



## Hip bone morphometrics of bottlenose dolphins (*Tursiops truncatus*) from Adriatic Sea: Sex determination and postnatal development

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### ABSTRACT

Bones are frequently the only cetacean samples available in the wild and may be useful for determination of sex, physical maturity, body length, and body mass. We analyzed the postnatal morphological development and morphometric characteristics of the cleaned and dried, paired hip bone (os coxae) from 131 bottlenose dolphins, *Tursiops truncatus*, (61 females, 70 males; total body length 117–322 cm) ranging in age from neonates to 28 yr, salvaged from Croatian waters of the eastern Adriatic Sea. Adult dolphins exhibited sexual dimorphism in their hip bone morphology and morphometrics: fully-formed hip bones from males were significantly longer, heavier, and more robust than those from females. Moreover, the fully formed hip bone from males featured a prominent bony tuberosity protruding dorsolaterally from the caudal half that was significantly wider and higher than in females. Morphometric measurements were used to generate classification functions for reliable sex determination (97%). In addition, hip bone length in both sexes correlated tightly with total body length, allowing one measurement to be calculated from the other. Only a single ossification developed in all hip bones although soft tissue attachments and comparative review suggests homological equivalence with the tripartite terrestrial mammal hip bone.

Key words: bottlenose dolphin, *Tursiops truncatus*, hip bone, os coxae, sexual dimorphism, Adriatic Sea.

Frequently cetaceans can be found in the field only as skeletal remains, so extracting as much useful information as possible from these specimens is critical for providing insights into their biology and ecology. Various studies have proposed methods for determining sex, physical maturity, body length, and body mass from cetacean bones (Hersh *et al.* 1990, Kemper and Leppard 1999, Murphy *et al.* 2006). Substantial sexual dimorphism in external appearance is well described in several cetaceans

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(Read *et al.* 1993, Tolley *et al.* 1995, Sanvicente-Añorve *et al.* 2004, Murphy and Rogan 2006) and in their skulls (Amano and Miyazaki 1992, Perrin *et al.* 2003, Turner and Worthy 2003, Frandsen and Galatius 2013), raising the question of whether other skeletal features may prove useful for sex determination. The hip bone is sexually dimorphic in at least three small cetacean species: the harbor porpoise (*Phocoena phocoena*) (van Bree 1973, Andersen *et al.* 1992, Galatius 2005), the finless porpoise (*Neophocaena phocaenoides*) (Yoshida *et al.* 1994, Tajima *et al.* 2004), and Commerson's dolphin (*Cephalorhynchus c. commersonii*) (Cáceres-Saez *et al.* 2014). It is reliable for sex determination in two: the finless porpoise (Yoshida *et al.* 1994) and the Commerson's dolphin (Cáceres-Saez *et al.* 2014).

The hip bone, anatomically known as os coxae (*Nomina Anatomica Veterinaria*, International Committee on Veterinary Gross Anatomical Nomenclature 2012), forms the bony girdle of the pelvic limb skeleton. In terrestrial mammals, this pelvic girdle, with the sacrum, forms the bony surround enclosing the organs of the pelvic cavity, and through the ilio-sacral joint transmits the locomotory propulsive force from the pelvic limbs to the vertebral column. The right and left hip bones meet mid-ventrally in a median joint, the pelvic symphysis, which is initially cartilage and usually ossifies within the lifetime of the mammal, resulting in ventral fusion of the left and right hip bones. Each hip bone is connected to the vertebral column at the sacrum *via* joints, ligaments and muscles (Evans and de Lahunta 2013). In marine mammals, however, the locomotor apparatus underwent significant adaptations to the aquatic environment, and with the prominent development of tail flukes for producing propulsion in manatees (Sirenia) and whales (Cetacea), pelvic limbs became vestigial with a much reduced girdle and the complete loss of all external elements. Even so, typical mammalian pelvic limb buds normally form during early embryogenesis, as documented in several whale species (Ogawa 1953, Thewissen *et al.* 2006). The external morphology of early-stage pelvic limb buds in spotted dolphin (*Stenella attenuata*) is comparable to that in other mammalian embryos. As development progresses, the pelvic limb buds gradually regress and are difficult to identify after prenatal day 48 (Sedmera *et al.* 1997). Hip bones occur in all modern whale species, whereas the more distal parts of the pelvic limb skeleton, such as vestigial femora and tibiae, are occasionally observed in odontocete and mysticete individuals (Ogawa and Kamyia 1957). Their major function in whales is to serve as attachment points for muscles supporting the genitalia, anus, and the abdominal body wall. Additionally, their reduction in size and changed position may facilitate dorso-ventral undulations of the peduncle (Tajima *et al.* 2004). In contrast to the extensive bony connection between the hip bones and vertebral column in terrestrial mammals, cetacean hip bones in extant species associate only loosely with the vertebral column by soft tissues.

The hip bones of cetaceans bear little resemblance to those of terrestrial mammals. The hip bone of a terrestrial mammal is a well-developed, complex of three bones—the ilium, the pubis, and the ischium, each of which develops from a separate ossification center within a single lateral cartilage plate (Dyce *et al.* 2010). A fourth bone, the acetabular bone, appears regularly in dogs (Evans and de Lahunta 2013). The hip bone anlage appears during embryologic life by condensation of the scleroblastema with a center formed by closely packed and very small mesenchymal cells being present for each of the three skeletal elements. Later, these centers each undergo chondrification, continue to grow and coalesce into a continuous plate of hyaline cartilage in which three ossification centers for each of the hip bone elements will appear (Ruth 1932). The cetacean hip bone, in contrast, is a small bone: we are unaware of specific

developmental data for extant species documenting separate presumptive bones (likewise noted by Gol'din, 2014) and/or establishing homology with the bony elements in terrestrial mammals. This is capably stated: "The use of the terms ischial, iliac, and pubic, applied to the different processes of the pelvic bone, diverging from the acetabulum, would be convenient . . . but as there is no evidence that the pelvic bone is developed from more than one centre of ossification, these terms are apt to mislead. The term innominate for like reason is objectionable." (Struthers 1881, p. 148).

Lack of developmental observations of the cetacean hip bone, and thus any possible homological investigations has contributed to nomenclatural discrepancies and confusion in the literature. Arvy (1976, 1979) strongly posited that each should be called "the abdominal bone." And phrases like "vestigial pelvic bone" (Tajima *et al.* 2004), "pelvic rudiments" (Perrin 1975), "pelvic vestiges" (Rommel 1990) persist, even though the small bones in question are homologous not to the pelvis of terrestrial mammals but to their hip bones. The bony pelvis refers to a substantial bony ring that comprises the left and right hip bones (*ossa coxarum*), the sacrum, and the first few caudal vertebrae (Getty 1975, Dyce *et al.* 2010, Evans and de Lahunta 2013). Therefore, based on standardized nomenclature we recommend "hip bone" or "os coxae" as the anatomically appropriate name for the pelvic girdle in cetaceans. Moreover, muscles of the abdominal wall, urogenital tract, and the tail (Meek 1918, Simões-Lopes and Gutstein 2004, Tajima *et al.* 2004, Cotten *et al.* 2008) attach to the hip bone of living cetaceans making it an important functional structure and not a vestigial remnant.

To help clarify the postnatal development of the cetacean hip bone and to examine whether its characteristics allow for reliable sex determination, we examined the hip bones of bottlenose dolphins, *Tursiops truncatus*, of various ages salvaged from a local, poorly defined small "subpopulation" of bottlenose dolphins in Croatian waters (Galov *et al.* 2011), which approximates the northern two-thirds of the eastern shore of the Adriatic Sea. Distribution, site fidelity (Natoli *et al.* 2005), and analysis of genetic markers (Galov *et al.* 2011) suggest this subpopulation is not isolated from the general pool of bottlenose dolphins in the Mediterranean Sea—and thus are not morphologically atypical of the taxon.

Our preliminary study showed remarkable sexual dimorphism in the bottlenose dolphin hip bone (Đuras *et al.*, unpublished data). As in other modern whales, the hip bones of bottlenose dolphins are all that exist of the pelvic limb skeleton. This pair of small bones is located just dorso-lateral to the anus and with the cranial tips in the same transverse plane with the first hemal arch. Their shape and size are not known in detail, in part because they are often lost during skeletal preparation. Nevertheless, Rommel (1990) reported that hip bones "may be larger" in male bottlenose dolphins than in females. Our morphometric findings of consistent sexual dimorphism (larger in males) and a strong relationship between total body and hip bone lengths should prove useful for extracting basic biological data from bottlenose dolphin carcasses and skeletons.

## MATERIAL AND METHODS

Cleaned and dried hip bones were collected from 131 bottlenose dolphins (61 females, 70 males; 1990–2011) that ranged in total body length (TBL) from 117 cm to 322 cm (TBL known for 121 dolphins, Fig. 1) and in age from neonates to 28 yr. Of these both right and left hip bones were available in 117, while only one member

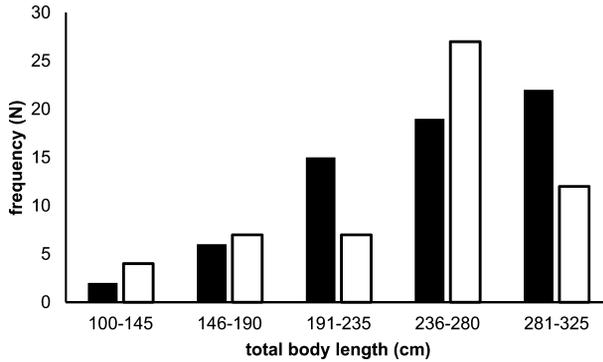


Figure 1. Total body length (TBL) distribution of 64 male (black bars) and 57 female (white bars) bottlenose dolphins salvaged from the eastern Adriatic Sea (1990–2011).

of the pair was available in 14 (10 left, 4 right); thus altogether 248 bottlenose dolphin hip bones were studied. Hip bones came from the collection at the Department of Anatomy, Histology, and Embryology of the Faculty of Veterinary Medicine at the University of Zagreb, which archives skeletons and postmortem reports of whales found dead stranded on the Croatian coast of the eastern Adriatic Sea.

Hip bones were carefully extracted from soft tissues following postmortem examinations, after which they were gently scraped with a dull knife and, if they were small (<5 cm in length), dried, or if larger, they were degreased by gently simmering in a detergent solution and then air-dried. In order to correctly determine the surfaces and topographical orientation of the hip bones, 31 hip bone pairs from different age classes were marked during the initial dissection. Immediately after the hip bone was removed from the carcass, a cotton filament was attached at the cranial end of the hip bone and fixed with a knot placed at the lateral surface of the hip bone. After degreasing and drying, the identified cranial and caudal ends and the lateral and medial surfaces were marked with a pen and the cotton filament removed. The correct topographical orientation of unmarked hip bones was determined according to the morphological characteristics of the marked hip bones of similar age class. Standard postmortem procedures were followed, including recording salvage date, sex, total body length (TBL), and other standard external measurements (Norris 1961). Estimated age was based on counts of dentinal growth layer groups (GLGs) in teeth sections (Hohn *et al.* 1989) prepared according to Slooten (1991), and assuming that one GLG in the dentine corresponded to one calendar year.

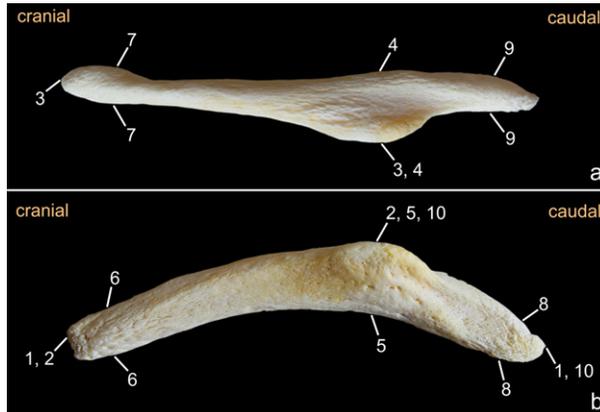
Hip bones were classified into one of three osseous developmental classes—neonate/yearling, juvenile, or fully formed—based on the estimated age of the individual dolphin combined with the morphological characteristics of the hip bone. Neonate and yearling hip bones originated from dolphins younger than one year of age and were composed of a slender bony central piece with elongate cranial and caudal cartilaginous tips. Juvenile hip bones came from dolphins more than one year old and still retained proportionately smaller cartilaginous cranial and caudal tips (preserved during preparation) or roughened end-surfaces for cartilage attachment. Fully formed hip bones came primarily from longer and older dolphins and were entirely bone, with a relatively smooth surface without any cartilage remaining on the tips.

Standard anatomical nomenclature and directional terminology was used based on *Nomina Anatomica Veterinaria* (International Committee on Veterinary Gross Anatomical Nomenclature 2012) to allow comparison between hip bones of the bottlenose dolphin and tetrapodal terrestrial mammals. Numeric data were analyzed using routine statistical methods in Microsoft Excel and the least squares method was used to fit the data to the Gompertz (user) equation in Statistica 12.1.<sup>2</sup> Correlations between hip bone measurements and total body length were examined.

The mass (in grams) of each hip bone was determined, and 10 straight-line/point-to-point measurements were made with a caliper (to a precision of 0.01 cm) (MEBA, Westerheim, Germany). Measurements of hip bone (Fig. 2):

- 1 Length, total.
- 2 Length from cranial tip to maximum height of tuberosity.
- 3 Length from cranial tip to middle of tuberosity.
- 4 Medial to lateral width at tuberosity (maximum).
- 5 Dorsoventral height at tuberosity (maximum).
- 6 Dorsoventral height at the cranial  $\frac{1}{8}$  of the length.
- 7 Medial to lateral width at the cranial  $\frac{1}{8}$  of the length.
- 8 Dorsoventral height at the caudal  $\frac{1}{8}$  of the length.
- 9 Medial to lateral width at the caudal  $\frac{1}{8}$  of the length.
- 10 Length from maximum height of tuberosity to caudal tip.

To analyze for sexual dimorphism the values for female and male hip bones were examined by ANCOVA with the total body length as covariate. In order to obtain normal distribution the original numeric data were transformed with the Box-Cox method (Box and Cox 1982). To develop classification functions for sex determination we used discriminant function analysis (Kachigan 1991). Prior to the discrimi-



*Figure 2.* Left fully formed hip bone of an adult male bottlenose dolphin (TBL 312 cm, age 16 yr) in (a) dorsal view and (b) lateral view. The point-to-point end points of the 10 specific measurements are represented by the same number and defined in text.

<sup>2</sup>Statistica (data analysis software system), Version 12. StatSoft, Inc. 2014. Available at <http://www.statsoft.com>.

nant function analysis normal distribution (Comogornov-Smiroff test) and homogeneity of variance (Levene's test) of the original data were verified. Normal distribution of data was determined at TBL > 200 cm. The data were standardized at mean = 0 and standard deviation = 1 because 10 measurements were expressed in centimeters and one (hip bone mass) in grams. The accuracy of the discriminant functions was tested using the posteriorly probabilities test. ANCOVA and discriminant function analysis were carried out in Statistica 12.1.<sup>3</sup>

## RESULTS

Hip bones changed remarkably in length (1.22–12.90 cm) and shape during post-natal development (Fig. 3) (TBL 117–322 cm). Significant differences between left and right hip bone measurements in any age class were not detected.

Neonates and yearlings ( $n = 10$ ; 6 females and 4 males; TBL 117–164 cm) featured a small hip bone somewhat cylindrical (1.22–3.52 cm), being slightly compressed mediolaterally, and bearing a cylindrical cartilage (0.4–0.9 cm) cranially and caudally. Only one center of ossification was present. The smallest hip bone (left: 1.22 cm, right: 1.5 cm) was in the shortest dolphin (female, TBL 117 cm), who was likely a late fetus or a newborn (see Fig. 4A) that died at or soon after birth. Cause of death could not be determined because the carcass was heavily decomposed.

Juvenile hip bones were salvaged from 49 bottlenose dolphins (19 females, 30 males; TBL 165–266 cm) aged 2–11 yr. Comparison with neonate hip bones revealed



Figure 3. Left hip bones of bottlenose dolphins at fully formed adult (male and female), juvenile and yearling stages salvaged from the eastern Adriatic Sea.

<sup>3</sup>See note 2 above.

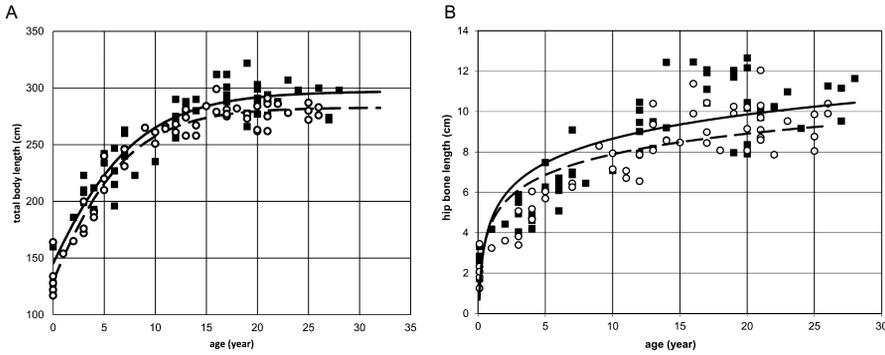


Figure 4. Growth curves of bottlenose dolphins from the eastern Adriatic Sea: (A) total body length vs. age (GLGs) for 55 females (white dots) and 57 males (black squares), and (B) hip bone length relative to age (GLGs) for 59 females (white dots) and 58 males (black squares).

that during development, ossification at the chondro-osseous interface progressed in cranial and caudal directions to elongate each hip bone. Cartilage was not present around the periphery of the body of the hip bone, thus its osseous growth in diameter was by subperiosteal ossification. The cylindrical hip bone of neonates transformed in the juveniles to a more dorsoventrally oval cross-section and longitudinally curved shape in lateral view (Fig. 3). In these the bony surface was smooth, the cranial portion was fully ossified, but the caudal tip was still cartilaginous. The length of the bony section was 3.32–8.7 cm.

Fully formed hip bones were recovered from 72 adult bottlenose dolphins (36 females, 36 males; TBL 249–322 cm) aged 7–28 yr. These hip bones (6.33–12.90 cm) were fully ossified, end-to-end, without any cartilage. They had a curved shape in lateral view with a convex dorsal edge and concave ventral edge (Fig. 3). A well-demarcated prominence developed on the dorsolateral aspect over the third longitudinal one-quarter of the hip bone. This tuberosity was significantly larger and more robust in males. The cranial half of the hip bone curved slightly medially and terminated as a mediolaterally flattened, slightly round end. The caudal one-quarter was cylindrical, more robust than the cranial half, and curved a little laterally (Fig. 3).

The growth of total body length of these bottlenose dolphins from the eastern Adriatic Sea slowed between 10 and 15 yr in both sexes (Fig. 4A). Their growth curves followed the Gompertz function expressed as:

$$X(t) = K \exp \left[ \log \left( \frac{X(0)}{K} \right) \exp(-\alpha t) \right]$$

where  $X(t)$  is a measure of total body length at a certain age,  $K$  asymptotic body length of adult bottlenose dolphins,  $X(0)$  total body length at birth,  $\alpha$  growth rate constant,  $t$  age (Table 1).

The total length of hip bones increased rapidly during the first years of life of these bottlenose dolphins (Fig. 4B) and afterwards continued to elongate slowly in both sexes.

Table 1. Growth parameters for Gompertz growth curves derived from our sample of female and male bottlenose dolphins from the eastern Adriatic Sea ( $K$  asymptotic body length of adult bottlenose dolphins,  $X(0)$  total body length at birth,  $\alpha$  growth rate constant,  $r$  correlation coefficient).

	Males ( $n = 57$ )	Females ( $n = 55$ )	All ( $n = 112$ )
$K$	297.1	282.8	289.3
$X(0)$	145.1	129.8	136.7
$\alpha$	0.184	0.211	0.201
$r$	0.94	0.97	0.96

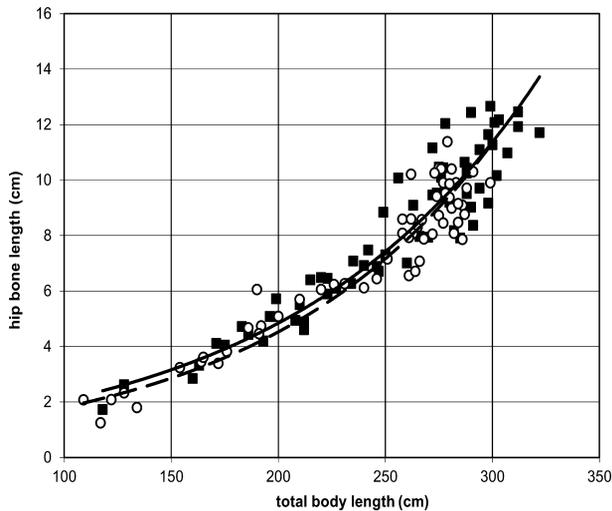


Figure 5. Correlation of hip bone length with total body length of 58 female (white dots) and 64 male (black squares) bottlenose dolphins from the eastern Adriatic Sea (see text for derived equations and correlation coefficients).

Hip bone length correlated well with total body length of both sexes ( $r^2 = 0.9218$  for females,  $r^2 = 0.9195$  for males; Fig. 5), allowing derivation of the following equations:

$$TBL_{\text{males}} = 125 \times [\ln(\text{hip bone length}) + 0.1301087]$$

$$TBL_{\text{females}} = 111.1 \times [\ln(\text{hip bone length}) + 0.3271161].$$

These equations demonstrated an exponential relationship between hip bone length and total body length, such that hip bone length increased faster in longer (older) bottlenose dolphins, and continued to elongate in the longest dolphins (TBL 322 cm, 28 yr) (Fig. 5). In males with a TBL  $\geq 257$  cm the hip bone was significantly longer (measurement #1) than in females of the same TBL (Table 2). In younger dolphins there was no significant difference in hip bone length between the sexes.

Table 2. Comparison of female (F) and male (M) hip bone values of bottlenose dolphins from the eastern Adriatic Sea by ANCOVA with the total body length (TBL) as covariate (g = differences between groups, s = differences between slopes, I = interaction, n.s. = not significant). Measurements represented by the number are defined in text and in Figure 2.

	Sexual dimorphism	P-values
Hip bone measurement (cm)		
m1	M > F if TBL ≥ 257 cm	s: $P < 0.000001$ ; i: $P < 0.01$
m2	M > F	g: $P < 0.05$ ; s: $P < 0.000001$
m3	M = F	g: n.s.; s: $P < 0.000001$
m4	M > F	g: $P < 0.01$ ; s: $P < 0.01$
m5	M > F if TBL ≥ 264 cm	s: $P < 0.000001$ ; i: $P < 0.02$
m6	F > M if TBL ≤ 181 cm; M > F if TBL ≥ 260 cm	s: $P < 0.000001$ ; i: $P < 0.001$
m7	M > F if TBL ≥ 234 cm	s: $P < 0.001$ ; i: $P < 0.05$
m8	M > F if TBL ≥ 207 cm	s: $P < 0.001$ ; i: $P < 0.05$
m9	M > F	g: $P < 0.000001$ ; s: $P < 0.000001$
m10	M = F	g: n.s.; s: $P < 0.000001$
Hip bone mass (g)	F > M if TBL ≤ 145 cm; M > F if TBL ≥ 206 cm	g: $P < 0.001$ ; s: $P < 0.000001$ ; i: $P < 0.001$

Sexual dimorphism was unmistakable in the development of the tuberosity of the hip bone, where the central section of the hip bone, including the tuberosity, broadened and protruded laterally to form the widest region of the hip bone (Fig. 6). Significant tuberosity width difference was observed in males of all body length classes, although tuberosity height (measurement #5) was significantly larger only in males with TBL ≥ 264 cm (Table 2).

In addition to sexual dimorphism in hip bone length and tuberosity width and height, male hip bones were significantly longer from the cranial tip to the maximum

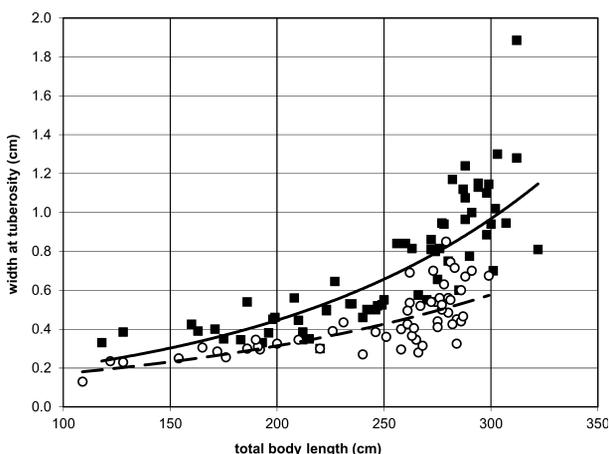


Figure 6. Correlation of width of hip bone at maximum protrusion of tuberosity (measurement #4) with total body length of 55 female (white dots) and 64 male (black squares) bottlenose dolphins from the eastern Adriatic Sea.

height of tuberosity (measurement #2) at all body length classes. Cranial tips were considerably higher (measurement #6) and wider (measurement #7) in males with  $TBL \geq 260$  cm and  $TBL \geq 234$  cm, respectively. Further, the sexual difference was prominent at caudal tips of the hip bones with males bearing wider caudal tips (measurement #9) than females at all body lengths and higher (measurement #8) caudal tips at  $TBL \geq 207$  cm. The hip bone mass of young dolphins ( $TBL \leq 145$  cm) was higher in females, whereas it was significantly higher in males at  $TBL \geq 206$  cm. The location of the tuberosity protruding dorsolaterally from the caudal half of the hip bone did not show sexual dimorphism (measurements #3 and #10) (length from cranial tip to middle of tuberosity and length from maximum height of tuberosity to caudal tip) did not differ significantly between the sexes (Table 2).

Finally, we developed classification functions for sex determination. We used data of 43 females and 51 males with a total body length  $>200$  cm. Four measurements that significantly contributed to the sexual discrimination of the bottlenose hip bones were hip bone mass (standard coefficient = 1.398), length from cranial to maximum height of tuberosity (m3) (standard coefficient = -1.371), maximal dorsoventral height of tuberosity (m5) (standard coefficient = -1.127) and hip bone length (m1) (standard coefficient = -1.002) (Table 3). Applying classification functions (Table 3) to hip bones originating from 94 bottlenose dolphins with a total body length  $>200$  cm led to correct sex determination of 91 of them (97%), whereas it failed in three males. One male (No. 195:  $TBL$  272 cm, 27 yr of age) had an exceptionally gentle hip bone when compared to males of the similar  $TBL$  and age. In this specimen the hip bone tuberosity protruded just slightly from the dorsal surface of the hip bone and the cranial and caudal tips were thinner and lower than in other adult males. The hip bones of the other two males (No. 124:  $TBL$  301 cm, 17 yr of age; No. 200:  $TBL$  298 cm, 28 yr of age) resembled typical male hip bones of bottlenose dolphins, but the width of their tuberosity was narrower when compared to males of similar  $TBL$  and age. In addition, the caudal tip of the hip bone of the bottlenose dolphin No. 200 was thinner than in other adult males.

## DISCUSSION

Our study has identified significant sexual dimorphism in the morphometrics and morphology of fully formed hip bones from 131 bottlenose dolphins salvaged from Croatian waters: those from males were significantly heavier, longer, and more robust. This now brings support to earlier general comments about the hip bone being larger in males of this species (Arvy 1979, Rommel 1990). Notably, a previously undescribed and prominent tuberosity protruded laterally from the convex dorsal edge over the third one-quarter of the hip bone. This tuberosity was significantly wider and higher in males than in females. Derived classification functions incorporating hip bone measurements presented herein proved reliable for sex determination (97%). Significant bilateral asymmetry was not observed for any of 10 hip bone measurements examined. For sex determination, we recommend nonetheless, choosing the side with the more robust hip bone, as Domning (1991) recommended for dugongs.

The cetacean hip bone (os coxae) serves as attachment site for several muscles of the abdominal wall and peduncle as well as muscles attaching to the base of the phallus, the terminal part of the reproductive tract and the anus—similar to homologous muscles in typical terrestrial mammals (Getty 1975). In terrestrial males, the crus

Table 3. Results of discriminant function analysis, discriminant and classification functions for sex determination from hip bone measurements of bottlenose dolphins (*Tursiops truncatus*). Measurements represented by the number are defined in text and in Figure 2.

Hip bone measurements	Wilks's lambda	Partial lambda	F-remove	P-value	Discriminant function		Classification function	
					Raw coefficient	Standard coefficient	Females	Males
m1	0.340	0.986	1.132	0.290	-0.998	-1.002	1.53528	-1.25314
m2	0.342	0.982	1.493	0.225	0.850	0.850	-1.29210	1.08310
m3	0.395	0.850	14.502	0.000	-1.363	-1.371	2.07669	-1.73030
m4	0.380	0.883	10.884	0.001	0.961	0.822	-1.43408	1.24962
m5	0.378	0.889	10.265	0.002	-1.139	-1.127	1.75499	-1.42712
m6	0.350	0.958	3.577	0.062	-0.466	-0.460	0.71021	-0.59081
m7	0.341	0.985	1.267	0.264	0.313	0.299	-0.47731	0.39796
m8	0.346	0.971	2.466	0.120	0.702	0.629	-1.04508	0.91678
m9	0.336	1.000	0.011	0.917	-0.045	-0.042	0.03996	-0.08616
m10	0.337	0.995	0.409	0.524	0.396	0.400	-0.59127	0.51602
Hip bone mass (g)	0.406	0.827	17.165	0.000	1.586	1.398	-2.49320	1.93712
Constant					0.019		-1.95922	-1.40397

penis of each side, surrounded by its companion large ischiocavernosus muscle takes origin from the caudally located ischial arch and the adjacent tuberosity on the caudolateral aspect of the ischium; while in females comparable structures exist but are smaller. Similar soft tissue relationships are reported in male cetaceans, *e.g.*, harbor porpoise (Meek 1918) and finless porpoise (Tajima *et al.* 2004). Preliminary dissections in adult male bottlenose dolphins reveal equivalent structures especially the crus penis firmly attached to the tuberosity and, surrounded by a prominent ischiocavernosus muscle broadly attached to the lateral and ventral surfaces of the hip bone (MÐ and AW, personal observation). A larger phallus and associated muscles in males would necessarily be supported by larger more robust hip bones—as determined in this study.

Relatively larger and more robust hip bones are also reported in male finless porpoises (Yoshida *et al.* 1994, Tajima *et al.* 2004), harbor porpoises (van Bree 1973, Galatius 2005), and in Commerson's dolphin (Cáceres-Saez *et al.* 2014). In contrast, individual sex could not be determined by the hip bone in the Antarctic minke whale (*Balaenoptera acutorostrata*) (Omura 1980). Care is required in interpretation of equivalence in reported “sexual dimorphism” of hip bones in cetaceans, for in some only length is recorded, in others, the shape, or weight maybe considered and not the length (see for example van Bree 1973).

We further propose that the tuberosity of the bottlenose dolphin hip bone is functionally and structurally homologous to the caudal part of the mammalian ischium and that the same factors that induce sexual maturity also induce final growth and differentiation of the hip bone tuberosity, particularly in males. An increase in length and weight of hip bones upon reaching sexual maturity was observed in the harbor porpoise (Andersen *et al.* 1992).

Our data suggest that analyzing the hip bone characteristics of bottlenose dolphins allows not only sex determination but also estimation of total body length. Even though the two lengths are not perfectly coupled, such that hip bones continue to elongate after body growth slows, the two measurements correlate strongly in adults in both sexes (Fig. 5). Whether this correlation also holds in other cetaceans is unclear. In right whales total body length correlates with other skeletal measurements: with greatest skull width, supraoccipital length, mandible length, scapula length, vertebra 7 centrum width, and baleen plate length in pygmy right whales (*Caperea marginata*) (Kemper and Leppard 1999); and with scapula length and width in bowhead whales (*Balaena mysticetus*) (Gerlach *et al.* 1993).

A much discussed question in cetacean evolutionary biology is whether their hip bones develop in a homologous manner to those of terrestrial mammals, in which separate ilium, pubis, and ischium ossify and then fuse together to form a unique bone (see especially Bejder and Hall 2002). Our analysis of hip bones from different developmental stages reveals that bottlenose dolphin neonates and yearlings have small hip bones composed of a cartilage model. A single ossification begins in the central portion of the cartilage plate and extends cranially and caudally. Similar soft tissue cranial and caudal portions occur in the hip bone of young finless porpoises (Tajima *et al.* 2004). Ossification was completed in the cranial part earlier than in the caudal part; as a result, juvenile hip bones had a cartilaginous caudal end for a relatively longer period of time, which transformed into bone in the adult. These cartilaginous tips provided the generative tissue and the ontogenetic process for elongation of the hip bone. We did not observe multiple ossification centers of the bottlenose dolphin hip bone. And as a concluding corollary “the existence of a skeletal element, however, does not depend on the presence of a separate ossific center”

(O’Rahilly and Gardner 1972), thus ontogenetic investigation in cetaceans may uncover the developmentally arrested separate chondrogenic or blastemal centers for the trimodal ossification of the hip bone as in terrestrial mammals. Radiographic data of the hip bone from the bottlenose dolphin with well-formed paired pelvic flippers living in the Taiji Aquarium/Whale Museum, Japan, (Ohsumi and Kato 2008) would be contributory. We conclude that the bottlenose dolphin hip bone developed from a single ossification, which we further suggest represents the functional ossification process within a single cartilage anlage, rather than being indicative of any particular phylogenetic antecedent(s).

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