RESEARCH ARTICLE

Hybridization in bottlenose dolphins—A case study of *Tursiops aduncus* × *T. truncatus* hybrids and successful backcross hybridization events

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Abstract

The bottlenose dolphin, genus *Tursiops* is one of the best studied of all the Cetacea with a minimum of two species widely recognised. Common bottlenose dolphins (*T. truncatus*), are the cetacean species most frequently held in captivity and are known to hybridize with species from at least 6 different genera. In this study, we document several intra-generic hybridization events between *T. truncatus* and *T. aduncus* held in captivity. We demonstrate that the F₁ hybrids are fertile and can backcross producing apparently healthy offspring, thereby showing introgressive inter-specific hybridization within the genus. We document that female F₁ hybrids can reach sexual maturity at 4 yr and 3 mo of age, and can become pregnant and give birth before being fully weaned. The information presented has implications for understanding hybrid reticulation among cetacean species and practical implications for captive facilities housing either *Tursiops* species or hybrids thereof.

Introduction

It is becoming increasingly clear that reticulation among species lineages is common [1], and can even support the establishment of new species radiations [2]. In her 2009 review of hybridization events in marine mammals, Bérube [3], summarises that 53 putative hybridization events have been reported within Cetacea, of which 28 hybrids have been identified within captive facilities. The evolutionary significance of hybridisation among cetacean species is not yet clear [4], however a better understanding of this process can be facilitated through investigations of hybridisation events in captivity.

The bottlenose dolphin (*Tursiops* spp.) is one of the best studied of all the cetaceans. However, there remains continued debate surrounding the number of *Tursiops* species recognised and the phylogenetic relationships between populations from which we have genetic
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Delphinus capensis hybrid back-crossed with T. truncatus and the resulting calf died shortly after birth [33].

Before the taxonomic definitions of the Tursiops genus were clarified, hybridization between T. t. gilli (now regarded as T. truncatus) and T. t. aduncus (now T. aduncus) was documented [21]. The F1 offspring survived 5+ years in good health in Okinawa Expo Memorial Park Aquarium, Japan. More recently, Martien et al., [34] found molecular evidence for a T. aduncus x T. truncatus hybridization event from samples of wild animals collected near Hawaii, with STRUCTURE [35–37] analysis suggesting the sampled animal had T. aduncus ancestors at least two generations past. However, as this study was based on molecular sampling from wild animals, no mating history was available to confirm the hybrid status of the sampled individual.

Our study documents several hybridization events between T. truncatus and T. aduncus held in a single captive facility in Durban, South Africa. Best [38] provides a short description of the captive colony of T. truncatus, T. aduncus and hybrids of the two species housed in this facility. The F1 hybrids can be identified by their external morphological characteristics [38], however the differences are subtle. Data from this captive setting are used to unambiguously demonstrate the ability for F1 hybrids to produce healthy backcross hybrid offspring that live into adulthood. The results have implications for understanding the evolution of cetacean species as well as practical implications for captive facilities housing either species or hybrids.

Methods

This study focuses on a captive colony of T. truncatus, T. aduncus and T. aduncus × T. truncatus hybrids held at uShaka Sea World (Durban, South Africa). The colony was established in 1976 within the Durban Sea World dolphinarium (a division of the South African Association for Marine Biological Research, SAAMBR). It moved to new facilities in 2004 under the name uShaka Sea World. For simplicity, we will use the current name (uShaka Sea World) to refer to the dolphinarium throughout time. It is currently the only captive facility housing dolphins in South Africa. The enclosure, some 7200 m³, encompasses an indoor and external holding facility and a large 3800m³ presentation pool. Although the seven pools in the holding facility can be separated by physical barriers, they allow visual and acoustic contact between groups. Configuration of the social groups has changed over time, and during the principle time of data collection in November 2016 the dolphins were held in three social groups, with most adult males and females held separately in two same-sex groups, and a mature T. truncatus and T. aduncus (Tt1 and Ta1) held together.

We here provide details on the breeding history, morphological characteristics (length, weight, ventral colouration pattern) and health status of this captive colony, detailing the existence of viable F1 Tursiops hybrids and a healthy backcross adult offspring. This study utilises historical medical and husbandry data collected through routine veterinary procedures and training records for the dolphins collated in November 2016. Photographs were taken in 2014 and November 2016. Updated length-weight data are summarised from March 2018, with length-weight data from the T. aduncus parent population included for comparison. No comparable length-weight data are available for the parent T. truncatus population.

Species assignment of the T. aduncus dam (Ta1) and T. truncatus sire (Tt1) of the first generation hybrids residing in uShaka Sea World was confirmed by phylogenetic analysis. DNA was extracted from blood samples preserved in 20% DMSO saturated with NaCl using a standard phenol chloroform method (after [39]). A 932bp fragment of the mtDNA control region was amplified using the forward 5’ TTC TAC ATA AAC TAT TCC 3’ primer and the reverse 5’ ATT TTC AGT GTC TTG CTT T 3’. PCR reactions were carried out in 25μl
containing 10mM Tris-HCL (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM dNTP, 2 mM of each primer, 10-15ng template DNA, and 0.625 U DNA Taq Polymerase (New England Bio-labs, USA). The PCR cycle was 2 min at 95˚C followed by 35 cycles of 40s at 95˚C, 40s at 44˚C, 45s at 72˚C and a final extension for 10 min at 72˚C. PCR products were then cleaned using the PureLink PCR Micro Kit (Invitrogen, USA). Sequencing was on an ABI 3730 and resulting sequences were analysed using Chromas 2.6.5 (https://technelysium.com.au/wp/chromas/). A neighbour joining tree was constructed using MEGA 5.2 with the Tamura-Nei evolution model (suitable given the rate variation observed across the control region) and 1000 bootstrap replications. Reference sequences were from Genbank including *T. truncatus* samples from the North Atlantic [40] and *T. aduncus* samples from South Africa and the tree was constructed using 488bp overlapping sequence from the control region Hypervariable Region 1. The out-group chosen was *Stenella attenuata* (from [41]).

**Ethics statement**

Dolphins are kept under human care under a South African Department of Environmental Affairs permit (DEA permit number withheld for confidentiality purposes). Blood samples for genetic analysis were collected during routine veterinary supervised preventative health screening procedures, performed in compliance with accredited best international welfare standards and conventions. They were collected in a voluntary manner during routine husbandry training. Other data are purely descriptive and therefore no ethics clearance was necessary. All data generated or analysed during this study are included in this published article, or are available on Genbank.

**Results**

The captive colony of *Tursiops* held at uShaka Sea World Durban includes wild stock of *T. truncatus* and *T. aduncus* captured in the southern African sub-region in the 1970s and early 1980’s and their offspring born at the facility since this time (see Fig 1 and Table 1 for details). Captures of *T. truncatus* took place in 1976 and 1983 in Walvis Bay, Namibia (22˚57’S, 14˚30’E) of which *Tt*1 (male) is the only surviving animal. A further two pure bred *T. truncatus* are held: *Tt*3 (male) born in captivity of a pregnant wild caught dam (*Tt*2, now deceased) and a wild sire, and *Tt*5 (female) the offspring of *Tt*1 and the female *Tt*4 (now deceased). The only pure bred *T. aduncus* (*Ta*1, female) was captured from the waters of Umhlanga (South Africa) in 1979. Species confirmation of *Ta*1 and *Tt*1 was confirmed by lineage assignment in the mtDNA control region phylogeny (Fig 2).

Periodically, since the inception of the dolphin programme, uShaka Sea World has allowed controlled breeding events to occur in the facility. In total, seven *F*1 hybrids and two backcross progeny have been born at the Sea World facilities. Of these, all the *F*1 hybrids and one calf from a backcross (paternal *T. truncatus*) have survived to adulthood. All *F*1 *T. aduncus* × *T. truncatus* hybrids held at the facility are the offspring of *Ta*1 and *Tt*1. Five out of the seven *F*1 hybrids were sired before 2000, when *T. truncatus* and *T. aduncus* were considered to be the same taxonomic species [6]. *Tt*1 and *Ta*1 are strongly bonded (as demonstrated by consistent affiliative behaviour, authors and trainers observations) and throughout time have been held together with their dependent offspring.

Two backcross progeny have been born at uShaka Sea World, with a third pregnancy documented. The first backcross hybrid offspring; BC1, is a female and was born on the 17th of July, 1993 to *Ta*-t1 (dam now deceased) with *Tt*1 the sire. The dam was an estimated 6 years and 3 months at the time of conception, based on back calculations from the date of birth (DOB) of BC1, using a gestation length of 12 months [42]. The BC1 adult is currently housed at uShaka.
Sea World, attaining an age of 23 years in 2016 and currently (2018) weighing 240.5 kg (Fig 3). Regular veterinary monitoring demonstrates that BC\textsubscript{1} is a healthy individual and ultrasound examinations indicate normal ovulation activity in this female.

Table 1. Background information on each bottlenose dolphin held at the uShaka Sea World.

| Code | Species | Sex | Date of Capture | Date of Birth | Current status (age on 1st November 2016 or age at death) |
|------|---------|-----|----------------|--------------|---------------------------------------------------------|
| Ta\textsubscript{1} | Ta | F | 26/06/1979 | ≤ 26/06/1974\textsuperscript{*} | Alive (42y, 4m) |
| Tt\textsubscript{1} | Tt | M | 08/12/1976 | ≤ 08/12/1971\textsuperscript{*} | Alive (44y, 10m) |
| Tt\textsubscript{2} | Tt | F | 20/10/1983 | 15/06/1973\textsuperscript{*} | Deceased (12y, 7m) |
| Tt\textsubscript{3} | Tt | M | Captive born | 22/01/1984 | Alive (32y, 9m) |
| Tt\textsubscript{4} | Tt | F | 20/10/1983 | 20/10/1978\textsuperscript{*} | Deceased (17y, 11m) |
| Tt\textsubscript{5} | Tt | F | Captive born | 12/05/1995 | Alive (21y, 5m) |
| Ta-t\textsubscript{1} | F1 Ta×Tt | F | Captive born | 23/04/1986 | Deceased (9y, 1m) |
| Ta-t\textsubscript{2} | F1 Ta×Tt | M | Captive born | 28/07/1990 | Deceased (24y, 9m) |
| Ta-t\textsubscript{3} | F1 Ta×Tt | F | Captive born | 23/05/1993 | Alive (23y, 5m) |
| Ta-t\textsubscript{4} | F1 Ta×Tt | M | Captive born | 07/09/1995 | Alive (21y, 1m) |
| Ta-t\textsubscript{5} | F1 Ta×Tt | F | Captive born | 09/12/1998 | Alive (17y, 10m) |
| Ta-t\textsubscript{6} | F1 Ta×Tt | M | Captive born | 22/05/2004 | Alive (12y, 5m) |
| Ta-t\textsubscript{7} | F1 Ta×Tt | F | Captive born | 25/11/2008 | Alive (7y, 11m) |
| BC\textsubscript{1} | Ta-t×Tt | F | Captive born | 17/07/1993 | Alive (23y, 3m) |
| BC\textsubscript{2} | Ta-t×Tt | M | Unborn | - | Deceased (>8 m in utero) |
| BC\textsubscript{3} | Ta-t×Tt | F | Captive born | 09/02/2014 | Deceased (9d) |

\textsuperscript{*} Estimated from age at capture.

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A second pregnancy was documented in Ta-t₁ (foetus hereby referred to as BC₂), representing another backcross event with Tt₃. Of note is that Ta-t₁ was lactating at the time of conception, with BC₁ who was two years old during this time period observed suckling. However, Ta-t₁ died on the 30th of May 1995 (at age 9 years) whilst pregnant with the unborn male calf in utero. She was estimated to be in the third trimester of pregnancy at the time of her death. The cause of death for Ta-t₁ and associated unborn calf (BC₂) was a peracute infection, possibly caused by the bacterium Clostridium chauvoei, resulting in toxaemia. The autopsy report states that the foetus and amniotic fluid appeared normal.
The second backcross (BC₃) offspring born at uShaka Sea World was born to Ta-t₇ on the 9th of February 2014. Ta-t₇ is estimated to have been 4 years and 3 months old at the time of conception (again back calculated from the DOB of BC₃) and demonstrated no obvious behaviour or physical signs to demonstrate reproductive receptivity. At the time of conception she was physically small, weighing around 222 kg (weight as of February 2013) and had no clear pattern of ventral speckling—a sign of physical maturation in some *Tursiops* species [6, 43]. Although fed on a diet of fish and squid from April 2009 onwards, she continued to suckle milk from her mother. As such, she was housed in a social unit consisting of Ta₁ and Th₁, her biological mother and father. Copulation was not observed but as they were housed together, it is most likely that Th₁ sired BC₃, as all other males were held together in adjacent pools, with no free intermixing between groups taking place. Pregnancy was confirmed in Ta-t₇ during a routine ultra sound examination on the 14th June 2013 and she was carefully monitored thereafter. Body length measured around this time in 2014 was estimated at 2.65 m i.e. longer than her thoroughbred mother (Ta₁) but shorter than the adult hybrids. Ta-t₇ continued to grow by an est. 26 cm in the following years, attaining an adult length of 2.91 m in 2018 (Fig 3).

No abnormal behaviour or physical symptoms were demonstrated during Ta-t₇’s pregnancy. When born, BC₃ was closely observed and appeared healthy, although for managerial reasons no individual medical examinations were conducted with BC₃. In the days following birth, BC₃ suckled from both her mother (Ta-t₇) and maternal grandmother (Ta₁). BC₃ died on the 18th of February at 9 days old. Post mortem examinations revealed BC₃ suffered nutritional complications, most likely resulting from a lack of sufficient colostrum intake in the days following birth and an associated undetermined infection.

The length-weight relationships of the hybrid and backcross offspring fall between the parent species (Fig 4). The first generation hybrid offspring (*i.e.* all Ta-t) have a length of 2.89 to

![Figure 4: Body length-weight relationship for dolphins housed at uShaka Sea World, as well as examples from the parent *T. aduncus* population. Data from three *T. aduncus* from KwaZulu Natal are by-caught specimens and the largest examples in the data-set from this region [44]. Growth curves for each species calculated by Best (2007) from 16 common bottlenose (Weight = 11.32 x Length²⁻⁰⁸⁶) and 41 Indo-Pacific bottlenose dolphin (Weight = 12.365 x Length²⁻⁰⁴⁹⁵) necropsies of animals within the study area.](https://doi.org/10.1371/journal.pone.0201722.g004)
3.02 m. (mean 2.95 m) and weigh between 231 to 273 kg (mean 247 kg), with BC1 falling within this range (2.99 m and 241 kg). The two pure bred male \textit{T. truncatus} held at uShaka Sea World are considerably larger (for instance \textit{Tt1} is 3.55 m in length and weighs 470 kgs). However, the pure bred female \textit{T. truncatus} (\textit{Tt3}) is unusual in this sample, by having a comparatively small length and weight for the species, attributed to premature maternal separation and restricted development (authors observations). All hybrids are longer and weigh more than \textit{Tt1} and the largest \textit{T. aduncus} specimens measured from the wild parent population where \textit{Tt1} originates (Fig 4).

Some \textit{T. aduncus} populations exhibit ventral speckling [43, 45], the degree of which increases with age and may indicate sexual maturation. We inspected the ventral surfaces of all dolphins within uShaka Sea World to determine the degree of ventral speckling. Ventral speckling was absent in \textit{Tt-t7} before conception and in 2016 (at age 7 yrs 11 mo) \textit{Tt-t7} still did not exhibit significant ventral speckling (Fig 5A and 5B). In 2016, some ventral speckling was present on the older hybrids held at uShaka Sea World (Fig 5D), although visual assessment indicated a much lesser degree of speckling than considered normal for mature individuals from the parent \textit{T. aduncus} or Shark Bay \textit{Tursiops} spp. species [6, 43] (compare Fig 5C and 5D). On the adult hybrids held at uShaka Sea World, the ventral speckles are faint and coverage of the ventral area is sparse (Fig 5D).

Observation and training with the \textit{F1} hybrids and the surviving backcross hybrid (BC1) is ongoing at uShaka Sea World. In all cases, the hybrids are fully incorporated into the daily activities of the facility and demonstrate social and cognitive functions, such as response rates during training for veterinary procedures and strong social bonding, similar to the thoroughbred dolphins housed at the same facility.

**Discussion**

To date, most hybridization events in wild cetaceans have been identified through morphological descriptions (e.g. [46, 47]) with the recent application of molecular techniques (e.g. [17, 28, 30, 48, 49]) used to identify hybrids and their parent species. Reports from captive facilities enable the tracking of breeding history (e.g. [50]), and as in our case, can provide important information on the breeding capabilities of dolphin species. Of the odontocetes, the common bottlenose dolphin is the species recorded most frequently to hybridize in captivity [3]. Although there are exceptions [33], the majority of hybrid offspring born in captivity do not survive [3, 21]. Here we demonstrate that \textit{F1} \textit{T. aduncus} and \textit{T. truncatus} can survive to adulthood, are healthy and can produce healthy backcross hybrid offspring in cases where the dam is the \textit{F1} hybrid and the sire is \textit{T. truncatus}.

The longevity of the hybrid offspring and most notably the BC1 hybrid at uShaka Sea World is unusual amongst captive facilities [3, 21]. This may be explained by the closer taxonomic relationship between \textit{Tursiops} species compared to species involved in inter-generic hybridization events, perhaps facilitating genetic compatibility. Breeding success may also be a reflection of good animal husbandry at the uShaka Sea World captive facility. The apparently normal ovulatory behaviour of the surviving backcross hybrid adult, suggests that subsequent generational hybrids may also be reproductively viable, though the lack of a test for F2 compatibility of hybrids is a limitation, especially since it is often the heterogametic sex (males) that shows hybrid sterility (‘Haldane’s rule’ [51]).

Although rare, there are documented cases of inter-generic hybridization involving \textit{T. truncatus}, resulting in fertile hybrids which have subsequently backcrossed with the parent \textit{T. truncatus} species. For example, Duffield [52] report that an \textit{F1} \textit{T. truncatus} x \textit{P. crassidens} hybrid backcrossed with \textit{T. truncatus} on two occasions. In another example, an \textit{F1} \textit{T. truncatus} x \textit{D.
survived (strong conclusions. We can note however that the parents were unrelated for the offspring that are too few to draw any backcross. Here we describe in some detail multiple intra-generic hybridization events between T. truncatus and T. aduncus and a successful backcross, supporting the potential for this type of reticulation in this genus and the consequent influence on evolution in the wild. We document backcross mating by two parental configurations, and so too few to draw any strong conclusions. We can note however that the parents were unrelated for the offspring that survived (Ta-t with Ta-t1, see Fig 1), while the offspring from the inbred mating (Ta-t with his daughter Ta-t2) did not.

Data on age at sexual maturity in female T. aduncus are sparse. Sexual maturity occurs before physical maturity, and earlier in females than males [6, 53]. Timing of maturity may also differ between captive and wild born animals [54] and between geographically separated populations [55], further complicating assessments of reproductive age. For example, mean ovulation age in captive killer whales (Orcinus orca) is 7.5 years and age at first conception 9.8 years, compared to the average first conception age of 12.1 years in wild, free ranging populations [56]. In the wild, ovulation in female T. aduncus from South African waters is reported to take place between 9.5 and 11 years of age [6]. However, reports of a stranded female from an earlier study suggest that sexual maturity can be attained under 9 yrs of age, and possibly as early as 6 yrs [42]. There are reports of sexual maturity as early as 3.5 years in Tursiops from Japan [57]. However, these data are derived from the examination of deceased dolphins, and it is unclear whether this minimum age is based on the occurrence of corpora lutea in the ovaries or observed pregnancies in animals of this young age (or both), with no further data on whether the outcome of pregnancy was a viable offspring [57]. Data from free ranging T. truncatus from Namibia are similarly sparse, although there is evidence from this population that first conception can take place around 5.5 years of age [58] and at approximately 2.8 m total length [38]. There are few data on the age at maturity of hybrids and whether, like other morphological [22, 25, 33, 38] and behavioural [25] characteristics, it is intermediate between that of the parent species. Zornetzer and Duffield [33], for example report the birth of a calf to a hybrid T.truncatus x D.capensis, born when the dam was 7.5 yrs and presumably conceived around 6.5 yrs of age. Our data on pregnancy in F1 T. aduncus x T. truncatus hybrids demonstrates that these animals can become pregnant early in life compared to the parent species. The estimated age of conception of 4 years and 3 months reported here for Ta-t1 may therefore be the youngest known viable pregnancy for either parent Tursiops species or hybrid thereof.

That Ta-t2 was still observed nursing during the period of conception is also of interest. Bottlenose dolphins can begin ingesting solid food between 4 and 11 months of age [59], with a combined solid and milk diet thereafter. At uShaka Sea World, Ta-t2 began eating solids from 4.5 months onwards. Bottlenose dolphins and other odontocetes are known to have prolonged lactation [59] and in South African T. aduncus milk remains have been documented in the stomachs of calves up to three years of age [60]. Although the majority of calves from bottlenose dolphins from Shark Bay, Western Australia were weaned before four years, some continued to suckle after this, with one animal only weaned at eight years of age [61]. Lactation in mammals, including dolphins, relies on close proximity and physical stimulation of the mammary area [62–64]. Captive studies have demonstrated that persistent suckling attempts can
induce lactation when orphaned calves are held in close proximity to previously non-lactating *Tursiops* females [65]. In the wild, pre-weaned animals maintain a close association with their mother, with weaning initiated during the females’ next pregnancy [61]. Therefore, the close association of mother and calf in the captive facility may have prolonged the lactation period of *Ta* to four years of age and beyond.

Morphological characteristics of hybrid cetacean offspring appear intermediate to the parent species [3, 33]. In the wild *T. aduncus* are smaller in length and estimated weight compared to *T. truncatus* [38]. Although limited, our length-weight data indicate that the size of hybrid offspring is intermediate to the biological parents, indicating it falls intermediate between the parent species (Fig 3). This observation might help identification of hybrids in the wild, however a greater sample size including unrelated individuals would clarify this relationship. The coloration patterns of hybrids can also differ from parent species, usually being somewhat intermediate [22, 33, 38]. Ventral speckling is absent in *T. truncatus* but is prominent in some populations of *T. aduncus* and the *Tursiops* spp. population found in Shark Bay, Western Australia which have had an uncertain taxonomic status but speckling patterns similar to *T. aduncus* [6, 43]. In the latter population, speckling develops with age, first appearing around the genital area around 10 years of age, but can occur as early as 7 years. The age of speckle onset around the genitalia usually correlates with the age of first parturition and is considered an honest sign of sexual maturation in the Shark Bay population [43]. The development of speckling has not yet been determined in hybrid *Tursiops* dolphins. Our observations indicate that the onset or degree of ventral speckling is not a reliable indicator of sexual maturity in *F* hybrids.

Karyological similarity within the Cetacea (most have the same number of chromosomes: 2n = 44 [3]) has been proposed as one explanation for the apparent ease with which distinctly related cetacean species hybridize [66]. Where their distributions overlap, new cetacean species can originate through hybridization, as demonstrated for the Clymene’s dolphin [4] and environmental pressures such as climate change may increase the frequency of introgressive hybridization, as recently suggested for pilot whales, genus *Globicephala* [32]. The distribution of *T. aduncus* and *T. truncatus* occur in parapatry throughout the Indo-Pacific region, with sympatric distributions in some areas such as the waters off South East China [8]. Given that we have demonstrated several hybridization events, it is somewhat surprising that other hybridization events have not been documented in wild populations and the genetic integrity of the parent species remains intact in areas where their distributions overlap such as in the Taiwan Strait [8, 67] and Australia [16]. Indeed, relatively high levels of genetic isolation have been documented in such areas [67]. Behavioural isolation mechanisms may be operating in the wild to reduce hybridization events. For example, *T. aduncus* and *T. truncatus* produce acoustic communication signals (whistles) with distinguishable frequency compositions [68, 69], which could assist in inter-species recognition thereby reducing intra-generic mating attempts.

**Conclusion**

We have demonstrated that *T. aduncus x T. truncatus* *F* hybrids can survive to adulthood, are healthy and can produce healthy backcross hybrid offspring. The documented hybridization in captivity may be an artefact of the close proximity and the limited mating opportunities afforded by captive situations, limiting mate choice and assortative mating. However, low levels of intra-generic hybridization in *Tursiops* may well be taking place in the wild [34], and may be revealed following more extensive molecular screening in the relevant geographic regions.
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References

1. Arnold ML. Divergence with Genetic Exchange. Oxford: University Press; 2015. 272 p.
2. Seehausen O. Hybridization and adaptive radiation. Trends Ecol Evol. 2004; 19(4):198–207. https://doi.org/10.1016/j.tree.2004.01.003 PMID: 16701254
3. Bérbé M. Hybridism. 2009. In: Encyclopedia of marine mammals [Internet]. New York: Academic Press. 2nd. [588–91].
4. Amaral AR, Lovewell G, Coelho MM, Amato G, Rosenbaum HC. Hybrid Speciation in a Marine Mammal: The Clymene Dolphin (Stenella clymene). PLoS One. 2014; 9(1):e83645. https://doi.org/10.1371/journal.pone.0083645 PMID: 24421898
5. Hershkovitz P. A catalog of living whales. United States National Museum Bulletin. 1966; 246:1–259.
6. Ross GJB, Cockcroft VG. Comments on Australian bottlenose dolphins and the taxonomic status of Tursiops aduncus (Ehrenberg, 1832). In: Leatherwood S, Reeves RR, editors. The Bottlenose Dolphin. San Diego: Academic Press; 1990. p. 101–28.
7. Natoli A, Peddemors VM, Hoelzel AR. Population structure and speciation in the genus Tursiops based on microsatellite and mitochondrial DNA analyses. Journal of Evolutionary Biology. 2004; 17(2):363–75. PubMed PMID: ISI:000188999500015. PMID: 15009270
8. Wang JY, Chou LS, White BN. Mitochondrial DNA analysis of sympatric morphotypes of bottlenose dolphins (genus: Tursiops) in Chinese waters. Molecular Ecology. 1999; 8(10):1603–12. PubMed PMID: ISI:000083466800005. PMID: 10583824
9. Moura AE, Nielsen SC, Vilstrup JT, Moreno-Mayar JV, Gilbert MT, Gray HW, et al. Recent diversification of a marine genus (Tursiops spp.) tracks habitat preference and environmental change. Syst Biol. 2013; 62(6):865–77. https://doi.org/10.1093/sysbio/syt051 PMID: 23929779
10. Wang JY, Chou LS, White BN. Differences in the external morphology of two sympatric species of bottlenose dolphins (genus Tursiops) in the waters of China. Journal of Mammalogy. 2000; 81(4):1157–65. PubMed PMID: ISI:000165637900024.
11. Rice DW. Marine mammals of the world: systematics and distribution. Society for Marine Mammalogy. 1998; Special Publication 4:1–231.
12. Charlton-Robb K, Gershwin L-A, Thompson R, Austin J, Owen K, McKechnie S. A new dolphin species, the burrunan dolphin *Tursiops australis* sp. nov., endemic to southern Australian coastal waters. PLoS One. 2011; 6(9):e24047. https://doi.org/10.1371/journal.pone.0024047 PMID: 21935372

13. Viaud-Martinez KA, Brownell RL, Komnenou A, Bohonak AJ. Genetic isolation and morphological divergence of Black Sea bottlenose dolphins. Biological Conservation. 2008; 141(6):1600–11. https://doi.org/10.1016/j.biocon.2008.04.004 PubMed PMID: ISI:000257536100015.

14. Chen I, Nishida S, Yang W-C, Isobe T, Tajima Y, Hoelzel AR. Genetic diversity of bottlenose dolphin (*Tursiops sp.*) populations in the western North Pacific and the conservation implications. Marine Biology. 2017; 164(10):202. https://doi.org/10.1007/s00227-017-3323-8 PubMed PMID: PMC5592193. PMID: 26983128

15. Charlton K, Taylor AC, McKechnie SW. A note on divergent mtDNA lineages of bottlenose dolphins from coastal waters of Southern Australia. Journal of cetacean research management. 2006; 8(2):173–9.

16. Allen SJ, Bryant KA, Kraus RH, Loneragan NR, Kopps AM, Brown AM, et al. Genetic isolation between coastal and fishery-impacted, offshore bottlenose dolphin (*Tursiops spp.*) populations. Mol Ecol. 2016; 25(12):2735–53. https://doi.org/10.1111/mec.13622 PMID: 27015516

17. Kingston SE, Adams LD, Rosel PE. Testing mitochondrial sequences and anonymous nuclear markers for phylogeny reconstruction in a rapidly radiating group: molecular systematics of the Delphininae (Cetacea: Odontoceti: Delphinidae). Bmc Evolutionary Biology. 2009; 9:19. doi: 245 https://doi.org/10.1186/1471-2148-9-19 PubMed PMID: ISI:000271884200001.

18. LeDuc RG, Perrin WF, Dizon AE. Phylogenetic relationships among the delphinid cetaceans based on full cytochrome B sequences. Marine Mammal Science. 1999; 15(3):619–48. PubMed PMID: ISI:000080863700001.

19. Vilstrup JT, Ho SY, Foote AD, Morin PA, Kreb D, Kruzen M, et al. Mitogenomic phylogenetic analyses of the Delphinidae with an emphasis on the Globicephalinae. BMC Evol Biol. 2011; 11(65):1471–2148.

20. McGowen M, R.. Toward the resolution of an explosive radiation—A multilocus phylogeny of oceanic dolphins (Delphinidae). Molecular Phylogenetics and Evolution. 2011; 60:345–57. https://doi.org/10.1016/j.ympev.2011.05.003 PMID: 21600295

21. Sylvestre J-P, Tasaka S. On the intergeneric hybrids in cetaceans. Aquatic Mammals. 1985; 11 (3):101–8.

22. Caballero S, Baker CS. Captive-born intergeneric hybrid of a Guiana and bottlenose dolphin: *Sotalia guianensis* × *Tursiops truncatus*. Zoo Biology. 2010; 29(5):647–57. https://doi.org/10.1002/zoo.20299 PMID: 20033990

23. Schaurich MdN Lopes FRV, de Oliveira LR. Hybridization phenomenon in cetacean and pinniped species. Neotropical Biology and Conservation. 2012; 7(3):199–209.

24. Glover KA, Kanda N, Haug T, Pastene LA, Òien N, Goto M, et al. Migration of Antarctic minke whales to the Arctic. PLoS One. 2010; 5(12):e15197. https://doi.org/10.1371/journal.pone.0015197 PMID: 21203557

25. Willis PM, Crespi BJ, Dill LM, Baird RW, Hanson MB. Natural hybridization between Dal’s porpoises (*Phocoenoides dalli*) and harbour porpoises (*Phocoena phocoena*). Canadian Journal of Zoology. 2004; 82:828–34.

26. Bérubé M, Aguilar A. A new hybrid between a blue whale, *Balaenoptera musculus*, and a fin whale, *B. physalus*: frequency and implications of hybridization. Marine Mammal Science. 1998; 14(1):82–98. https://doi.org/10.1111/j.1748-7692.1998.tb00692.x

27. Reyes JC. A possible case of hybridism in wild dolphins. Marine Mammal Science. 1996; 12(2):301–7. https://doi.org/10.1111/j.1748-7692.1996.tb00581.x

28. Brown AM, Kopps AM, Allen SJ, Bejder L, Littleford-Colquhoun B, Parra GJ, et al. Population Differentiation and Hybridisation of Australian Snubfin (*Orcaella heinsohni*) and Indo-Pacific Humpback (*Sousa chinensis*) Dolphins in North-Western Australia. PLoS One. 2014; 9(7):e101427. https://doi.org/10.1371/journal.pone.0101427 PMID: 24988113

29. Glover K, Kanda N, Haug T, Pastene L, Òien N, Sellassen B, et al. Hybrids between common and Antarctic minke whales are fertile and can back-cross. BMC Genetics. 2013; 14(1):25. https://doi.org/10.1186/1471-2156-14-25 PMID: 23586609

30. Spilliaert R, Vikingsson G, Arnason U, Palsdottir A, Sigurjonsson J, Arnason A. Species hybridization between a female blue whale (*Balaenoptera musculus*) and a male fin whale (*B. physalus*): molecular and morphological documentation. J Hered. 1991; 82(4):269–74. PMID: 1679066

31. Miralles L, Lens S, Rodriguez-Folgar A, Carrillo M, Martin V, Mikkelsen B, et al. Interspecific Introgression in Cetaceans: DNA Markers Reveal Post-F1 Status of a Pilot Whale. PLoS One. 2013; 8(8):e69511. https://doi.org/10.1371/journal.pone.0069511 PMID: 23990883
32. Miralles L, Oremus M, Silva MA, Planes S, Garcia-Vazquez E. Interspecific Hybridization in Pilot Whales and Asymmetric Genetic Introgression in Northern Globicephala melas under the Scenario of Global Warming. PLoS One. 2016; 11(8):e0160080. https://doi.org/10.1371/journal.pone.0160080 PMID: 27508496

33. Zometzer HR, Duffield DA. Captive-born bottlenose dolphin × common dolphin (Tursiops truncatus × Delphinus capensis) intergeneric hybrids. Canadian Journal of Zoology. 2003; 81(10):1755–62. https://doi.org/10.1139/z03-150

34. Martien KK, Baird RW, Hedrick NM, Gorgone AM, McSweeney DJ, et al. Population structure of island-associated dolphins: Evidence from mitochondrial and microsatellite markers for common bottlenose dolphins (Tursiops truncatus) around the main Hawaiian Islands. Marine Mammal Science. 2012; 28(3):E208–E32. Epub 2011. https://doi.org/10.1111/j.1748-7692.2011.00506.x

35. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155(2):945–59. PMID: 10835412

36. Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 2003; 164(4):1567–87. PMID: 12930761

37. Hubisz MJ, Falush D, Stephens M, Pritchard JK. Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour. 2009; 9(5):1322–32. https://doi.org/10.1111/j.1755-0998.2009.02591.x PMID: 21564903

38. Best PB. Whales and dolphins of the Southern African Subregion. Cambridge: Cambridge University Press; 2007. 338 p.

39. Hoelzel AR. Molecular Genetic Analysis of Populations; A Practical Approach. Oxford: Oxford University Press; 1992.

40. Natoli A, Birkun A, Aguilar A, Lopez A, Hoelzel AR. Habitat structure and the dispersal of male and female bottlenose dolphins (Tursiops truncatus). Proc R Soc B-Biol Sci. 2005; 272(1569):1217–26. PubMed PMID: IS1:000230563200004.

41. Oremus M, Leqata J, Baker CS. Resumption of traditional drive hunting of dolphins in the Solomon Islands in 2013. Royal Society Open Science. 2015; 2(5):140524. https://doi.org/10.1098/rsos.140524 PubMed PMID: PMC4453245. PMID: 26064656

42. Ross GJB. The taxonomy of bottlenosed dolphins Tursiops species in South African waters, with notes on their biology. Annals of the Cape Provincial Museum (Natural History). 1977; 11:135–94.

43. Krzyseckzyk E, Mann J. Why become speckled? Ontogeny and function of speckling in Shark Bay bottlenose dolphins (Tursiops sp).1. Marine Mammal Science. 2012; 28(2):295–307. https://doi.org/10.1111/j.1748-7692.2011.00483.x

44. Plön S, Albrecht K, Cliff G, Fromenman PW. Organ weights of three dolphin species from South Africa-implications for ecological adaptation?2012. 265–76 p.

45. Amir OA, Jiddawi NS, Berggren P. The occurrence and distribution of dolphins in Zanzibar, Tanzania, with comments on differences between two species of Tursiops. Western Indian Ocean Journal of Marine Science. 2005; 4(1):85–93.

46. Hodgins NK, Dolman SJ, Weir CR. Potential hybridism between free-ranging Risso’s dolphins (Grampus griseus) and bottlenose dolphins (Tursiops truncatus) off north-east Lewis (Hebrides, UK). Marine Biodiversity Records. 2014; 7. https://doi.org/10.1017/s175526721400089x

47. Heide-Jørgensen MP, Reeves RR. Description of an anomalous monodontid skull from West Greenland: A possible hybrid? Marine Mammal Science. 1993; 9(3):258–68. https://doi.org/10.1111/j.1748-7692.1993.tb00454.x

48. Baird RW, Willis PM, Guenther TJ, Wilson PJ, White BN. An intergeneric hybrid in the family Phocidae. Canadian Journal of Zoology. 1998; 76(1):198–204. https://doi.org/10.1139/z97-175a

49. Arnason U, Spilliaert R, Palsdottir A, Arnason A. Molecular identification of hybrids between the two largest whale species, the blue whale (Balaenoptera musculus) and the fin whale (Balaenoptera physalus). Hereditas. 1991; 115(2):183–9. PMID: 1687408

50. Dohl TP, Norris KS, Kang I. A Porpoise Hybrid: Tursiops × Steno. Journal of Mammalogy. 1974; 55 (1):217–21. https://doi.org/10.2307/1379276 PMID: 4819596

51. Haldane JBS. Sex ratio and unisexual sterility in hybrid animals. Journal of Genetics. 1922; 12(2):101–9. https://doi.org/10.1007/bf02983075

52. Duffield DA, editor Examples of captive hybridization and a genetic point of view. World Marine Mammal Science Conference; 1998; UK.

53. Kemper CM, Trentin E, Tomo I. Sexual maturity in male Indo-Pacific bottlenose dolphins (Tursiops aduncus): evidence for regression/pathological adults. Journal of Mammalogy. 2014; 95(2):357–68. https://doi.org/10.1644/13-mamm-a-007.1
54. O'Regan HJ, Kitchener AC. The effects of captivity on the morphology of captive, domesticated and feral mammals. Mammal Review. 2005; 35(3–4):215–30. https://doi.org/10.1111/j.1365-2907.2005.00070.x

55. Hale PT, Barreto AS, Ross GJB. Comparative morphology and distribution of the aduncus and truncatus forms of bottlenose dolphin Tursiops in the Indian and Western Pacific Oceans. Aquatic Mammals. 2000; 26(2):101–10.

56. Robeck TR, Willis K, Scarpuzzi MR, O'Brien JK. Comparisons of life-history parameters between free-ranging and captive killer whale (Orcinus Orca) populations for application toward species management. Journal of Mammalogy. 2015; 96(6):1055–70. https://doi.org/10.1093/mammal/gyv113 PubMed PMID: PMC4668992. PMID: 26937049

57. Kasuya T. Fishery-dolphin conflict in the Iki Island area of Japan. 1985. In: Marine Mammals and Fisheries [Internet]. London: George Allen & Unwin; [253–72].

58. Peddemors VM. Minimum age at sexual maturation of a female south east Atlantic bottlenose dolphin Tursiops truncatus. South African Journal of Marine Science. 1989;(8):345–8.

59. Ofstad OT. Lactation in whales and dolphins: evidence of divergence between baleen- and toothed-species. J Mammary Gland Biol Neoplasia. 1997; 2(3):205–30. PMID: 10882306

60. Cockcroft VG, Ross GJB. Age, growth, and reproduction of bottle-nosed dolphins Tursiops truncatus from the East Coast of Southern Africa. Fish Bull. 1990; 88(2):289–302. PubMed PMID: ISI:A1990DX59600006.

61. Mann J, Connor RC, Barre LM, Heithaus MR. Female reproductive success in bottlenose dolphins (Tursiops sp.): life history, habitat, provisioning, and group-size effects. Behavioral Ecology. 2000; 11(2):210–9. https://doi.org/10.1093/beheco/11.2.210

62. Peddemors VM, Fothergill M, Cockcroft VG. Feeding and growth in a captive-born bottlenose dolphin Tursiops truncatus. South African Journal of Zoology. 1992; 27(2):74–80. https://doi.org/10.1080/02541858.1992.11448265

63. McClellan HL, Miller SJ, Hartmann PE. Evolution of lactation: nutrition v. protection with special reference to five mammalian species. Nutr Res Rev. 2008; 21(2):97–116. https://doi.org/10.1017/S095442240800749 PMID: 19087365

64. Nowak R, Porter RH, Levy F, Orgeur P, Schaal B. Role of mother-young interactions in the survival of offspring in domestic mammals. Rev Reprod. 2000; 5(3):153–63. PMID: 11006165

65. Ridgway S, Kamolnick T, Reddy M, Curry C, Tarpley RJ. Orphan-induced lactation in Tursiops and analysis of collected milk. Marine Mammal Science. 1995; 11(2):172–82. https://doi.org/10.1111/j.1748-7692.1995.tb00516.x

66. Arnason U, Gullberg A. Comparison between the complete mtDNA sequences of the blue and the fin whale, two species that can hybridize in nature. J Mol Evol. 1993; 37(4):312–22. PMID: 8308901

67. Yang GA, Ji GQ, Ren WH, Zhou KY, Wei FW. Pattern of genetic variation of bottlenose dolphins in Chinese waters. Raffles Bull Zool. 2005; 53(1):157–64. PubMed PMID: ISI:000231709900018.

68. Gridley T, Berggren P, Cockcroft VG, Janik VM. Whistle vocalizations of Indo-Pacific bottlenose dolphins (Tursiops aduncus) inhabiting the south-west Indian Ocean. Journal of the Acoustical Society of America. 2012; 132(6):4032–40. https://doi.org/10.1121/1.4763990 PMID: 23231132

69. Erbs F, Elwens SH, Gridley T. Automatic classification of whistles from coastal dolphins of the southern African subregion. The Journal of the Acoustical Society of America. 2017; 141(4):2489–500. https://doi.org/10.1121/1.4978000 PMID: 28464668