones (Murdy, 1989), the posterior vertebral column and the caudal skeleton of mudskippers (Ghanbarifardi *et al.*, 2020a) have good fossilization potential and could therefore facilitate recognition of fossil species of mudskippers, which are currently unknown. Moreover, these examinations proved that otolith shape analysis along with body shape analysis could be regarded as informative phylogenetic traits that are useful for taxonomic and systematic surveys.

Acknowledgments

References

Agorreta, A., San Mauro, D., Schliewen, U., Van Tassell, J.L., Kovačić, M., Zardoya, R. and Rüber, L., 2013. Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies. *Molecular Phylogenetics and Evolution*, 69(**3**), 619-633. DOI: 10.1016/j.ympev.2013.07.017

Bănaru, D., Morat, F. and Creteanu, M., 2017. Otolith shape analysis of three gobiid species of the Northwestern Black Sea and characterization of local populations of *Neogobius melanostomus*. *CYBIUM*, 41(**4**), 325-333. DOI: 10.26028/cybium/2017-414-00

Campana, S.E. and Casselman, J.M., 1993. Stock discrimination using otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 50(**5**), 1062-1083. DOI: 10.1139/f93-123

Doustdar, M., Kaymaram, F., Seifali, M., Jamili, S. and Bani, A., 2019. Stock identification of Arabian yellow fin sea bream (*Acanthopagrus arabicus*) using shape of otolith in the Northern Persian Gulf and Oman Sea. Iranian *Journal of Fisheries Sciences*,18(**1**), 60-70. DOI: 10.22092/IJFS.2018.116366

Ferson, S.F., Rohlf, F.J. and Koehn, R.K., 1985. Measuring shape variation of two-dimensional outlines. *Systematic Zoology*, 34, 59-68. DOI: 10.1093/sysbio/34.1.59

Ghanbarifardi, M., Esmaeili, H.R., Gholami, Z., Aliabadian, M. and Reichenbacher, B., 2016. Molecular phylogeny of three mudskippers (Gobiidae) from the Persian Gulf and Gulf of Oman (Makran). *Journal of Applied Ichthyology*, 32, 416-420. DOI: 10.1111/jai.12999

Ghanbarifardi, M., Gut, C., Gholami, Z., Esmaeili, H.R., Gierl, C. and Reichenbacher, B., 2020a. Osteology of the posterior vertebral column and caudal skeleton of marine amphibious gobies (mudskippers) (Teleostei: Gobioidei). *Journal of Applied Ichthyology*, 36(**5**), 713-723. DOI: 10.1111/jai.14071.

Ghanbarifardi, M., Gut, C., Gholami, Z., Esmaeili, H.R., Gierl, C. and Reichenbacher, B., 2020b. Possible link between the structure of otoliths and amphibious mode of life of three mudskipper species (Teleostei: Gobioidei) from the Persian Gulf. *Zoology in the Middle East*, 66(**4**), 311-320. DOI: 10.1080/09397140.2020.1805140.

Gholami, Z., Esmaeili, H.R., Erpenbeck, D. and Reichenbacher, B., 2014. Phylogenetic analysis of *Aphanius* from the endorheic Kor River Basin in the Zagros Mountains, South-western Iran (Teleostei: Cyprinodontiformes: Cyprinodontidae). *Journal of Zoological Systematics and Evolutionary Research*, 52(**2**), 130-141. DOI: 10.1111/jzs.12052

Gholami, Z., Esmaeili, H.R., Erpenbeck, D. and Reichenbacher, B., 2015. Genetic connectivity and phenotypic plasticity in the cyprinodont *Aphanius farsicus* from the Maharlu Basin, south-western Iran. *Journal of Fish Biology*, 86, 882-906, DOI: 10.1111/jfb.12599.

Gierl, C., Liebl, D., Anda, R., Vukić, J., Esmaeili, H.R. and Reichenbacher, B., 2018. What can goby otolith morphology tell us? Cybium: *International Journal of Ichthyology*, 42(**4**), 349-363. DOI: 10.26028/cybium/2018-424-006

Gut, C., Vukić, J., Šanda, R., Moritz, T. and Reichenbacher, B., 2020. Identification of past and present gobies: distinguishing *Gobius* and *Pomatoschistus* (Teleostei: Gobioidei) species using characters of otoliths, meristics and body morphometry. *Contributions to Zoology*, 89(**3**), 282-323. DOI: 10.1163/18759866-bja10002

Hammer, Ø., Harper, D.A. and Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(**1**), 1-9.

Jaafar, Z. and Murdy, E.O., 2017. *Fishes out of water: biology and ecology of mudskippers*, 1st ed. Taylor and Francis Publishing, Oxfordshire, UK.

Karahan, A., Borsa, P., Gucu, A.C., Kandemir, I., Ozkan, E., Orek, Y.A., Acan, S.C., Koban, E. and Togan, I., 2014. Geometric morphometrics, Fourier analysis of otolith shape, and nuclear-DNA markers distinguish two anchovy species (*Engraulis* spp.) in the Eastern Mediterranean Sea. *Fisheries Research*, 159, 45-55. DOI: 10.1016/j.fishres.2014.05.009

Lombarte, A., Palmer, M., Matallanas, J., Gomez-Zurita, J. and Morales-Nin, N., 2010. Ecomorphological trends and phylogenetic inertia of otolith sagittae in Nototheniidae. *Environmental Biology of Fishes*, 89: 607-618. DOI: 10.1007/s10641-010-9673-2

Lombarte, A., Miletić, M., Kovačić, M., Otero-Ferrer, J.L. and Tuset, V.M., 2018. Identifying sagittal otoliths of Mediterranean Sea gobies: variability among phylogenetic lineages. *Journal of Fish Biology*, 92(6), 1768-1787, DOI: 10.1111/jfb.13615.

Lord, C., Morat, F., Lecomte-Finiger, R. and Keith, P., 2012. Otolith shape analysis for three Sicyopterus (Teleostei: Gobioidei: Sicydiinae) species from New Caledonia and

Vanuatu. *Environmental Biology of Fishes*, 93(**2**), 209-222. DOI: 10.1007/s10641-011-9907-y

Maisey, J.G., 1987. Notes on the structure and phylogeny of vertebrate otoliths. *Copeia*, 1987(**2**), 495-499. DOI: 10.2307/1445791

Murdy, E.O., 1989. A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae: Oxudercinae). Records of the Australian Museum Supplement, 11, 1-93. DOI: 10.3853/j.0812-7387.11.1989.93

Murdy, E.O., 2011. Systematics of Oxudercinae. In: *The Biology of Gobies.* Patzner R.A., Van Tassell J.L., Kovačić M. and Kapoor B.G. editors. CRC Press Boca Raton, USA: 99-106.

Nolf, D., 1985. Handbook of paleoichthyology, Volume 10, Otolithi piscium. Dr. Friedrich Pfeil Verlag Publishing, München, Germany.

Nolf, D., 2013. The diversity of fish otoliths, past and present. Brussels: Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

Polgar, G., Ghanbarifardi, M., Milli, S., Agorreta, A., Aliabadian, M., Esmaeili, H.R., and Khang, T.F., 2017. Ecomorphological adaptation in three mudskippers (Teleostei: Gobioidei: Gobiidae) from the Persian Gulf and the Gulf of Oman. *Hydrobiologia*, 795(**1**), 91-111. DOI: 10.1007/s10750-017-3120-8

Popper, A.N. and Fay, R.R., 2011. Rethinking sound detection by fishes. *Hearing Research*, 273, 25-36. DOI: 10.1016/j.heares.2009.12.023

Reichenbacher, B., Feulner, G.R. and Schulz-Mirbach, T., 2009. Geographic variation in otolith morphology among freshwater populations of *Aphanius dispar* (Teleostei, Cyprinodontiformes) from the southeastern Arabian Peninsula. *Journal of Morphology*, 270(**4**), 469-484. DOI: 10.1002/jmor.10702

Rohlf, F.J. and Slice, D., 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology*, 39, 40-59. DOI: 10.2307/2992207

Rohlf, F.J., 2006. tpsUtil v1.38. Retrieved from http://life.bio.sunysb.edu/morph.

Rohlf, F.J., 2013. tpsDig v2.17. Retrieved from http://life.bio.sunysb.edu/morph.

Stransky, C. and MacLellan, S.E., 2005. Species separation and zoogeography of redfish and rockfish (genus *Sebastes*) by otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 62, 2265–2276. DOI: 10.1139/f05-143

Taravati, S. and Darvish, J., 2010. GMTP: Geometric Morphometric Tools Package. Version 2.0 Beta Program.

Teimori, A., Schulz-Mirbach, T., Esmaeili, H.R. and Reichenbacher, B., 2012a. Geographical differentiation of *Aphanius dispar* (Teleostei: Cyprinodontidae) from

southern Iran. *Journal of Zoological Systematics and Evolutionary Research*, 50(**4**), 289-304. DOI: 10.1111/j.1439-0469.2012.00667.x

Teimori, A., Esmaeili, H.R., Gholami, Z., Zarei, N. and Reichenbacher, B., 2012b. *Aphanius arakensis*, a new species of tooth-carp (Actinopterygii, Cyprinodontidae) from the endorheic Namak Lake basin in Iran. *ZooKeys*, 215, 55-76. DOI: 10.3897/zookeys.215.1731

Teimori, A., Esmaeili, H.R., Hamidan, N. and Reichenbacher, B., 2018. Systematics and historical biogeography of the *Aphanius dispar* species group (Teleostei: Aphaniidae) and description of a new species from Southern Iran. *Journal of Zoological Systematics and Evolutionary Research*, 56, 579-598, DOI: 10.1111/jzs.12228.

Torres, G.J., Lombarte, A. and Morales-Nin, B., 2000. Variability of the sulcus acusticus in the sagittal otolith of the genus *Merluccius* (Merlucciidae). *Fisheries Research*, 46: 5-13. DOI: 10.1016/S0165-7836(00)00128-4

Tuset, V.M., Lombarte, A. and Assis, C.A. 2008. Otolith atlas for the western Mediterranean, north and central eastern Atlantic. *Scientia Marina*, 72(**S1**), 7-198.

Tuset, V.M., Otero-Ferrer, J.L., Gómez-Zurita, J., Venerus, L.A., Stransky, C., Imondi, R., Orlov, A.M., Ye, Z., Santschi, L.,

Afanasiev, P.K., Zhuang, L., Farré, M., Love, M.S. and Lombarte, A., 2016. Otolith shape lends support to the sensory drive hypothesis in rockfishes. *Journal of evolutionary biology*, 29(**10**), 2083-2097. DOI: 10.1111/jeb.12932

Vignon, M. and Morat, F., 2010. Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. *Marine Ecology Progress Series*, 411, 231-241. DOI: 10.3354/meps08651

Volpedo, A. and Echeverria, D.D., 2003. Ecomorphological patterns of the sagitta in fish on the continental shelf off Argentine. *Fisheries Research*, 60(**2-3**), 551-560. DOI: 10.1016/S0165-7836(02)00170-4

Wang, Y., Ye, Z., Liu, Q. and Cao, L., 2011. Stock discrimination of spotted tail goby (*Synechogobius ommaturus*) in the Yellow Sea by analysis of otolith shape. *Chinese Journal of Oceanology and Limnology*, 29(**1**), 192-198. DOI: 10.1007/s00343-011-9087-9

Yu, X., Cao, L., Liu, J., Zhao, B., Shan, X. and Dou, S., 2014. Application of otolith shape analysis for stock discrimination and species identification of five goby species (Perciformes: Gobiidae) in the northern Chinese coastal waters. *Chinese Journal of Oceanology and Limnology*, 32(**5**), 1060-1073. DOI: 10.1007/s00343-015-4022-0