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Osteological variation does not support the occurrence of three species of bottlenose dolphins (*Tursiops* spp.) in Australia

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Introduction

The Delphinidae is the most diverse living cetacean family (Barnes 1990, Berta & Sumich 1999, Fordyce 2009). It has experienced a rapid speciation (McGowen *et al.* 2009, Steeman *et al.* 2009), which has led to taxonomic difficulties separating several of the species within the family (LeDuc *et al.* 1999, McGowen 2011, Perrin 2013). The *Delphinus-Stenella-Tursiops* clade appears especially problematical (LeDuc *et al.* 1999). This may be due to slow molecular evolution, the difficulty of resolving short branches produced by cladistic analyses and the identification of clear diagnostic morphological characters (Buchholtz & Schur 2004, Amaral 2009, McGowen 2011). There have been several attempts to resolve the taxonomic status of *Tursiops* in Australian waters. Ross and Cockcroft (1990) examined external morphology and skeletons from around the continent. They concluded that there was a gradual increase in size from north to south that might be associated with decreasing water temperatures and that the lack of clear morphological boundary led the authors to conclude that a single species, *T. truncatus*, occurred around Australia. Hale *et al.* (2000) described two morphologically distinct forms of *Tursiops* in south-eastern Queensland; a large, unspotted form (*T. truncatus*) living in water > 30 m and a smaller, spotted form (*Tursiops* cf. *aduncus*) in waters < 30 m. Genetic studies have since confirmed the presence of *T. aduncus* in New South Wales (Möller & Beheregaray 2001) and Shark Bay, Western Australia (Krutzen *et al.* 2001), although both studies only used a single marker (mtDNA). Using skeleton measurements and features, Kemper (2004) found support for two morphotypes of bottlenose dolphins in South Australia that had affinities with *T. aduncus* and *T. truncatus*. Two *Tursiops* species are currently recognized in Australian waters: *T. truncatus* and *T. aduncus*, with a third species, *T. australis*, recently described. Due to the overlap in metric characters, low molecular support, lack of comparison to other bottlenose dolphins in the world, *T. australis* has not yet been accepted worldwide (Committee on Taxonomy 2014). In order to clarify the taxonomy of the genus *Tursiops* in the Australian region, the relationships between *Tursiops* spp. and to other Delphininae have to be elucidated. We compared *Tursiops* spp. to other species within the subfamily Delphininae occurring in Australian waters, and looked at morphological relationships between species within the genus *Tursiops*. We also reexamined the validity of the proposed new species using a large number of broadly distributed morphological samples of Australian *Tursiops* to date. This document summarizes our morphological results to date.

Methods

All available cranial from cranially mature specimens of bottlenose dolphins (*Tursiops* spp.) were measured using 2D and 3D methods. A small number of nine other Delphinidae species (*Stenella coeruleoalba* (Striped dolphin), *Stenella attenuata* (Pantropical spotted dolphin), *Stenella longirostris* (Spinner dolphin), *Delphinus delphis* (Short-beaked common dolphin), *Steno bredanensis* (Rough-toothed dolphin), *Lagenodelphis hosei* (Fraser's dolphin) and *Sousa sahulensis* (Australian humpback dolphin) within the subfamily Delphininae occurring in Australian waters were also measured (Table 1).

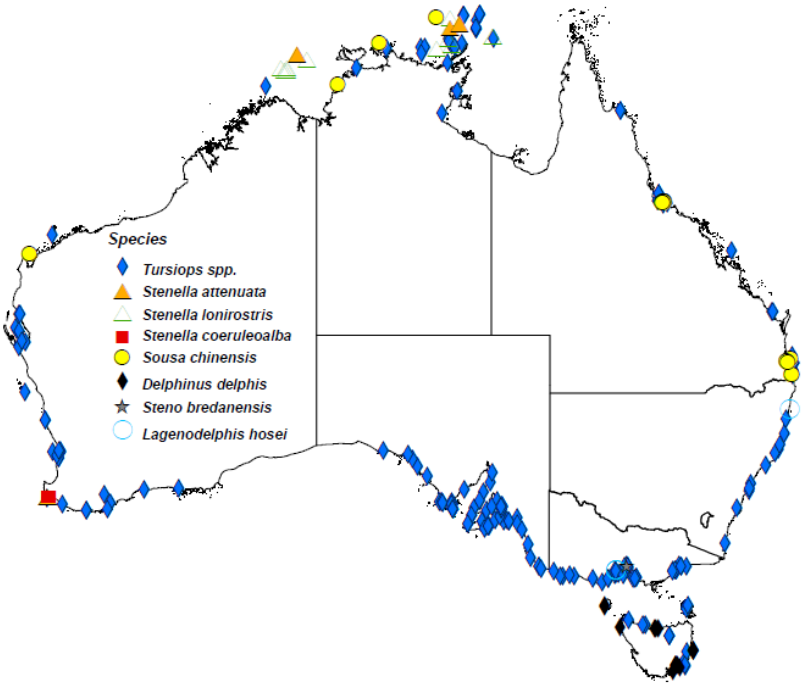

Figure 1. Map of Australia showing collecting localities for the specimens and species used for the study.

The holotype specimens of *T. truncatus* (*Delphinus truncatus*, Montagu 1821), *T. aduncus* (*Delphinus aduncus*, Ehrenberg 1832), syntype specimens from previously identified species found in Australian waters of *Delphinus catalania*, Gray 1862, and *Tursiops maugeanus*, Iredale and Troughton 1934, one of which was renamed *Tursiops australis* were also examined.

Table 1. Collecting location and number of cranially mature skulls used in the study.

Species	Females	Males	Unknown sex	Total
Tursiops spp.	69	60	129	258
Delphinus truncatus (holotype, *T. truncatus*)	0	0	1	1
Delphinus aduncus (holotype, *T. aduncus*)	0	0	1	1
Tursiops australis (holotype)	1	0	0	1
Tursiops maugeanus (syntype)	0	1	0	1
Delphinus catalania (syntype)	2	0	0	2
Stenella attenuata	1	6	11	18
Stenella longirostris	4	8	3	15
Stenella coeruleoalba	0	8	0	8
Sousa chinensis	1	5	11	17
Delphinus delphis	0	0	15	15
Steno bredanensis	2	1	2	5
Lagenodelphis hosei	2	1	2	5
Total	**82**	**90**	**175**	**347**

We carried out 2-D- (54 cranial measurements, six categorical variables, six tooth counts, one tooth measurement, and vertebral count (only available for *Tursiops* spp.) and 3-D-geometric morphometrical analyses (73 landmarks, MicroScribe G2X, Immersion Corporation). Cranial measurements (2D) were taken with anthropometers and spreading calipers (cranial height) to the nearest millimeter, and 3-D-geometric morphometrical analyses (3GM) using a 3D-digitizer (MicroScribe G2X, Immersion Corporation).

Principal Component Analyses (PCA, JMP, version 9, SAS Institute Inc., Cary, NC, estimation methods REML, ML, Robust, Row-wise and Pairwise), discriminant function analyses (SPSS, version 20.0 Armonk, NY:IBM Corp., default settings), hierarchical cluster analyses (SPSS, Euclidian distance) and k-mean analyses (SPSS, default settings) were conducted on the 2D cranial data.

Results

Comparing Tursiops spp. to other Australian delphinids

Two-dimensional cranial measurement, count and categorical data

Multivariate analyses (principal component, discriminant function, cluster and k-mean analyses) of skull data revealed a clear separation of *Tursiops* spp. and the other species (including type specimens). In the PCA, *Tursiops* was the only genus clearly separated from the other six genera (*Tursiops*, *Stenella*, *Sousa*, *Delphinus*, *Steno* and *Lagenodelphis*). This was based on the non-overlapping 95% confidence eclipse (Figure 2). The first four PCs accounted for 84.2% of the total variation. Width variables (rostrum width at midlength and rostrum width at 3/4 of rostrum length from base) contributed to most of the variation in PC1 and primarily length variables (length of upper tooth row to tip of rostrum and rostrum length) in PC2. At a species level, S. *longirostris* was the only other species that could be separated from other species. *Tursiops aduncus* and *T. truncatus* specimens clustered close together and did not form separate groups. *Sousa sahulensis* clustered with *S. bredanensis*, *S. attenuata* overlapped with *D. delphis*, and *L. hosei* clustered adjacent to *S. coeruleoalba* (Figure 2).

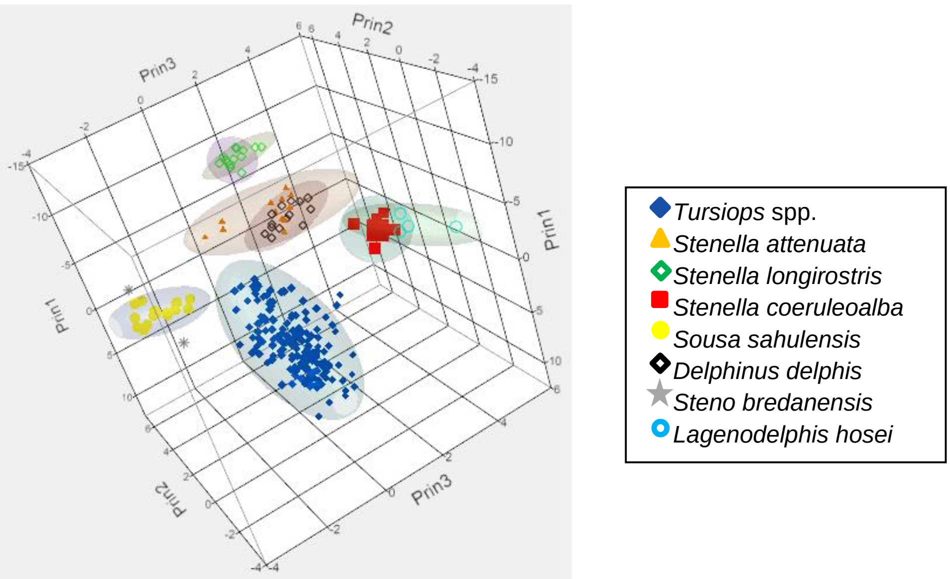

Figure 2. Plot of PC1, PC2 and PC3 for all species used in the study. A 0.95 confidence ellipses illustrated for each taxon.

The overlapping species showed similarities in rostrum width and length, but could be separated by the number of teeth. *Tursiops* spp. were significantly different to the other taxa in tooth count ($p<0.001$) and some categorical data (pterygoid shape, arch at premaxilla, and development of the nuchal crest, $p<0.001$).

Three-dimensional data

The results from the PCA performed on the 3GM data, showed very similar patterns to the 2D data. The data were separated into five main clusters where *Tursiops* (including type specimens) separated from all other genera, S. *longirostris* the only other species to separate from other species, *S. attenuata* overlapped with *D. delphis*, *S. sahulensis* clustered with *S. bredanensis*, and *L. hosei* clustered adjacent to *S. coeruleoalba*. The relative size of the cranium and rostrum were the main differences along the PC1 axes. Along the PC2 axes, there was a gradual skull shape from O to A. The skulls of *S. bredanensis* and *S. sahulensis* had a more round shaped cranium (O-shaped), the skulls of *S. coeruleoalba* and *L. hosei* were more square-shaped (A-shaped), while the *Tursiops* spp., *S. attenuata* and *S. longirostris* skulls had a shape between O and A (Figure 3).

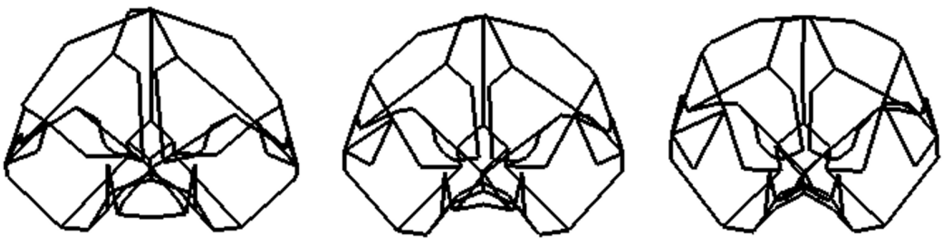

Figure 3. Posterior view of the skull shapes showing the gradual difference from A- to O-shape. The skulls on the left represent *S. coeruleoalba* and *L. hosei*, the one in the middle represents *Tursiops* spp., *S. attenuata* and *S. longirostris*, and the one to the right *S. bredanensis* and *S. sahulensis*.

Results

Defining patterns within Tursiops spp.

Two-dimensional cranial measurement, count and categorical data

Multivariate analyses (Principal component, hierarchical cluster, k-mean and discriminant function analyses) revealed two morphological groups (2D-1 and 2D-2). Consistent results between the four analyses were obtained for most specimens (89.5%). But the separation between the two groups was not complete (Figure 4), as about 11% of the individuals could not be assigned to either group, because they clustered in different groups depending on the analysis performed, and these were defined as intermediates. The intermediate specimens were not a hybridisation of *T. aduncus* and *T. truncatus* but had intermediate features between the two species (Table 2), and did not come from a specific geographical area. In the PCA (Figure 4), both width and length variables had a large influence on PC1 and PC2 to separate groups. The discriminant function analyses weighted mandible length, condylobasal length and rostrum length as important length variables, and least supraorbital width, greatest postorbital width of skull and greatest preorbital width of skull as important width variables for the separation of groups.

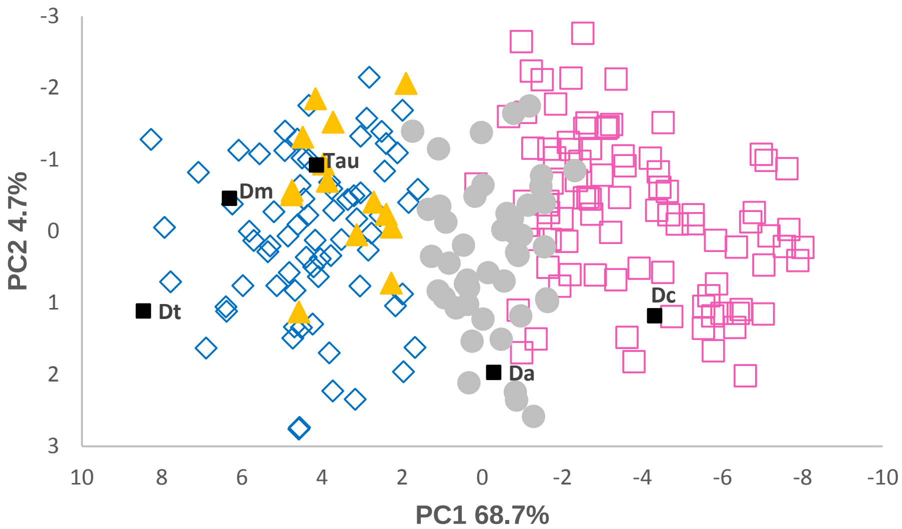

Figure 4. Plot of PC1 and PC2 for 2D data, hollow square=2D-1, hollow diamond=2D-2, solid grey circles=intermediate *Tursiops* spp., solid orange triangles= *T. australis*, Da=*D. aduncus* type (BZM 66400), Dt=*D. truncatus* type (NHMUK 353a), Tau=*T. australis* type (QVM 1365), Dm=*D. mageanus* type (QVM 1360) and Dc=*D. catalania* type (NHMUK 1862.6.6.13).

The 2D-1 group represented *T. aduncus* and contained the smaller specimens. The type skull of *D. aduncus* (from the Red Sea) clustered in the middle of the two groups. Compared to other osteological studies conducted in China (Wang *et al.* 2000) and South Africa (Ross 1977, Ross 1984), the Australian 2D-1 specimens were comparable in size range to other *T. aduncus* specimens. They were smaller in size compare to the other studies, had the lowest vertebrae count but had the widest rostrum width and most variation in number of teeth (Table 2). The 2D-2 group represented *T. truncates* and contained the larger specimens, including the types of *D. truncatus*, *T. australis* and the *T. australis* skulls (Figure 4). The Australian 2D-2 group were comparable in size (Table 2) to *T. truncatus* specimens found in China (Wang *et al.* 2000) and South Africa (Ross 1977, Ross 1984). They were similar in size to the *T. truncatus* in Chinese waters and slightly smaller compare to the ones in South Africa (Table 2). The Australian 2D-2 specimens had the widest rostrum, most variation in number of teeth and the lowest vertebrae count compare to the other countries (Table 2). None of the *T. australis* specimens included in the present study clustered amongst the intermediate skulls, but instead clustered well within the *T. truncatus* group. The skull measurements for *T. australis* also overlap with *T. truncatus* specimens from China and South Africa (Table 2).

In northern Australia (the Northern Territory), where the continental shelf is the widest, only *T. aduncus* specimens were found, and only *T. truncatus* specimens occurred in the south-eastern part of Australia (the states of Victoria and Tasmania), where the continental shelf is narrow and water is deep.

Table 2. Comparison of osteological variables (min-max) between Australian, China (Wang *et al.* 2000) and South Africa (Ross 1977, Ross 1984). CBL= condylobasal length, ML= mandible length, RL= rostrum length, ZW= zygomatic width of skull, MH= mandible height, RWM= rostrum width at midlength, total number of teeth and total number of vertebrae.

	T. aduncus			***T. truncatus***			***T. australis***	**Intermediate**
Variable	**China**	**South Africa**	**Australia**	**China**	**South Africa**	**Australia**		
CBL	451-529	433-507	381-504	394-561	504-578	469-561	470-513	466-506
ML	386-461	373-422	312-429	341-481	426-498	403-475	405-441	393-444
RL	258-317	250-297	212-290	204-320	283-335	260-315	266-295	256-290
ZW	209-251	198-251	176-244	189-290	257-313	223-292	209-243	214-246
MH	77-93	72-90	62-87	61-104	90-110	80-107	87-97	79-90
RWM	41-61	34-60	45-71	45-80	56-85	60-101	71-88	61-77
Total teeth	96-111	97-111	88-114	80-106	88-96	81-110	86-107	83-108
Vertebrae	64-67	59-62	53-61	64-67	64-65	58-66	N/A	58-65

The specimens in the 2D-1 group had more teeth in the lower left mandible but the teeth were smaller in diameter. No teeth differences could be found between the 2D-2 group and the *T. australis* specimens. T-tests (SPSS) performed on the categorical data showed that the 2D-1 group had slightly more damage to the frontals and pterygoids, had a lower occipital crest, more likely had the occipital crest as the highest point of the skull and never had the pterygoid and palatine of the same length compare to the 2D-2 group. The 2D-1 group also had fewer vertebrae. No significant difference could be found between *T. australis* and group 2D-2 for the ratio between the length of the pterygoids and paletine, the smooth transition between the maxilla and premaxilla region or any of the other count or categorical data.

Three-dimensional data

The PCA performed on the 3GM data also revealed two adjacent clusters (Figure 6). When plotting the 2D-1 and 2D-2 groups from the 2D measurement results in the 3D PCA plot, the 2D-1 cluster on one side together with the type skull of *D. catalania*, while the *D. aduncus* type cluster between the two groups, the 2D-2 group cluster on the other side with all *T. australis* skulls, and the type skulls of *D. truncatus, T. australis* and *D. mageanus*. The separation of the two groups is clearer compare to the 2D data, and the intermediate skulls are clustering well within the groups rather than between (Figure 6).

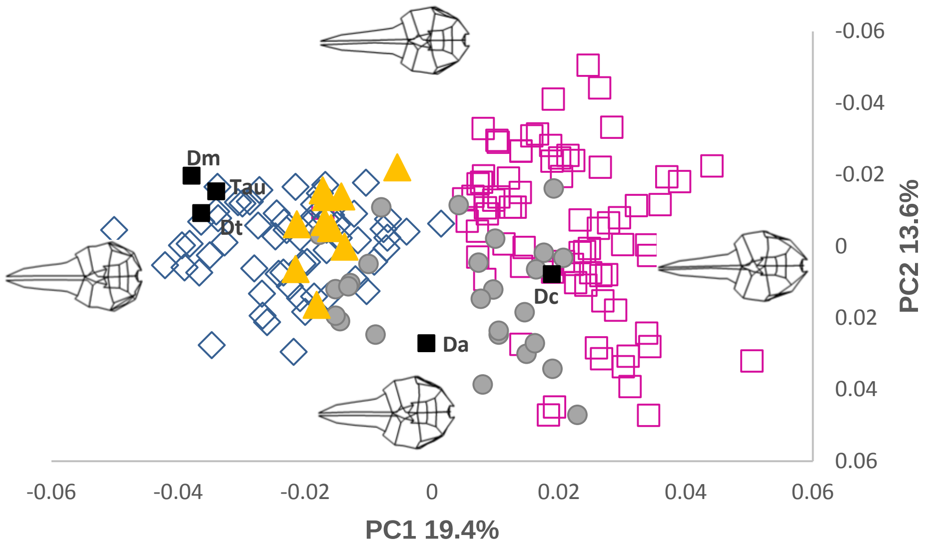

Figure 6. PC1 plotted against PC2 for the 3GM data including all Tursiops spp. The specimens labelled according to the 2D results, hollow squares=2D-1, hollow diamonds=2D-2, circles=Intermediate *Tursiops* spp., triangles=*T. australis*, Da=*D. aduncus* type (BZM 66400), Dt=*D. truncatus* type (NHMUK 353a), Tau=*T. australis* type (QVM 1365), Dm=*D. mageanus* type (QVM 1360) and Dc=*D. catalania* type (NHMUK 1862.6.6.13).

The specimens in the 3D-1 group had a more round O-shaped skull along the PC1 axis, while the skulls in the 3D-2 group had a more A-shaped skull (Figure 7). The differences along the PC2 axes were mainly in the width and length of the rostrum, where the 3D-1 group had a longer and narrow rostrum while 3D-2 had a shorter and broader rostrum.

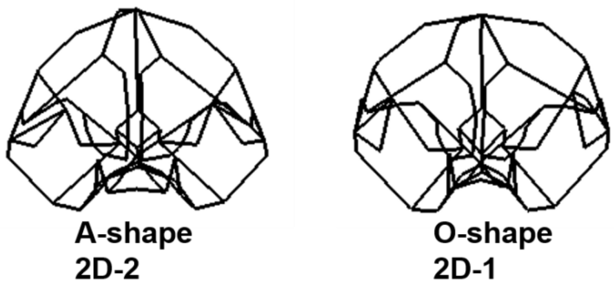

Figure 7. The 3D shape differences between (posterior views) 2D-1 and 2D-2 groups along the PC1 axes, showing the A- vs. O-shape.

Discussion

Summary and future research

The taxonomic distinction of the *Delphinus*-*Stenella*-*Tursiops* clade have proven difficult to disentangle genetically (LeDuc *et al.* 1999, McGowen 2011, Perrin 2013) (LeDuc *et al.* 1999, McGowen 2011, Perrin 2013). Morphologically, *Tursiops* has been distinguished from some species within *Delphinus* and *Stenella* (Amaral 2009), and they have been separable using diagnostic characters (Barnes 1990, Rommel 1990). But a comprehensive morphological comparison has not been conducted, and diagnostic characters have not been defined (Perrin 2013). The present study showed a clear morphological separation of *Tursiops* spp. and other genera and species, showing that morphological methods (2D and 3D) can be used effectively when distinguishing *Tursiops* spp. from other taxa within the subfamily Delphininae. Species with overlapping size were not possible to distinguish using multivariate analyses, but were distinguishable in combination with using the number of teeth. The sample size for some of the species (e.g. *L. hosei*, *S. bredanensis* and *S. coeruleoalba*) were very low, but no more specimens were available at Australian museums so an extended analysis for Australian specimens is therefore not possible.

Several morphological studies have been conducted on the genus *Tursiops*, and been able to distinguish them as separate species using size, in South Africa (Ross 1977, Ross 1984), Indian and western Pacific Ocean (Kurihara & Oda 2007), China (Wang *et al.* 2000), Japan (Kakuda *et al.* 2002) and Australia (Hale *et al.* 2000, Kemper 2004). In the present study, support was found for two bottlenose dolphin species in Australian waters, represented to *T. aduncus* and *T. truncatus*. The distinction was not absolute between the two species, showing some morphological overlap. But support for more than two species were not possible and no morphological distinction could be made between *T. truncatus* and *T. australis*. This study was the first attempt in Australian water to compare species within Delphininae on a large geographical scale. But a lot of work is still to be done to resolve the taxonomy of species within Delphinidae and Delphininae. A world-wide comparison of taxa within Delphinidae is therefore necessary.

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