Received: 12 June 2016 | Accepted: 27 August 2016

DOI: 10.1111/jai.13283

SHORT COMMUNICATION

WILEY Journal of Applied Ichthyology DWK

First record of the honeycomb stingray *Himantura leoparda* (Manjaji-Matsumoto & Last, 2008) (Myliobatoidei: Dasyatidae) in the Mediterranean Sea, confirmed by DNA barcoding

N. Yucel[1] | **A. Sakalli**[1] | **A. Karahan**[2]

[1]Faculty of Marine Sciences & Technology, Iskenderun Technical University, Iskenderun, Hatay, Turkey

[2]Institute of Marine Sciences, Marine Biology and Fisheries, Middle East Technical University, Erdemli-Mersin, Turkey

Correspondence
Nebil Yucel, Faculty of Marine Sciences & Technology, Department of Water Resources Management and Organization, Iskenderun Technical University, Iskenderun, Hatay, Turkey.
Email: nebilyucel@gmail.com

Funding information
DEKOSIM; Turkish Ministry of Development

1 | INTRODUCTION

The honeycomb stingray *Himantura uarnak* (Forskal, 1775) belongs to the family Dasyatidae, and is widely distributed throughout the Indian and Pacific oceans. It is the only species of the *Himantura* genus also found in the Mediterranean Sea, where it was first recorded by Ben-Tuvia (1966) in the eastern Mediterranean along the Israeli and Turkish coasts (Mersin). *Himantura uarnak* has been recorded along other Mediterranean coasts by Mouneimne (1977, in Lebanon), El Sayed (1994, in Egypt), Basusta, Erdem, and Kumlu (1998, in Turkey) and by Ali, Saad, Ben Amor, and Capapé (2010), Ali, Saad, Reynaud, and Capapé (2013), in Syria). However, *Himantura leoparda* is a recently recorded viviparous species of whip-ray described by Manjaji-Matsumoto and Last (2008) as closely related to *H. uarnak*, with both species having a similar shape and dorsal disc pattern. The taxonomic identification of these two relatively close species is usually achieved by using the DNA barcoding technique (Arlyza et al., 2013).

In this paper we identify for the first time a *Himantura* species, namely *H. leopard*, in the Mediterranean by using both morphological and molecular techniques as well as providing the first DNA barcode record of *H. leoparda* in the Mediterranean Sea.

2 | MATERIALS AND METHODS

2.1 | Sampling and location

A live specimen of the stingray *Himantura leoparda* was caught by atrawl vessel on a sandy bottom at 130–150 m depth, on 24 January 2016 along the Turkish coast (36°10′54.78″N; 35°48′1.96″E). The captured pregnant female gave birth to seven live pups, six of which were returned the sea, with one specimen frozen for morphometric measurements and molecular analyses. Unfortunately, the adult female did not survive but its morphological characteristics were measured onboard. The pup was carefully examined, photographed and measured with vernier calipers (precision 1 mm) in the laboratory.

2.2 | DNA isolation, PCR and data submission

Genomic DNA extraction was carried out according to Paz, Douek, Mo, Goren, and Rinkevich (2003). The cytochrome oxidase I (COI) gene (~650 bp) was amplified following Ward, Zemlak, Innes, Last, and Hebert (2005) using primers (FishF1, FishR1). The PCR outcome was screened on 1.3% agarose gel for the existence of PCR products. Sequencing was performed by Macrogen Inc., The Netherlands, for both directions (forward and reverse). Sequence data, trace files, primer details, specimen collection data, voucher codes and specimen images were submitted to the BOLD system (http://www.boldsystems.org, see Ratnasingham & Hebert, 2007: IMSMETU-HL-01/BOLD:AAB7830).

2.3 | Data analysis

COI sequences in both directions were edited and aligned using BioEdit v.7.0.9.0 (Hall, 1999) software and checked for stop codons, insertions or deletions. The relationships between haplotypes and the species in the present and previously studied samples, downloaded from the NCBI and BOLD system databases, were estimated using the median joining algorithm with default settings for constructing the network (weight = 10 $\varepsilon = 0$) in the program NETWORK version 5.0.0.0. (Bandelt, Forster, & Röhl, 1999). The accession numbers of

TABLE 1 Results of morphometric measurements of *Himantura leoparda* captured at the Turkish coast (January 2016)

Parameters	Pregnant female	Pup
Sex	Female	Female
Disc width (mm)	1352	261
Disc length (mm)	1212	272
Total length (mm)	3002	991
Tail length (mm)	1773	751
Disc thickness (mm)	175	32
Length pelvic fin (mm)	203	55
Weight (kg)	55	0.722

the NCBI and BOLD system mined sample barcodes are provided in Table S1.

3 | RESULTS

Morphometric measurements of both the adult female and the pup are given in Table 1. Forward and reverse sequences of the mitochondrial COI gene, obtained from a *H. leoparda* specimen, were 602 bp in length after trimming. Mean GC and AT % values were calculated as 44.02 and 55.98, respectively.

The COI sequences in the present study, in Cerutti-Pereyra et al. (2012), Arlyza et al. (2013), and Lim, Lim, Chong, and Loh (2015) and in the sequences of four unpublished Indian *H. leoparda* samples (KF899500, KF899501, KF899502, and KF899353) from Indonesia, India (Bineesh), Tanzania (Zanzibar), Malaysia (Kean-Chong) and South Africa (KwaZulu-Natal, Cape Videl) were used to build the network (see Table S1). During the analysis only two base pair (bp) differences were observed between the samples in the present study and two previously-studied *H. leoparda* samples from South Africa [Arlyza et al. (2013), H27, see Figure 1], and five bp differences observed for three H1samples from India (KF899500, KF899501, and KF899502). On the other hand, mutation numbers between the present study and previously studied *H. leoparda* specimens from Indonesia, Malaysia and Tanzania were observed as being above 10 bp (see Figure 1).

4 | DISCUSSION

Himantura leoparda is recorded here for the first time in the Mediterranean Sea. Our findings show that *H. leoparda* inhabits and reproduces on the Turkish coasts of the Northeastern Mediterranean.

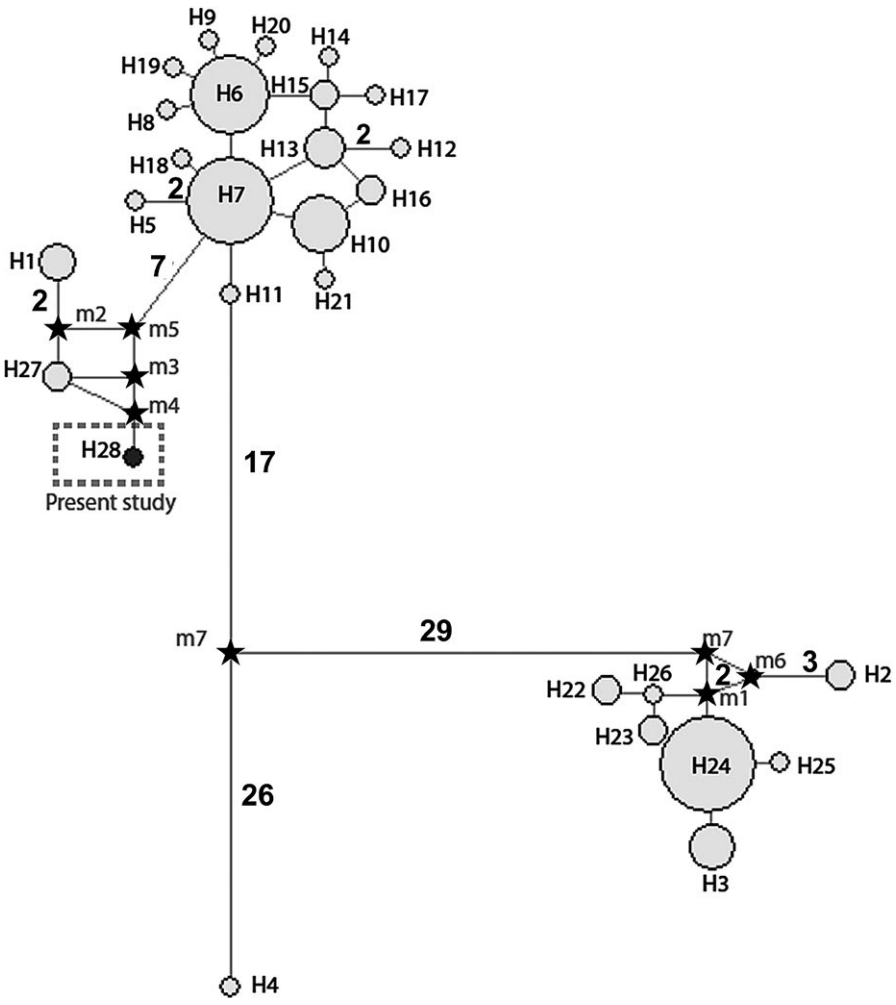

FIGURE 1 Median-joining network for COI haplotypes in present and previosly-studied samples. Circle size is proportional to the number of samples. Bold-Black numbers = mutation step between haplotypes. Stars = median vectors

The DNA barcode of the present study sample produced a 99% match with the *H. leoparda* species of Arlyza et al. (2013) from South Africa.

Samples of the '*Himantura uarnak*' species complex (*H. leoparda*, *H. uarnak*, *H. undulata*) were analyzed using nuclear markers and mitochondrial DNA sequences by Arlyza et al. (2013). According to their study, the COI sequences showed four major mitochondrial lineages and both Clades I and IV clustered with sequences of reference *H. leoparda* specimens. When the results of Arlyza et al. (2013) and the present study were combined, it was clearly seen that the Mediterranean Sea samples were clustered as in Clade IV Arlyza et al. (2013). Since this species is of Indo-pacific origin, we assume that it migrated into the Mediterranean Sea through the Suez Channel, just like *H. uarnak* (Serena, 2005).

ACKNOWLEDGEMENTS

The authors wish to thank the local fishermen from Samandag-Hatay for providing the specimens. The molecular studies supported by DEKOSIM (Deniz Ekosistem vs Iklim Arastirmalari Merkezi-BAP-08-11-DPT2012K120880) Project of Middle East Technical University, Institute of Marine Science, Erdemli/Mersin-Turkey, funded by the Turkish Ministry of Development. We thank Mrs Alison Kideys for correcting the manuscript.

REFERENCES

Ali, M., Saad, A., Ben Amor, M. M., & Capapé, C. (2010). First records of the honeycomb stingray, *Himantura uarnak* (Forskål, 1775), off the Syrian coast (eastern Mediterranean) (Chondrichthyes: Dasyatidae). *Zoology in the Middle East*, *49*(1), 104–106.

Ali, M., Saad, A., Reynaud, C. M., & Capapé, C. (2013). Additional records of honeycomb stingray *Himantura uarnak* (Chondrichthyes: Dasyatidae) off the Syrian coast (eastern Mediterranean). *Tishreen University Journal for Research and Scientific Studies-Biological Sciences Series*, *35*(4), 215–221.

Arlyza, I. S., Shen, K. N., Solihin, D. D., Soedharma, D., Berrebi, P., & Borsa, P. (2013). Species boundaries in the *Himantura uarnak* species complex (Myliobatiformes: Dasyatidae). *Molecular Phylogenetics and Evolution*, *66*, 429–435.

Bandelt, H. J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, *16*(1), 37–48.

Basusta, N., Erdem, U., & Kumlu, M. (1998). Two new fish records for the Turkish seas: Round stingray *Taeniura Grabata* and skate stingray *Himantura uarnak* (DASYATIDAE). *Israel Journal of Zoology*, *44*(1), 65–66.

Ben-Tuvia, A. (1966). 1966: Red Sea fishes recently found in the Mediterranean. *Copeia*, *2*, 254–275.

Cerutti-Pereyra, F., Meekan, M. G., Wei, N. W. V., O'Shea, O., Bradshaw, C. J. A., & Austin, C. M. (2012). Identification of rays through dna barcoding: An application for ecologists. *PLoS One*, *7*(6), e36479.

El Sayed, R. S. (1994). *Check-list of Egyptian Mediterranean Fishes*. Alexandria, Egypt: Institute of Oceanography and Fisheries. 77 pp.

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *Oxford University Press*, *41*, 95–98.

Lim, K. C., Lim, P. E., Chong, V. C., & Loh, K. H. (2015). Molecular and morphological analyses reveal phylogenetic relationships of stingrays focusing on the family Dasyatidae (Myliobatiformes). *PLoS One*, *10*(4), e0120518.

Manjaji-Matsumoto, B. M., & Last, P. R. (2008). *Himantura leoparda* sp. nov., a new whipray (Myliobatoidei: Dasyatidae) from the Indo-Pacific. In: P. R. Last, W. T. White & J. J. Pogonoski (Eds.), *Descriptions of new Australian Chondrichthyans* (pp. 293–301). Hobart: CSIRO Marine and Atmospheric Research Paper No 022.

Mouneimne, N. (1977). Liste des poissons de la côte du Liban (Méditerranée orientale) (Check-list of fishes from the coast of Lebanon (eastern Mediterranean)). *CYBIUM*, *1*, 37–66.

Paz, G., Douek, J., Mo, C., Goren, M., & Rinkevich, B. (2003). Genetic structure of *Botryllus schlosseri* (Tunicata) populations from the Mediterranean coast of Israel. *Marine Ecology Progress Series*, *250*, 153–162.

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The barcode of life data system (http://www.barcodinglife.org). *Molecular Ecology Notes*, *7*(3), 355–364.

Serena, F. (2005). Field identification guide to the sharks and rays of the Mediterranean and Black Sea. In *FAO species identification guide for fishery purposes* (97 pp). Rome: FAO.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*, 1847–1857.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Yucel N, Sakalli A, and Karahan A. First record of the honeycomb stingray *Himantura leoparda* (Manjaji-Matsumoto & Last, 2008) (Myliobatoidei: Dasyatidae) in the Mediterranean Sea, confirmed by DNA barcoding. *J Appl Ichthyol*. 2017;33:530–532. https://doi.org/10.1111/jai.13283