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RESEARCH ARTICLE

Prevalence of Epidermal Conditions in Critically Endangered Indo-Pacific Humpback Dolphins (*Sousa chinensis*) from the Waters of Western Taiwan

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ABSTRACT

The prevalence of epidermal conditions in a small critically endangered population (<100 individuals) of coastal Indo-Pacific humpback dolphins (*Sousa chinensis*) from the waters of western Taiwan was assessed during a photo-identification study conducted between 2006 and 2010. Of 97 individuals photographically examined, 37% were affected by one or multiple conditions. Besides, mature individuals had significantly higher prevalence than immature ones. Five different skin condition categories were considered, including pox-like lesion, pale lesion, orange film, prolonged ulcer lesion, and nodule on body. This first study to investigate epidermal conditions on *S. chinensis* in the world offers data for comparison with other studies in the future and new ground for discussion on the health of these animals and the potential impact of anthropogenic activities.

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INTRODUCTION

Epidermal diseases have been described in many cetacean species (Maldini *et al.,* 2010). Meyer *et al.* (2008) suggested the malfunction of the host's defense mechanisms at the skin surface of cetaceans could cause infections, and a variety of pathogens could penetrate through the host via the infections. Many naturally occurring microorganisms including viruses, bacteria, fungi and ciliated protozoa in the marine environment were reported as pathogens (Van Bressem *et al.,* 2007). The factors such as water salinity, temperature, pollution and contaminants were linked to the skin conditions of bottlenose dolphin (*Tursiops truncatus*) populations worldwide (Wilson *et al.,* 1999). Contaminants, especially, increase the severity of clinical signs because the long-term exposure of contaminants can suppress the immune system of cetaceans (De Guise *et al.,* 1995; Levin *et al.,* 2007).

Indo-Pacific humpback dolphins (*Sousa chinensis*) inhabit tropical and subtropical near-shore waters of the Indian and Pacific Oceans from central Australia, southern Mainland China and southeastern Asia, and around the rim of the Indian Ocean to southern Africa (Wang *et al.,* 2004). The species is red-listed by IUCN as 'Near Threatened' driven primarily by 'heavy fishing pressure (incidental mortality) and habitat loss in coastal and estuarine areas (Ross *et al.,* 2010). The population of *S. chinensis* from the coastal waters of western Taiwan was first reported scientifically in 2004 (Wang *et al.,* 2004). Based on line-transect data from 2002 to 2004, Wang *et al.* (2007) reported that the initial estimate of population size for western Taiwan *S. chinensis* was 99 (CV = 51.6%; 95% CI = 37 to 266). The western Taiwan population is red-listed by IUCN as 'Critically Endangered' as a result of its geographical isolation, small population size and presumed ongoing decline due to existing and anticipated threats (Ross *et al.,* 2010).

From 2006 to 2009, a variety of epidermal conditions were observed on western Taiwan population that raises concerns about the health status and potential negative effects on this critically endangered population. Here we report the types of skin conditions found in the western Taiwan population of *S. chinensis*. It offers data for comparison with other future studies in different areas, and new ground for discussion on the health of these endangered animals and the potential impact of anthropogenic activities. Besides, the information obtained in this study would be important to coastal dolphins and ecosystem health of western Taiwan. To the authors' knowledge, this is the first study to investigate epidermal conditions on *S. chinensis* in the world.

MATERIALS AND METHODS

Data collection: Study area includes the western Taiwan coastline between Miaoli to Chiayi (24˚44'N 120˚50'E to 23˚22'N 120˚00'E) (Fig. 1). The study area included coastal waters with depths ≤ 15 m, which is the limit of *S. chinensis* distribution along western Taiwan coasts. The study area is also of conservation interest, because it is subject to urban runoff and pollution from the adjacent cities of western Taiwan. Boat-based photo-identification survey was done at an average speed of 6-10 nm per hour. Surveys covered 200 km of coastline from Miaoli to Chiayi and were only conducted in ideal conditions (Beaufort ≤ 2). Data were collected during 370 coastal surveys in the study area between August 2006 and November 2010, mostly during summer months (18 in 2006, 20 in 2007, 114 in 2008, 154 in 2009 and 64 in 2010). Dolphins were vertically photographed using digital technology (Olympus, Canon, Pentax equipped with 70-300 mm zoom lens or 400 mm fixed lens). All images were compiled into a photo-identification catalog with individual dolphins identified from distinctive markings and scars. Images were cataloged using PhotoImpact© software and by using techniques modified from Mazzoil *et al.* (2004).

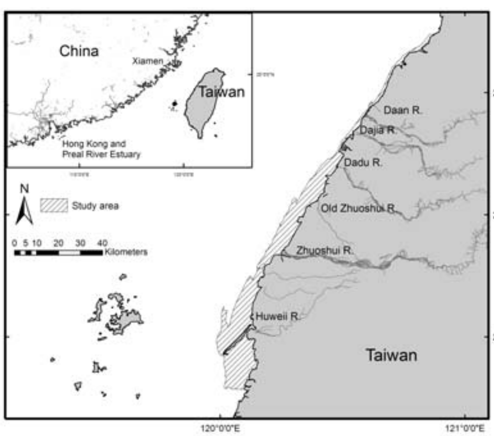

Fig. 1: Map of the study area that included coastal waters with depths ≤ 15 m, which is the limit of Indo-Pacific humpback dolphins (*Sousa chinensis*) distribution along western Taiwan coasts.

Photo identification: A total of 64,109 photos were collected. The dorsal fin and the body above the sea surface was cut by PhotoImpact©. Quality (Q) of dolphin images was scored on a range from Q1-100 (derived from Friday *et al.* (2000) and Karczmarski *et al.* (2005)), based on clarity of focus, color contrast, shot angle, and the coverage of full dorsal fin. Photographs with a Q score over Q80 were used for further identification analysis. Non-calf dolphins were identified individually according to distinctive characteristics, such as notches on the dorsal fin or spots on the body. It was difficult to apply the same criteria to the identification of calves because they seldom carried enough identifiable physical characteristics (*i.e.*, scars or spots). The tight maternal connection and fast somatic growth during the lactation period of a mammalian life history, however, could provide an alternative method to identify individual calve indirectly. For each photograph, the types and locations of condition were recorded. Body scars that clearly suggested injury or trauma such as rake-marks and bleeding wounds were excluded from the study. To ensure consistency, two independent observers evaluated each photo.

Coloration-stage determination: Dolphins were then identified and divided by coloration pattern derived from Jefferson (2000): the unspotted dark grey or grey individuals were classified into calves and young juveniles; spotted juvenile and spotted sub-adult phase were classified into groups called mottled and speckled, respectively; less spotted individuals were included into group called spotted, and the unspotted pink or white individuals were considered as unspotted age group.

Skin condition classification: Skin conditions were assigned into 5 categories (Fig. 2). Four categories have been described by Wilson *et al.* (1997), Bearzi *et al.* (2009), Van Bressem *et al.* (2009a) and Maldini *et al.* (2010): i) pale lesion, ii) orange film, iii) pox-like lesion, iv) nodule. The fifth category is prolonged ulcer, which is new to the literature. Prolonged ulcer lesions only included long-term (>2 mo) wound with pinkish or reddish color. They were present along the edges of dorsal fin, flanks and flakes. Prevalence of each condition was expressed as a proportion. Skin conditions were classified into three coverage levels: low, medium and high (Bearzi *et al.,* 2009). Low was defined as the coverage smaller or equal to 20% of visible epidermis, medium was defined as coverage was between 21% and 50% of visible epidermis and high was defined as coverage was larger than 50% of visible epidermis. Statistical analysis was done by applying the independent *t* test using Microsoft Excel 2008 software. Statistical significance was declared if P<0.05.

RESULTS

The effort of analyzing ~60,000 photos resulted in the photo-identification of 97 *S. chinensis* including 26 calves, 25 in mottled stage, 29 in speckled stage, 13 in spotted stage and 4 in unspotted stage. The 5 types of skin conditions in the population were in the following overall proportions, listed by prevalence: nodule (15.5%), orange film (11.3%), pale lesion (10.3%), pox-like lesion (3.1%), and prolonged ulcer (3.1%) (Table 1). Thirty-six individuals (37.1%) were affected by one (29.9%) or two (7.2%) skin conditions. Six of the 7 individuals with two conditions were mature (spotted/unspotted), while the other one was a calf. Most of the conditions (78.6%) were belonged to low-level coverage. High prevalence was found in mature individuals (spotted: 92.3%; unspotted: 75%), while moderate to low prevalence was found in young animals (32% and 37.9%) and calves (7.7%). The differences in prevalence of animals affected overall between calves and the other coloration stages were significant (P=0.029~<0.001). The differences between mottled or speckled to spotted were also significant (P<0.001). The difference between immature (pooled calves/mottled/speckled) and mature (pooled spotted/ unspotted) animals was significant (P<0.001). Among the skin conditions, the only one with high-level coverage was

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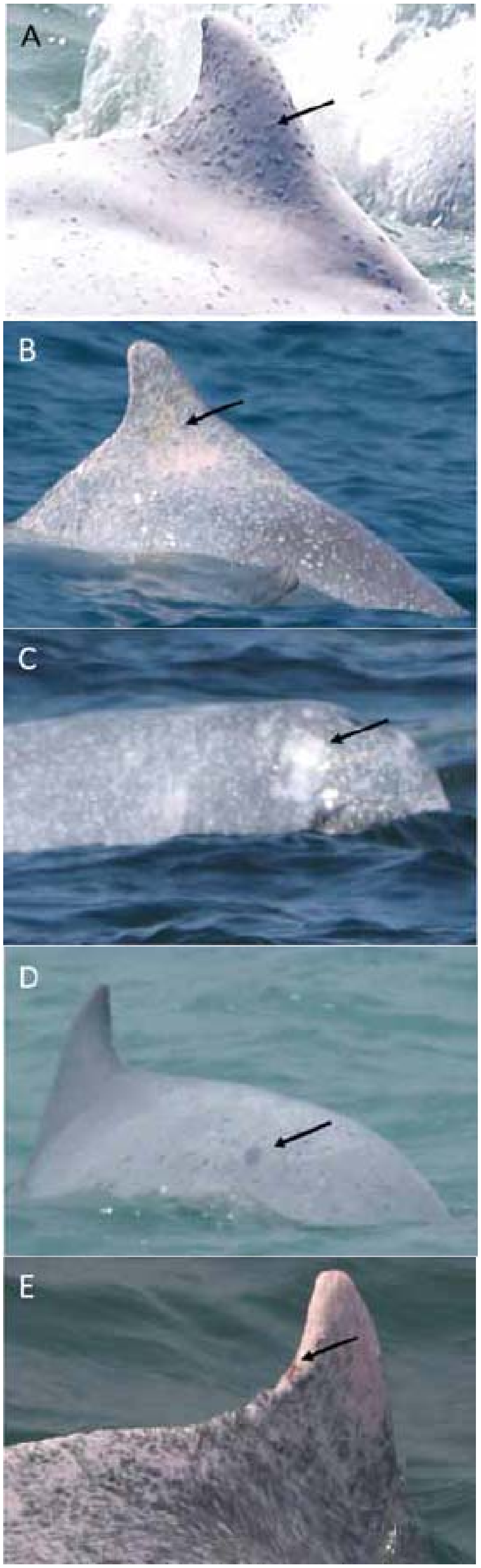

Fig. 2: Examples of skin condition categories found in the Indo-Pacific humpback dolphins (*Sousa chinensis*) from the waters of western Taiwan: A) Nodule; B) Orange film; C) Pale lesion; D) Pox-like lesion and E) Prolonged ulcer.

Table 1: Number of affected individuals and extent of skin conditions in different coloration stages. L, M, H: the number of individuals with low/medium/high extent of coverage

	Affected individuals (L,M,H)					
Coloration stage	Nodule	Orange film	Pale	Pox like	Prolonged ulcer	Total
Calves	0(0,0,0)	0(0,0,0)	1(1,0,0)	2(2,0,0)	0(0,0,0)	2
Mottled	0(0,0,0)	1(0,1,0)	6(5,1,0)	1(1,0,0)	0(0,0,0)	8
Speckled	2(2,0,0)	3(3,0,0)	3(2,1,0)	0(0,0,0)	3(3,0,0)	11
Spotted	10(6,2,2)	6(6,0,0)	0(0,0,0)	0(0,0,0)	0(0,0,0)	12
Unspotted	3(1,2,0)	1(1,0,0)	0(0,0,0)	0(0,0,0)	0(0,0,0)	3
Total	15	11	10	3	3	36

nodule, and it was more prevalent in mature animals. Only speckled individuals were found with long lasting ulcer and only calves and mottled individuals had pox-like lesions.

DISCUSSION

Harzen and Brunnick (1997) reported that 85% of the resident bottlenose dolphins inhabiting the estuary showed signs of skin disorders and they suggested that habitat degradation may play an important role causing immune system disorder and subsequent skin conditions. Ninety-five percent of the bottlenose dolphins in the Moray Firth, Scotland, showed one or more types of skin lesion on the back or dorsal fin, and a wide variety of factors was supposed to cause high prevalence of skin lesions (Wilson *et al.,* 1997). In a comparative investigation of 10 geographically separated bottlenose dolphin populations, the prevalence ranged between 67% in Florida and 100% in England (Wilson *et al.,* 1999). In California, 73.4% (Santa Monica Bay, Bearzi *et al.,* 2009) and 81% (Monterey Bay, Maldini *et al.,* 2010) of the coastal bottlenose dolphins exhibited at least one type of skin condition and pollution from many sources may explain the high prevalence. The prevalence among dolphins from three sites along the southeast United States coast ranged from 38 to 59%, and this observed geographic difference may be due to seasonal or environmental fluctuations, exposure to anthropogenic influences, or differences in population demographics (Hart *et al.,* 2012). Compared with the prevalence of skin conditions in these coastal cetacean populations across the globe mentioned above, which ranged between 38 and 100%, the prevalence in *S. chinensis* from the waters of western Taiwan was low (37.1%). However, it is needed to assess that certain skin condition is indicative of a specific etiology that may be important to population health. Besides, local environmental factors such as water temperature, salinity and water contamination may lead to the variability in prevalence of skin conditions (Wilson *et al.,* 1999), and different susceptibility to anthropogenic impacts and environmental factors between *S. chinensis* and other species.

Nodule was the most prevalent condition (15.5%) in this study, which is characterized by circumscribed and raised skin lump. The color of the nodules found in this study was nearly identical to the adjacent skin. This type of skin lesion is similar to the early stage of nodular skin disease (NSD) in Guiana dolphins (*Sotalia guianensis*) (Van Bressem *et al.,* 2009a). NSD is suggested to be an early form of lobomycosis-like disease (LLD), which grossly resembles lacaziosis (lobomycosis, LD) caused by the fungus *Lacazia loboi* (Van Bressem *et al.,* 2009a). LD and LLD have been reported in dolphins from waters surrounding South America, Madagascar, France, and along the Gulf of Mexico coast and Atlantic coast of the USA (Hart *et al.,* 2011). Bottlenose dolphins affected by LD from the Indian River Lagoon, Florida, were found to have significant impairment in adaptative immunity possibly related to chronic exposure to environmental stressors, while variation in salinity and water temperature may also play a role in the infection (Reif *et al.,* 2009). In addition, at least four other pathogens are known to cause skin nodules in small cetaceans: *Streptococcus iniae*, papilloma virus, *Fusarium* spp. and *Trichophyton* spp. (reviewed in Van Bressem *et al.,* 2009a), although the aspects of lesions caused by them do not highly resemble the nodules found in our study. The role of these pathogens in the etiology of the skin nodule in *S. chinensis* should be further investigated.

Orange film was the second most prevalent condition (11.3%). The most likely cause of this coloration pattern is diatom infestation, which has been documented in other cetacean species (Maldini *et al.,* 2010). Although diatom attachment was not suggested to be a skin lesion type that has an infectious disease etiology (Hart *et al.,* 2012), the accumulation of diatoms indicates slower skin regeneration or swimming speed that may reflect compromised physical condition of the affected dolphins.

Pale lesion was the third most prevalent skin condition (10.3%), which is either circular or amorphous with rounded edge and white to matte appearance, and it was limited to immature individuals. The prevalence of pale lesion may be underestimated because it could be difficult to observe the lesion when the dolphin is mature (spotted and unspotted stages). Hart *et al.* (2012) reported that the histological findings of pale lesion revealed indications of traumatic scarring, ectoparasite attachment, prior viral infection and inflammation, and several lesion samples were positive for herspesvirus by PCR. Since herpesviruses cause persistent infections in their hosts, latent virus can be reactivated and the recrudescence is likely associated with stress and immunosuppression (Soto *et al.,* 2012). Last, only 3 immature individuals were found with prolonged ulcer and pox-like lesion, respectively. The cause of prolonged ulcer is supposed to be microorganism infection. The pox-like lesion was not implied a known etiology but simply revealed the similarity in appearance to lesions described in previous studies (Van Bressem *et al.,* 1999, 2009b). Whether prolonged ulcer and pox-like lesion are significant to the population health of *S. chinensis* cannot be assessed currently. For elucidating if the 5 different skin conditions observed in *S. chinensis* from the waters of western Taiwan are stages of certain diseases or are indicative of many different disorders, it is needed to use tissue samples from live or freshly stranded animals to determine the causative agents by histological examination and molecular diagnostics.

Age data was not consistently available for dolphins examined in the previous studies on skin conditions, and age-class identification from photo-ID data was often limited to adult or calf distinctions that may not provide useful information for diseases that commonly occur among sub-adults (Hart *et al.*, 2012). *S. chinensis* is uniformly black at birth, and mottled and speckled animals with heavy to moderate spotting are presumably juveniles and sub-adults, while spotted and unspotted animals with pinkish white body color are presumably adults (Jefferson, 2000). Compared with most species of dolphins in which body coloration does not change dramatically with age, the age-class information in *S. chinensis* provides a unique opportunity to shed light on epidemiological factors influencing the prevalence of skin conditions and different condition types in coastal dolphins. The intriguing finding in this study is that high prevalence was found in mature individuals, while moderate to low prevalence was found in immature animals. *S. chinensis*, like other coastal cetacean species, is the apex predator and vulnerable to indirect threats, such as fisheries bycatch, habitat destruction (land reclamation), pollution, water diversions (reduced flow into estuaries), underwater noise and disturbance (Ross *et al.*, 2010). Any of the above-mentioned factors may be responsible for the prevalence and extent of skin conditions on *S. chinensis*. The hypothesis that the variations in salinity and water temperature are factors in the skin conditions in *S. chinensis* does not explain the disparity in prevalence among the ages. Similar conditions in other dolphin populations have been linked to harmfully high-level accumulations of contaminants in fatty tissues (Reif *et al.,* 2009). Hung *et al.* (2004) reported alarmingly high concentration of methylated mercury in *S. chinensis* in Hong Kong. The concentrations of PCBs and DDT in *S. chinensis* in Hong Kong were higher than established thresholds showed in marine mammals (Parsons, 2004; Jefferson *et al.,* 2006). Mercury (Chen *et al.,* 2002) and residues of PCBs (Chou *et al.,* 2004) have been investigated in cetaceans from Taiwan waters, although only a single *S. chinensis* has been examined for PCBs, suggesting that setting up programs to monitor pollutant concentrations in *S. chinensis* and its habitats would be essential to protect the species and coastal ecosystem. Since the total population was estimated at about only 100 individuals, the conservation measures reducing the environmental stressors are considered imperative.

We used photo-ID data to obtain minimum skin condition prevalence estimates, as the detection of conditions was restricted to body parts that are routinely photographed. Although restrained for establishing the etiology of skin diseases, photo-ID data can be regarded as a random sample of the population and can yield longitudinal data allowing for assessing the development, remission and relapse of skin conditions. Furthermore, because capture-release projects may not be feasible for health monitoring of this small critically endangered *S. chinensis* population, the data obtained from photo-ID studies provide a relatively inexpensive and non-invasive tool to assess the health of free-ranging populations. This investigation could be extended to a larger scale to include comparisons of the prevalence of skin conditions in other populations of *S. chinensis* such as Mainland China and southeastern Asia. This would allow assessing changes in skin lesion presence and extent over time at both the population and individual levels, and identifying natural and anthropogenic factors that correlate with skin conditions, which should be continued for the benefit of both dolphins and humans because coastal dolphins can act as a sentinel organism in coastal environments.

This study demonstrates the first record of skin conditions in *S. chinensis* using a non-invasive and cost-effective approach. Since skin conditions have been found among several dolphin species globally, the continuous monitoring of these conditions on free-ranging dolphins may function as a signal of other latent health implications or environmental risks. Besides, more research is needed to confirm the roles of environmental fluctuations, population demographics, and anthropogenic influences on skin condition development.

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