

First Documented Courtship Behavior between *Mobula birostris* and *M. alfredi* at a Coral Reef Cleaning Station in Misool, Raja Ampat

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Abstract: Though a previous study in Sudan confirmed the existence of a living hybrid of an oceanic manta ray (*Mobula birostris*) and a reef manta ray (*M. alfredi*), courtship behaviors between the two closely related species have never been documented. Here we report the first observation of courtship behaviors between two male *M. birostris* and a female *M. alfredi* at a manta ray cleaning station on a shallow coral reef seamount in Misool, Raja Ampat, Indonesia. The observed courtship event lasted for approximately 45 min and involved one male oceanic manta actively chasing the mature reef manta female around the cleaning station and repeatedly bumping her on the left wing tip with his right cephalic lobe, while the second male continuously flanked the female to seemingly prevent her retreat from the cleaning station and potential evasion of the pursuing male. Notably, the female exhibited a rapid coloration change, likely indicative of courtship-related stress, transitioning from the striking pale hue she exhibited throughout the courtship activity to a normal chevron pattern once the males departed.

Keywords: mobulids; endangered species; marine megafauna; coral reefs; reproductive biology; aggregation site; population dynamics



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The taxonomic classification of manta rays underwent a significant revision in 2009 [1], resulting in the division of these enigmatic creatures into two distinct species: the oceanic manta ray (*Mobula birostris*) and the reef manta ray (*M. alfredi*). This differentiation was initially based on morphological and meristic data [1] and was subsequently supported by genetic analyses in 2012 [2]. Despite generally displaying habitat segregation across their ranges, both species occasionally exhibit mosaic sympatry, particularly in some productive coastal waters [3]. Micropatry, characterized by co-occurrence within 2–100 km, has been observed in eight regions, while microsypatry, involving co-occurrence at the same sites, has been documented only from a few locations (including in Raja Ampat, Indonesia) [3,4]. Despite this microsypatric occupancy, instances of known or suspected hybridization between *M. birostris* and *M. alfredi* are exceedingly rare, with only a single documented case from Dunganob Bay, Sudan [5]. There, the existence of a living *M. birostris* × *M. alfredi* hybrid was confirmed through genetic analysis.

Our understanding of the reproductive behaviors of manta rays in their natural habitats remains considerably constrained. Courtship behaviors, a crucial aspect of their reproductive strategies, have been regularly observed in various regions, with the majority of observations involving *M. alfredi* and sparingly for *M. birostris* [4,6–12]. While studies have reported the presence of fresh mating scars (caused by the male manta ray biting the left wing tip of the female manta ray to gain leverage before initiating copulation [11]) as indicative evidence of mating events, documented observations of mating events, including copulation, remain extremely rare. Such observations have been reported for free-living

manta rays in only three areas of the globe: Japan's Ogasawara Islands and the Galapagos for *M. birostris* [11,12] and French Polynesia [11], Hawaii [11], Mozambique [9] and the Maldives [11] for *M. alfredi*. Additionally, copulation in *M. alfredi* has been observed repeatedly with captive individuals in Japan's Okinawa Churaumi Aquarium [13].

Despite the existence of living *M. birostris* × *M. alfredi* hybrids and a growing body of observations on the reproductive behaviors of these two distinct species [11], documentation of courtship behaviors culminating in hybridization remains absent from the scientific record. In addressing this knowledge gap, we present the first record of courtship behavior between *M. birostris* and *M. alfredi* observed in Misool, Southern Raja Ampat, Eastern Indonesia. This region serves as a haven for both species of manta ray, hosting thriving and robust populations documented in numerous microparapatric and microsympatric sites scattered throughout the archipelago [4,6,14,15].

The courtship event was observed on 14 December 2016 at Magic Mountain, a shallow coral reef seamount renowned for harboring multiple cleaning stations frequented by both *M. birostris* and *M. alfredi* [4]. The courtship event was observed for approximately 45 min and involved three individuals. This ensemble comprised two sexually mature male *M. birostris*, discernible by their conspicuous and calcified claspers, and a sexually mature female *M. alfredi*. The latter exhibited evident mating scars on her left pectoral fin, indicative of prior reproductive activities, and displayed physical characteristics consistent with mid-term pregnancy (Figure 1, Video S1) [16]. The female *M. alfredi* was estimated to have a disc width (DW) of 3.6 m (from wing tip to wing tip), whereas the two male *M. birostris* were slightly larger in DW than the female *M. alfredi*. This contrasts with *M. alfredi*, where courtship typically occurs between a larger female and smaller male(s) [11]. In Raja Ampat, drone-measured sexually mature female *M. alfredi* ranged from 324 to 372 cm DW, while sexually mature males ranged from 275 to 316 cm DW [17].

In the course of the observed courtship event, only the initial three stages, as delineated by Stevens et al. [11], were evident—comprising initiation, endurance, and, to a limited extent, evasion. One male *M. birostris* took an active role in pursuing and courting the female *M. alfredi*. By contrast, the second male *M. birostris* maintained a distinctive “wingman” position throughout the courtship proceedings. Intriguingly, this male consistently flanked the female *M. alfredi*, effectively limiting her ability to depart from the cleaning station and evade the pursuing male (Video S1).

The courtship event was started when one of the two male *M. birostris* slowly approached the female *M. alfredi*, which had been hovering over a cleaning station and was actively being cleaned by a number of wrasses, from her left side. Upon realizing the male's presence, the female ceased cleaning and initiated swimming in an apparent attempt to evade the approaching male. Simultaneously, the second male *M. birostris*, positioned nearby, strategically blocked the escape path of the female, ensuring she remained at the cleaning station and within the proximity of the pursuing male. The flanking behavior exhibited by the male indirectly involved in the courtship event was observed on multiple occasions, with the escorting male continuously pursuing the female.

Utilizing its cephalic lobes, the pursuing male made several attempts to establish physical contact with the female's left pectoral fin from above. As the female gradually slowed down, hovering over the reef, the male successfully bumped the female's left pectoral fin using its right cephalic lobe, precisely at the location of a large white mating scar (Figure 1B). In response, the female increased its swimming speed, executed a sharp left turn, and then returned to the cleaning station, facing the departing male that gradually distanced himself from the female before shortly returning and repeating the behavior six times.

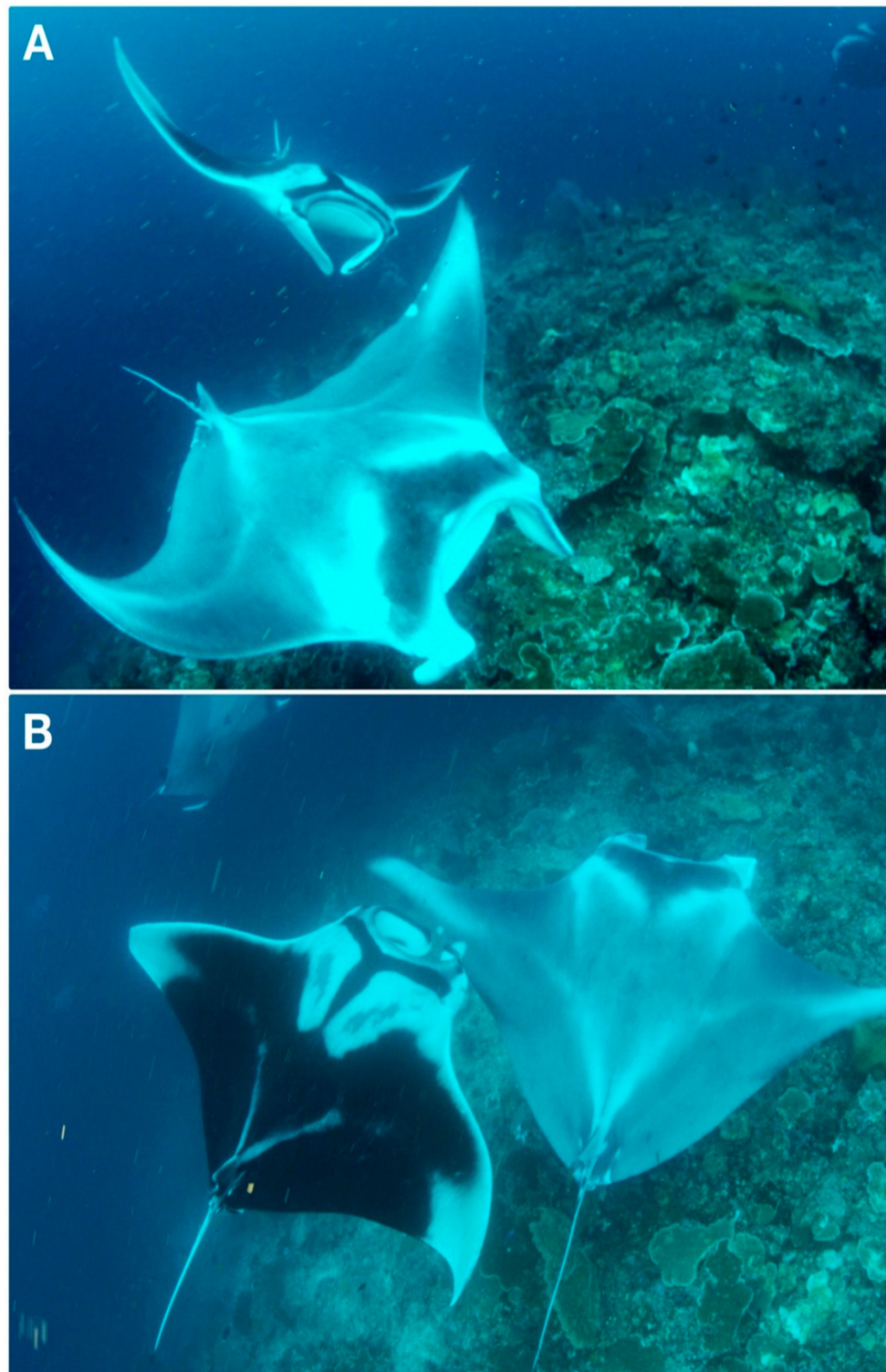


Figure 1. Courtship behavior between a male oceanic manta ray (*M. birostris*) and a female reef manta ray (*M. alfredi*) at a cleaning station, Magic Mountain, Southern Raja Ampat. **(A)** A male oceanic manta ray (*M. birostris*) pursues a female reef manta ray (*M. alfredi*) in a courtship behavior. **(B)** Detailed interaction showing the male using its right cephalic lobe to make contact with the female's left pectoral fin, precisely at the site of a previous mating scar.

The observation concluded when author S.H. ascended to the boat for a surface interval, replacing his SCUBA tank, and subsequently returned to continue the observation.

Upon S.H.'s return, both male *M. birostris* had moved away from the reef, leaving the female *M. alfredi* alone at the cleaning station. Intriguingly, in the moments after the male oceanic manta rays left, this female underwent a rapid coloration change, transitioning from the pale hue seen throughout the courtship event to the normal dark hue commonly seen in chevron individuals. The pale coloration initially observed in this female *M. alfredi* was hypothesized to be associated with courtship-related stress (Figure 2). Rapid coloration changes in manta rays have previously only been documented in individuals held in captivity [18].

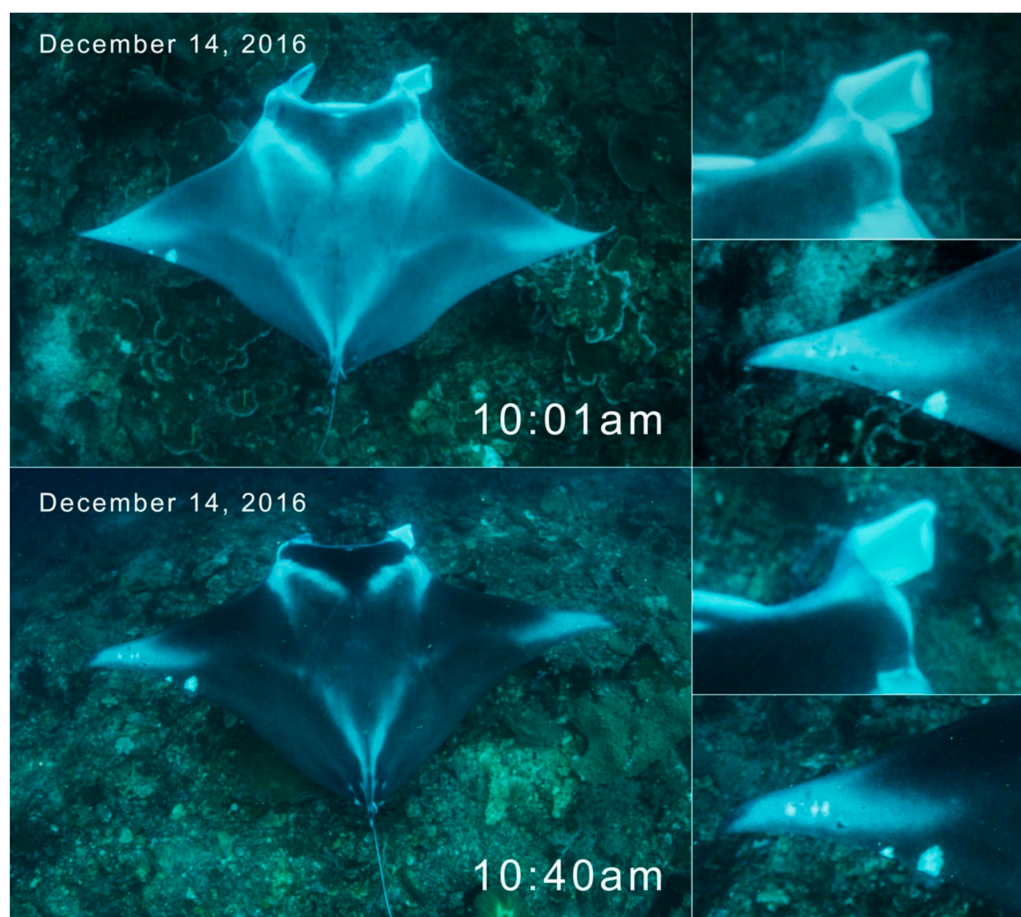


Figure 2. Dorsal surface view illustrating coloration changes from pale hue to normal dark hue in a chevron-patterned female reef manta ray (*M. alfredi*) at the conclusion of the courtship event on 14 December 2016, at Magic Mountain, Misool, Southern Raja Ampat, Eastern Indonesia.

This observation represents the first record of courtship behaviors between the closely related species *M. birostris* and *M. alfredi*. In a distinctive interspecies interaction, we observed a coordinated mating attempt involving two sexually mature male *M. birostris*, one strategically flanking the female *M. alfredi* to prevent her departure from the cleaning site while the second male pursued and repeatedly used his right cephalic lobe to bump the female in the exact position of her previous mating scars. Furthermore, this study provides the first documented evidence of a rapid coloration change in *M. alfredi* observed in its natural habitat, presumably as a result of courtship-related stress. Our findings contribute to the expanding understanding of elasmobranch reproductive behaviors, highlighting previously undocumented intricacies in the courtship dynamics of manta rays.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d16060319/s1>, Video S1: Raja Ampat oceanic and reef manta ray courtship behaviour_Blue Sphere Media.mov.

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Conflicts of Interest: Author Shawn Heinrichs was employed by the company Blue Sphere Media. All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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