Mar Biodiv
DOI 10.1007/s12526-013-0183-2

OCEANARIUM

First record of living *Manta alfredi* × *Manta birostris* hybrid

R. P. Walter · S. T. Kessel · N. Alhasan · A. T. Fisk · D. D. Heath · T. Chekchak · R. Klaus · M. Younis · G. Hill · B. Jones · C. D. Braun · M. L. Berumen · J. D. DiBattista · M. A. Priest · N. E. Hussey

Received: 4 July 2013 / Revised: 27 August 2013 / Accepted: 30 August 2013

Following a recent taxonomic revision, two manta species (*Manta alfredi* and *Manta birostris*) have been advocated based on meristic and morphological characteristics (Marshall et al. 2009). Subsequent genetic analyses of the two species using mitochondrial and nuclear markers confirmed two distinguishable genetic groups (Kashiwagi et al. 2012). Using the above taxonomic and genetic criteria, we provide evidence for the first record of a living *Manta alfredi* × *Manta birostris* hybrid.

The *Manta* individual (Fig. 1a, b) was non-lethally sampled from a known manta aggregation site in Dunganab Bay, Sudan, Red Sea, in October 2012. The individual was identified in the field as *Manta alfredi* based on distinguishing morphological criteria: dorso-ventral colouration/spot patterns, mouth and pectoral fin colouration, and absence of remnant spine (Fig. 1b; Marshall et al. 2009). DNA was recovered from a fin clip using standard molecular genetic protocols. The mitochondrial gene *ND5* (1,154 bp) and nuclear gene *RAG1* (646 bp) were amplified following Kashiwagi et al. (2012), and sequenced. Recovered sequences (GenBank accession nos. KF574269-KF574270) were aligned with those previously reported (Kashiwagi et al. 2012) and a haplotype network was constructed in TCS 1.21 (Clement et al. 2000).

The *Manta* specimen was confirmed to carry a new *Manta alfredi* mtDNA haplotype (*red* in Fig. 1). However, the *RAG1* sequence indicates that this individual is an interspecific hybrid. The two *Manta* species are reciprocally monophyletic at the *RAG1* locus, which contains two species-specific single nucleotide polymorphisms (SNPs): at position 73 (*M. alfredi*: G; *M. birostris*: A) and position 507 (*M. alfredi*: A; *M. birostris*: C) following Kashiwagi et al. (2012). The hybrid individual is heterozygous at both SNPs (see *inset* chromatograms). Heterozygosity was confirmed by sequencing this individual in triplicate in both forward and reverse directions at *RAG1*.

Our finding indicates that reproductive isolation among *M. alfredi* and *M. birostris* may be less complete than previously thought, or alternatively, that species-specific taxonomic and genetic differentiation is not as unambiguous as currently suggested. Given the designation of the two discrete species as vulnerable on the IUCN Red List and their recent listing on CITES appendix II following concern over increasing global

R. P. Walter (✉) · S. T. Kessel · A. T. Fisk · D. D. Heath · N. E. Hussey
University of Windsor – GLIER, Windsor, Canada
e-mail: rwalter@uwindsor.ca

N. Alhasan
WCGA, Port Sudan, Sudan

S. T. Kessel · T. Chekchak · R. Klaus · M. Younis · N. E. Hussey
Equipe Cousteau, Paris 75017, France

G. Hill · B. Jones
The Deep Aquarium, Hull HU1 4DP, UK

C. D. Braun · M. L. Berumen · J. D. DiBattista · M. A. Priest
KAUST, Thuwal, Saudi Arabia

Published online: 27 September 2013

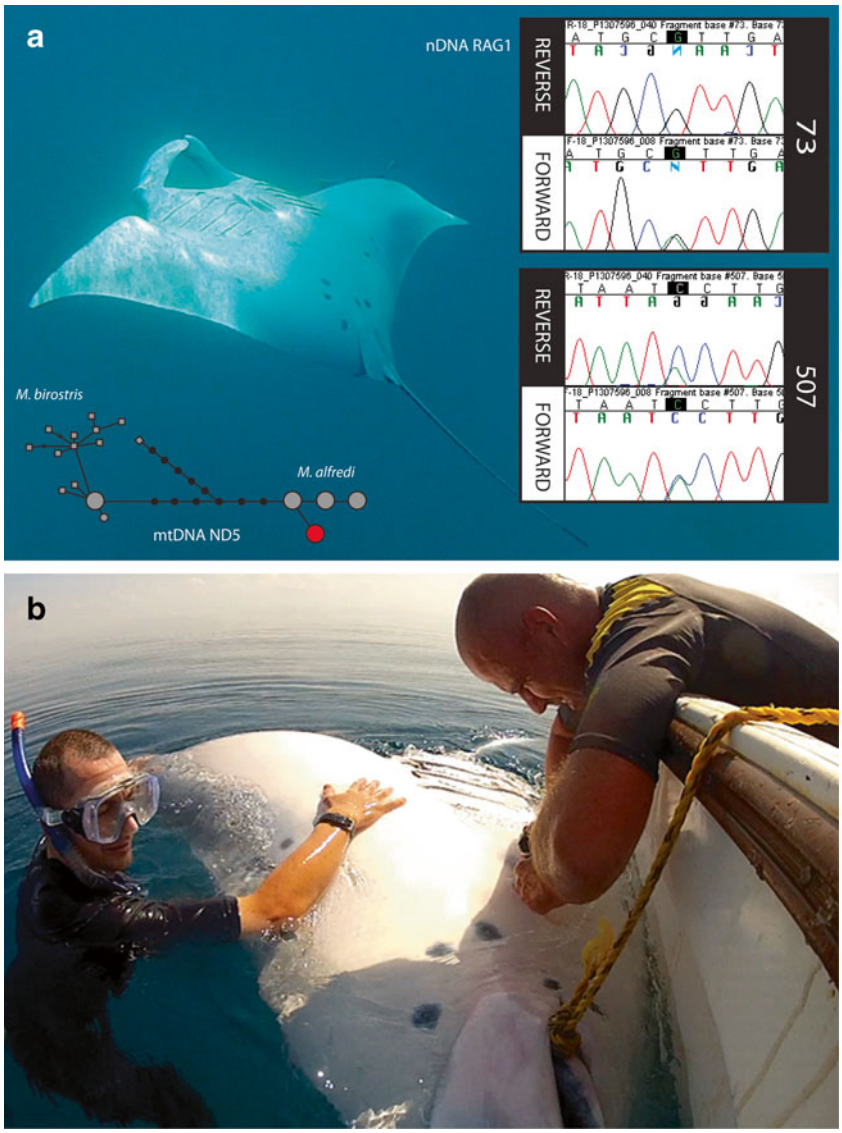

Fig 1 **a** Hybrid manta individual showing mtDNA *ND5* haplogroup membership (based on Kashiwagi et al. 2012, *red circle* indicates hybrid individual) and chromatogram traces indicate heterozygosity (position 73: A and G; position 507: A and C) for species-specific nDNA *RAG1* polymorphisms. **b** Ventral colouration is consistent with *M. alfredi* designation following Marshall et al. (2009): (1) blue-grey to black spots of variable sizes across most of ventral surface, (2) no remnant dorsal spine, (3) pale to dark charcoal bands present on the posterior edge of each pectoral fin margin, (4) small dark spots posterior to the fifth gill slits, (5) mouth—white to light grey colouration and (6) ventral surface predominantly black with no defined shoulder patches

exploitation rates, the occurrence of hybridisation underscores implications for monitoring, conservation and management of threatened species.

Acknowledgments This research forms part of the Equipe Cousteau Sudan Shark and Ray Conservation and Management Program. We thank N. Stroh (Equipe Cousteau), Claudio Scarpellini and regional dive operators, the Wildlife Conservation General Administration (WCGA), The Red Sea State Government and The Red Sea University.

References

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659

Kashiwagi T, Marshall AD, Bennett MB, Ovenden JR (2012) The genetic signature of recent speciation in manta rays (*Manta alfredi* and *M. birostris*). Mol Phylogenet Evol 64:212–218

Marshall AD, Compagno LJV, Bennett MB (2009) Redescription of genus *Manta* with resurrection of *Manta alfredi* (Krefft, 1868) (Chondrichthyes; Myliobatoidei; Mobulidae). Zootaxa 2301:1–2