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On the Resident "Bottlenose Dolphins" from Mikura Water.

By

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Introduction

Systematics is a product of various contemporary competing ideas. Ramping vs. splitting, morphology vs. molecular, and cladistic vs. conventional are some of the representatives of the opposing pairs. Here we discuss systematic position of the dolphin from one geographic area, based on both morphology and molecular phylogeny.

"Bottlenose dolphins" inhabit in the water around Mikura Jima (Mikura Island) are seen almost throughout the year. Mikura Jima is a small island in the Pacific Ocean, located about 200 km to the south south-east from Tokyo, where Kuroshio current often crosses Izu Shoto (Izu Islands) (Fig. 1). During the seasons when climate is milder and sea is calmer, dolphin watch and dolphin swim are popular among the visitors. These dolphins have been known as "bottlenose dolphins", with ventral spots in adults (Shirakihara *et al*., 2002; Kasuya *et al*., 1997). They are somewhat smaller than the offshore bottlenose dolphins, and with proportionately long and slender beak (Kasuya *et al*., 1997) which looks upturned. Tip of the beak is often white in older individuals. Bottlenose dolphins of similar appearances are also known from the seas around Amakusa Shoto, Amami Gunto, and

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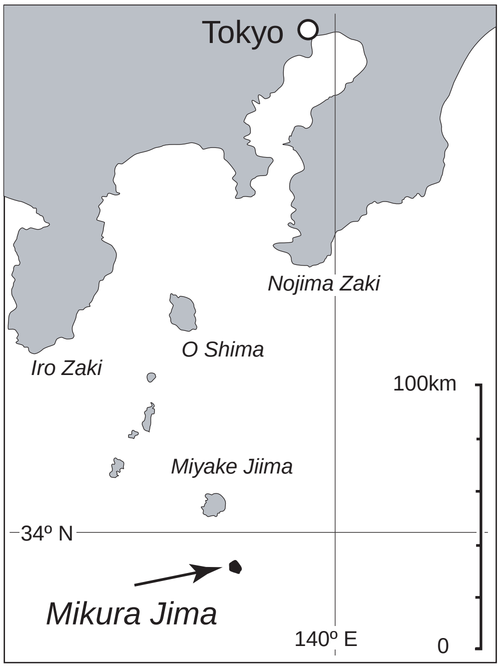

Fig. 1. Map showing the position of Mikura Jima.

Ogasawara Gunto (Bonin Islands) (Miyazaki & Nakayama, 1989; Uchida,1994; Kasuya *et al*., 1997; Shirakihara *et al*., 2002). Although *Tursiops truncatus* (Montagu, 1821) had been assigned as a single cosmopolitan species for the bottlenose dolphins, validity of *Tursiops aduncus* (Ehrenberg, 1833) has repeatedly been claimed for the spotted bottlenose dolphins of Indo-Pacific waters (van Bree, 1966; Pilleri & Gihr, 1972, 1973; Ross, 1977, 1984; Zhou & Qian, 1985; Hale *et al*., 2000). While the issue remains still controversial (Ross & Cockroft, 1990; Wang *et al*., 1999, 2000a), Rice (1998) listed *Tursiops aduncus* as the second species in the genus, and analyses based on mitochondrial DNA sequences resolved two species as independent (LeDuc & Curry, 1998; Wang *et al*., 1999). We analyzed mitochondrial DNA of two individuals from the Mikura water and discussed the systematic position of the spotted "bottlenose dolphins" of Mikura. Basic biological data available are also listed in the present paper for reference.

Materials and Methods

Materials used are comprised of two Mikura "bottlenose dolphins" (*Tursiops* sp.) and seven offshore bottlenose dolphins (*Tursiops truncatus*) (Table 1). The latter seven specimens were identified as *Tursiops truncatus* based on external morphology and osteological features. These samples were donated by Enoshima Marine Land, Shimoda Floating Aquarium and Marine World Uminonakamichi.

Six mitochondrial DNA sequences were obtained from GenBank as comparative sequences, namely; three for *aduncus*-type *Tursiops* (Accession Nos. AF049100, AF056239, and AF056240) and another three for *truncatus*-type *Tursiops* (Accession Nos. AF056223, AF056232, and AF056224),

Table 1. List of materials. Two "bottlenose dolphins" of Mikura Jima and seven offshore *truncatus*-type bottle nose dolphins were used for the present study.

Reg. No.	Found Date	Species	Sex	Body Length	Remarks	
M30133	May28 1996	*Tursiops* sp.	F	220cm	Stranding	Mikura Jima
M32733	Jul.20 2001	*Tursiops* sp.	M	216.5cm	Stranding	Mikura Jima
M30117	Nov.18 1995	*Tursiops truncatus*	M	316.8cm	Shimoda Aquarium	Futo, Shizuoka-ken
M32417	Jul.04 1998	*Tursiops truncatus*	F	277cm	Shimoda Aquarium	Captive born
M32418	Jun.30 1998	*Tursiops truncatus*	F	274cm	Marine World Uminonakamichi	Iki, Nagasaki
M32431	Jan.04 1999	*Tursiops truncatus*	F	268cm	Enoshima Marineland	Futo, Shizuoka-ken
M32469	Jun.07 1999	*Tursiops truncatus*	F	286cm	Shimoda Aquarium	Futo, Shizuoka-ken
M32530	Oct.20 1999	*Tursiops truncatus*	F	287.2cm	Shimoda Aquarium	Futo, Shizuoka-ken
M32763	Feb.02 2002	*Tursiops truncatus*	F	268.8cm	Enoshima Marineland	Futo, Shizuoka-ken

both from Chinese waters (Wang *et al*., 1999). For the out-group comparison, two sequences, one for *Delphinus delphis* and the other for *Delphinus capensis*, from Californian waters (Rosel *et al*. 1994) were also taken from GenBank (Accession Nos. U02656, and U02649).

Two of the Mikura dolphins (NSMT M30133 and M32733) were found dead by local people, M30133 on the 28th of May 1996 and M32733 on the 20th of July 2001, reported to Mikura Jima Iruka Kyokai (Mikura Jima Dolphin Association), and were frozen after several hours the carcasses were secured. These were necropsied for gross pathology and skin samples were taken for molecular phylogenetic analyses. M30133 was examined at the National Science Museum, Tokyo on the 25th of July 1996 and M32733 in Mikura Jima on the 30th of October 2001. Flensed skeletons were boiled for a few days at 80℃, washed, dried and stored as un-mounted specimens for osteology.

One each tooth was collected from both carcasses, after the skeleton was boiled, from the middle of lower jaw (tooth number 12 on the right) for age determination (Hohn & Fernandez, 1999). The teeth were cut using a diamond band saw EXAKT E300 longitudinally along paramedian plane and thin sections which are 30 to 50 μm thick were prepared. The sections were decalcified in 5% formic acid for 8 hours, rinsed in running water for 10 hours, stained by Mayer's hematoxylin for 3 hours and blued in running water for 1 hour following Kasuya (1976) and Hohn *et al*. (1989). The specimens were temporarily mounted in 50% glycerin for Growth Layer Group (GLG) count.

The external (Table 2) and the skull (Table 5) measurements were made nine individuals listed in Table 1., based on Norris (1961), Perrin (1975), Ross (1977), and Wang *et al*. (2000a, 2000b). Skull measurements that were stated as useful in discriminating *aduncus*-type from *truncatus*-type bottlenose dolphins by Wang *et al*. (2000a) were added to the measurement list. GWBS (greatest width of basisphenoid) of Wang *et al*. (2000a), however, was impossible with our specimens, where basisphenoid is not visible and the drawing of GWBS in Wang *et al.* (2000a, p. 150, Fig. 2) corresponds to the width of vomer at the posterior end. Minimum width of pterygo-palatine complex was added as Measurement No. 44. Osteological examinations were made on the macerated bone specimens.

In order to assess systematic position of Mikura "bottlenose dolphins" in the genus *Tursiops,* sequences of mitochondrial DNA control region of two Mikura dolphins and seven *Tursiops truncatus* were determined and compared.

Total DNA was extracted from the skin tissue sampled from the dolphins listed above, according

Table 2 A. List of external measurements used in Table 2B

1	Total length, from tip of upper jaw to deepest part of notch on flukes
2	Tip of upper jaw to center of eye
3	Tip of upper jaw to apex of melon
4	Tip of upper jaw to angle of gape
5	Tip of upper jaw to external auditory meatus
6	Center of eye to external auditory meatus
7	Center of eye to angle of gape
8	Center of eye to center of blowhole
9	Tip of upper jaw to blow whole
10	Tip of upper jaw to anterior insertion of flipper
11	Tip of upper jaw to anterior insertion of dosal fin
12	Tip of upper jaw to tip of dosal fin
13	Tip of upper jaw to midpoint of umbilicus
14	Tip of upper jaw to midpoint of genital aperture
15	Tip of upper jaw to center of anus
16	Projection of lower jaw beyond upper
17	Girth on a transverse plane intersecting axilla
18	Maximum girth
19	Girth on a transverse plane intersecting anus
20	Length of eye
21a	Length of mammary slits a) left
21b	Length of mammary slits b) right
22a	Length of genital slit
22b	Length of anal opening
22c	Length of anogenital slit
23	Width of blow whole
24	Length of flipper, anterior insertion to tip
25	Length of flipper, axilla to tip
26	Maximum width of flipper
27	Height of dosal fin
28	Length of dosal fin base
29	Width of flukes, tip to tip
30	Distance from nearest point on anterior border of flukes to notch
31	Depth of notch between flukes

to the standard procedure using phenol. Partial sequence of mitochondrial DNA control region was amplified by Polymerase Chain Reaction (PCR). The primer set, tt505f and tt505r were designed from the highly conserved portions of the control region of *Tursiops truncatus*. The amplification was carried out for 30 cycles as follows: 94℃ for 30 seconds, 54℃ for 30 seconds, and 72℃ for 1 minute. The direct sequencing reaction (sequence PCR) was carried out for 25 cycles: 96℃ for 10 seconds, 54℃ for 5 seconds, and 60℃ for 4 minutes, using dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems Inc.). The sequencing primers for forward and for reverse reaction were same as those used for amplification. PCR products were sequenced in ABI Prism 310 genetic analyzer (ABI). Resultant sequences were aligned by computer program package Clastal X (Thompson *et al*., 1997), and genetic relationship among Mikura dolphins and two types of *Tursiops* defined by Wang *et al*. was analyzed by the maximum likelihood estimation (Nei & Li, 1979). Phylogenetic analysis was

Table 2 B. Measurement values and %Md of seven bottlenose dolphins.

	Offshore bottlenose dolphins				Mikura dolphins			%Md
	M30117	M32469	M32530	Av	M30133	M32733	Av	
1	316.8	286	287.2	296.7	220	216.5	218.3	
2	36.3	35	33.4	34.9	34	33.5	33.8	0.27
3	8.8	11.3	11.4	10.5	12	14	13.0	0.50
4	32	30	27.5	29.8	32	27.5	29.8	0.30
5	45.9	43	47.9	45.6	38.2	39.5	38.9	0.14
6	-	6.7	12.2	-	4.9	6.8+	-	-
7	-	6.2	6	-	5.8	5.6+	-	-
8	-	20.9	24	-	19.6	17.8	18.7	-
9	31.5	35	28.4	31.6	31	33	32.0	0.31
10	62.4	58	56.2	58.9	54	51.3	52.7	0.19
11	-	127	120.4	-	101.5	94.5	98.0	-
12	182	-	-	-	-	122	-	-
13	142	162	131.2	145.1	105	105	105.0	-0.02
14	196.8	200	197	197.9	143	134.5	138.8	-0.05
15	224	207	205.5	212.2	152	153	152.5	-0.02
16	1	0.3	1	0.8	-	-	-	-
17	-	134	129.8	-	115.9	-	-	-
18	-	136.5	136	-	129	120.8	124.9	-
19	90.2	75.5	76.2	80.6	75	65	70.0	0.17
20	-	2.8	3.8	-	3.9	2.2	3.1	-
21a	-	-	-	-	-	-	-	-
21b	-	-	-	-	-	-	-	-
22a	-	-	-	-	-	-	-	-
22b	224	207	205.5	212.2	152	153	152.5	-0.02
22c	-	-	-	-	-	-	-	-
23	-	4.3	4.6	-	3	3	3.0	-
24	46.6	43	46.8	45.5	41.2	39.5	40.4	0.19
25	35	33	34	34.0	31.6	29.5	30.6	0.20
26	18.4	15.7	15	16.4	14.5	14.5	14.5	0.19
27	31	25.8	23.3	26.7	24.5	28	26.3	0.29
28	51	45	42	46.0	35	39	37.0	0.09
29	71	64.5	67	67.5	56.5	62.5	59.5	0.18
30	23	-	-	-	-	18	18.0	-
31	-	-	6.3	-	-	-	-	-

performed using Neighbor-joining (NJ) procedure (Saito & Nei, 1987) provided in computer program package MOLPHY Ver. 2.2 (Adachi & Hasegawa, 1996). Sequence data were bootstrapped 1000 times using MOLPHY.

Results

Results of the examinations on the two Mikura dolphins (M30133 and M32733)

External appearance and measurements. External appearance and measurements of two Mikura dolphins are shown in Fig. 2 and Table 2. Body color is basically light gray all over, with darker gray cape on the dorsal side and the ventral region is lighter gray. Darker portion starts from the upper jaw, and the ascending boundary passes almost at the level of the eye to the region dorsal to the flipper base, then shift descending to the area between the umbilicus and the genital area. Both these dolphins had no ventral spotting. They have several cookie cutter shark (*Isistius* sp.) bites and scars caused by teeth

Fig. 2. External appearance of the bottlenose dolphins of Mikura water. A. M30133, B. M32733

of probably similar-sized dolphins. The beak looks much longer in proportion, compared to that of the *truncatus* type bottlenose dolphin. Measurements that have large difference between Mikura and off shore bottlenose dolphins are Nos. 3 (tip of upper jaw to apex of melon), 4 (tip of upper jaw to angle of gape), 9 (tip of upper jaw to blow whole), 27 (height of dorsal fin), 2 (tip of upper jaw to center of eye), and 25 (length of flipper, axilla to tip), according to the magnitude of difference $\%Md=(Xo-Xm)\times 2/(Xo+Xm)>0.2$ (where Xo and Xm are mean value of percentage of measurements to standard body length in offshore bottlenose dolphins and Mikura dolphins). Second group of items with $Md>0.1$ are Nos.10 (tip of upper-jaw to anterior insertion of flipper), 24 (length of flipper, anterior insertion to tip), 26 (maximum width of flipper), 29 (width of flukes, tip to tip), 19 (girth on transverse plane intersecting axilla), and 5 (tip of upper jaw to ext. auditory meatus).

Necropsy. Both these two necropsied Mikura dolphins had no significant pathological findings other than severe pulmonary edema, which suggests that the death was caused by drowning. Stomach contents were fairly rich and fresh, indicating these individuals had been actively foraging just before they died (Table 3). Weight of the major visceral organs were tabulated in Table 4. The thymus looked active in both individuals and weighed 66 g in M30133 and 64 g in M32733. *Philobothlium* sp. larvae were found in blubber mainly in the ano-genital region. In M32733 numerous nematodes possibly *Crassicauda* sp. were found from the pterygoid sinuses and the middle ear cavity, as well as in the spaces between the periosteum and skull in the circum-orbital regions.

Age determination. The pulp cavities of both specimens were open and 7.5 and 4.5 GLGs were counted in the dentine layers of M30133 and M32733 teeth, respectively (Fig. 3). In M30133, strongly contrasted layers were observed in the 7th. GLG.

Table 3 List of stomach contents obtained from two Mikura dolphins.

	Species	n	Identified by
M30133	*Cypselurus agoo* (Temminck ＆ Schlegel, 1846)	7	Shinohara, Gento
M32733	*Albunea symnista* (Linnaeus, 1758)	several	Takeda, Masatsune
	Parexocoetus mento (Valenciennes, 1847)	2 heads, 2 postcranials	Shinohara, Gento
	Todarodes pacificus Steenstrup, 1880	2	Kubodera, Tsunemi
	Enoploteuthis chunii (Ishikawa, 1914)	2	Kubodera, Tsunemi
	Onychoteuthis borealijaponica Okada, 1972	1	Kubodera, Tsunemi
	Cranchiidae sp. or *Galiteuthis pacifica* (Robson, 1948)？	3	Kubodera, Tsunemi

Table 4 List of major visceral organ weight.

Organs	M30133	M32733	Organs	M30133	M32733
Liver	2145.0	2500.0	Epidydymidis R	-	14.0
Esophagus	339.0	400.0	Lung R	-	1400.0
Stomach	1405.0	1900.0	Lung L	-	1100.0
Fore stomach	885.0	800.0	Thyloid gland	-	24.0
Main stomach	416.0	300.0	Adrenal gland R	7.9	5.0
Pyloric stomach	106.0	21.0	Adrenal gland L	6.0	5.0
Intestine	3987.0	4200.0	Spleen	65.0	130.0
Pancreas	294.0	190.0	Thymus	66.0	64.0
Urinary bladder	-	82.0	Mesenteric lymph	182.0	-
Gonad R	-	19.0	Pulmonary lymph	18.4	-
Epidydymidis R	-	6.0	Superficial cervical lymph	-	24.0
Gonad L	-	20.0	Mammaly gland	55.0	

Genetic analysis. About 400 base pairs (bps) of the control region of the mitochondrial DNA were sequenced. The Sequences of two Mikura dolphins were the same in length; insertions or deletions were not observed, and these sequences were all identical (Fig. 4). Comparison of the sequences of Mikura dolphins with those of *aduncus*-type dolphins of Chinese waters, 1 to 8 substitutions were found. All substitutions were transition, and no insertions nor deletions were observed. On the other hand, within the consensus of Mikura dolphin's and *truncatus*-type's sequence, 22 to 25 substitutions that included 19 to 22 transitions and two transversions, one deletion were found. Fourteen of these substitutions were phylogenetically informative. Within the sequences of *truncatus*-type bottlenose dolphins of both the Japanese and Chinese Waters, 1 to 11 substitutions (all transition) and 7 to 16 substitutions (all transition) were found, respectively.

Molecular Phylogeny. The division of the two types of *Tursiops* was supported by neighbor-joining analyses of control region sequence data (Fig. 5). The bootstrap value at the branching point of two types is high (93%), and two clusters were clearly distinct. Seven *truncatus*-type specimens of Japanese waters were all included in the same cluster as those from the *truncatus*-type dolphins of Chinese water. On the contrary, two Mikura specimens were both clustered with the *aduncus*-type of Chinese water.

Skull morphology and measurements. Skull photographs are shown in Fig. 6. Miyake dolphin skulls are much more slender in appearance than the offshore bottlenose dolphins. Five cranial characters

A-1 **A-2**

B-1 **B-2**

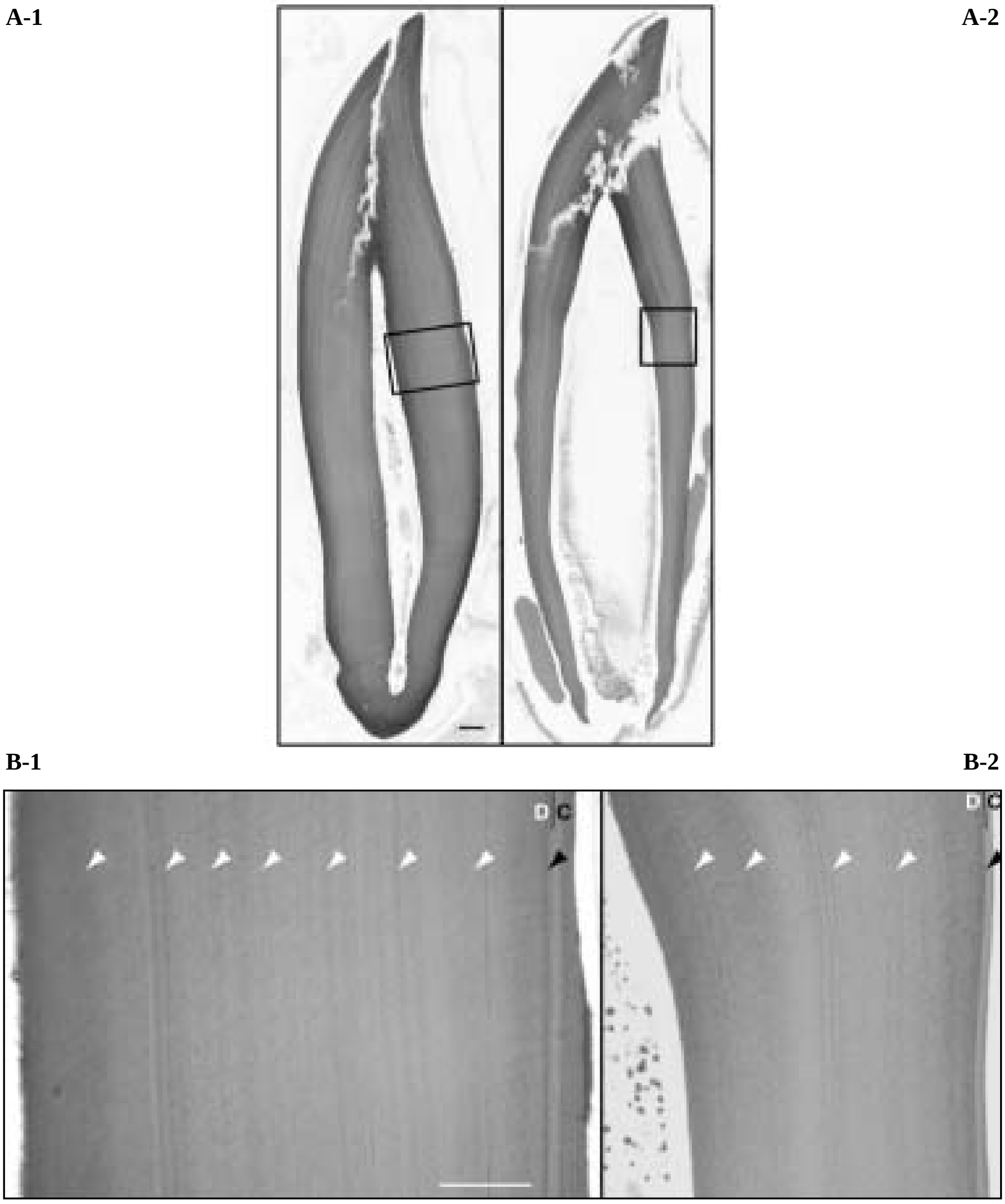

Fig. 3. Decalcified and stained thin sections of teeth. (A) Low power view of the sections; M30133 on the left (1) and M32733 on the right (2). Scale bar: 1 mm. (B) High power view of the sections; in dentin region (D), seven GLGs (white arrow heads) are seen in M30133 on the left (1) and four GLGs in M32733 on the right (2). Scale bar: 50 μm. Black arrowhead next to cementum (C) indicates neonatal line.

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                                 1  1  1  1  1  1  1  2  2  2  2  2  2  2  3  3  3  3  3  3
                                    1  2  7  7  8  9  9 60 63 63 65 68 69 79 80 93 93 05 15 45
                              6  8  9  7  8  3  5  6  0  4  5  3  8  0  8  8  0  5  0  0  2
                              *        *                 *  *  *           *  *        *  *
M30133  (MIKURA)     A  T  T  C  A  C  T  A  A  T  A  C  C TC TT CT TC CG CG T- AT CA T  G  A  T
M32733  (MIKURA)     .  .  .  .  .  .  .  .  .  .  .  .  . .. .. .. .. .. .. .- .. .. .  .  .  .
Taiwan_A1                  .  .  .  .  .  .  .  .  G  .  .  T  T  .  C  .  .  .  G  T  .  .  .  .
Taiwan_A7                  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  -  .  .  .  .
Taiwan_A8                  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  C  T  .  .  -  .  .  .  .
Taiwan_T5                  G  C  C  T  .  T  C  G  G  .  G  C  C  T  A  C  T  A  C  A  C  .  .  C
Taiwan_T6                  G  .  C  T  .  T  .  G  G  .  .  T  .  T  A  C  T  .  T  A  C  .  G  C
Taiwan_T7                  G  C  .  T  .  T  C  G  G  .  .  C  C  T  A  C  T  .  C  A  C  .  .  C
M30117                        G  .  C  T  .  .  .  G  G  .  C  C  T  C  A  C  .  G  C  A  C  G  C
M32417                        G  .  C  T  .  .  .  G  G  .  C  C  T  C  A  C  .  G  C  A  C  G  C
M32418                        G  .  C  T  .  T  .  G  G  C  .  T  T  .  A  C  C  G  T  A  C  .  C
M32431                        G  C  .  T  .  T  C  G  G  .  C  C  T  C  A  C  .  .  C  A  C  .  C
M32469                        G  C  C  T  G  T  C  G  G  .  .  C  T  C  A  C  T  A  C  A  C  .  C
M32530                        G  C  C  T  .  T  C  G  G  .  G  T  T  C  A  C  T  .  C  A  C  .  C
M32763                        G  C  .  T  .  T  C  G  G  .  C  C  T  C  A  C  .  G  C  A  C  .  C
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Fig. 4 Partial sequences of control region in mitochondrial DNA of Mikura dolphins and two types of *Tursiops* (*aduncus* and *truncatus*). Designation with “M” plus seven digits indicates the sequences of the specimens that stored in National Science Museum, Tokyo. “MIKURA” indicate the sequence of Mikura dolphins. Sample number starting with “TAIWAN” is those from Chinese waters. “A” plus number indicate reference sequences of *aduncus*-type and and “T” plus number *truncatus*-type in Chinese waters, respectively. Identity with top reference sequence denoted by dots, dash and asterisks indicate that deletion and fixed different site between two types, respectively. Site Numbers correspond to the positions in the control region sequence of the *truncatus*-type that determined in this study.

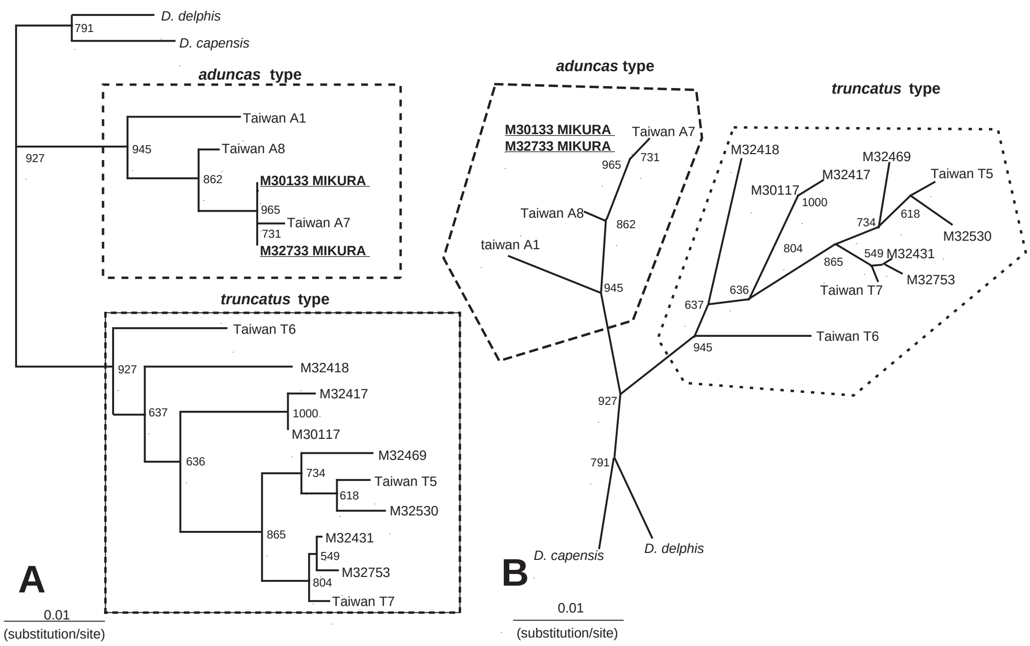

Fig. 5 Phylogenetic relationships among Mikura dolphins and two types of *Tursiops* (*aduncus* type and *truncatus* type) constructed by NJ method in Phylogram shape (A) and Radial shape (B). These genetic trees were produced from the same data. “M30133 MIKURA” and “M32733 MIKURA” indicate samples of Mikura dolphins. *D. delphis* and *D. capensis* are out group. See other sample explanation at Fig. A. Branch length indicates genetic distance from each sample. Bootstrap values are shown at the node.

listed by Ross (1977) that can be useful in identifying *Tursiops truncatus* and *Tursiops aduncus* were observed: 1) Posterior end of vomer in ventral view is wide and somewhat flared in Mikura dolphins, almost similar to those of the offshore bottlenose dolphins; 2) Pterygoid notch in lateral view looks similar in two groups; 3) Squamosal visible in lateral view at the lower wall of the temporal fossa is smaller in Mikura dolphins but the apex is directed dorso-caudally in M32733 and dorsally in M30133; 4) Premaxillary convexity is prominent in Mikura dolphins; 5) Lateral margin of premaxilla has no cancellous bone in Mikura dolphins. Other than the above characters, the followings are perceivable differences between Mikura dolphins and offshore bottlenose dolphins: a) In ventral view at the base of rostrum, palatine-pterygoid complex is pinched much more strongly at the anterior portion of the pterygoid in Mikura dolphins; b) At the vertex, nasals and postero-medial portion of both frontals form a complex which is much more clearly demarcated and elevated in offshore bottlenose dolphins; c) Supraoccipital has bilateral bulges on the dorso-caudal aspect in Mikura dolphins; d) Maxilla has an antero-mediad ridge (linea terminalis rostrae; terminal line of beak) from antorbital notch obliquely to almost middle of the beak. This ridge is longer and less prominent in Mikura dolphins whereas shorter and robust in offshore bottlenose dolphins; e) The lateral contour lines of maxillae are more straight in Mikura dolphins and outwardly curved in offshore bottlenose dolphins; f) Muscular ridge along the suture between frontals and supraoccipital is more prominent in offshore bottlenose dolphiins. The basihyoid and thylohyoid were fused on the right side in M30133 and not fused at all in M32733.

Skull measurements. Skull measurements are listed in Table 5. According to the %Md value (calculated as percentage to condylobasal length), Nos. 24 (distance from foremost end of junction between nasals to hind most point of margin of supraoccipital crest), 32 (length of upper left tooth row-from hindmost margin of hindmost alveolus to tip of rostrum), 26 (length of antorbital process of left lacrimal), 44 (minimum width of pterygo palatine complex), 4 (width of rostrum at 60 mm anterior to line across hindmost limits of antorbital notches), 5 (width of rosrum at midlength), 28 (greatest length of left pterygoid), 41 (JW/GWBS), 42 (WAS/JW), 7 (width of rostrum at 3/4 length, measured from posterior end), 43 (JW/TPC), 6 (width of premaxllaries at midlength of rostrum), 31 (greatest length of periotic of left tympanoperiotic), 27 (greatest width of internal nares), and 30 (greatest length of bulla of left tympanoperiotic) show marked differences between Mikura and off shore bottlenose dolphins.

Postcranial osteology. Vertebral counts are listed in Table 6. They have 12 pairs of ribs and no floating ribs were found. As is indicated in Table 6., epiphyses of vertebrae were not fused in Th1 to Ca12, and in Ca13 to Ca16 only fused on the caudal ends in M30133, and not fused in C7 to Ca16, only the cranial end of C6 was fused in M32733.

Discussion

Spotted "bottlenose dolphins" in Mikura water. As is seen in Fig. 7., "bottlenose dolphins" of Mikura water are known to be spotted ventrally in adult (or older) individuals. Two carcasses of the dolphins investigated in the present study (M30133 and M32733) were found from the bottom, along the Mikura Jima coasts, and had external body characters that are very similar to the resident Mikura bottlenose dolphins such as the long and slender beak, which is curled up. M30133 had been identified by the Mikura Jima Bandoiruka Kenkyukai (Mikura Island Bottlenose Dolphin Research Group) and nicknamed as "Kushidango". "Kushidango" had been continuously sighted among the pods of typical Mikura bottlenose dolphins and recognized as "sub-adult' for two years before the animal died. Considering these conditions, we inferred that these two dolphins were members of the resident Mikura

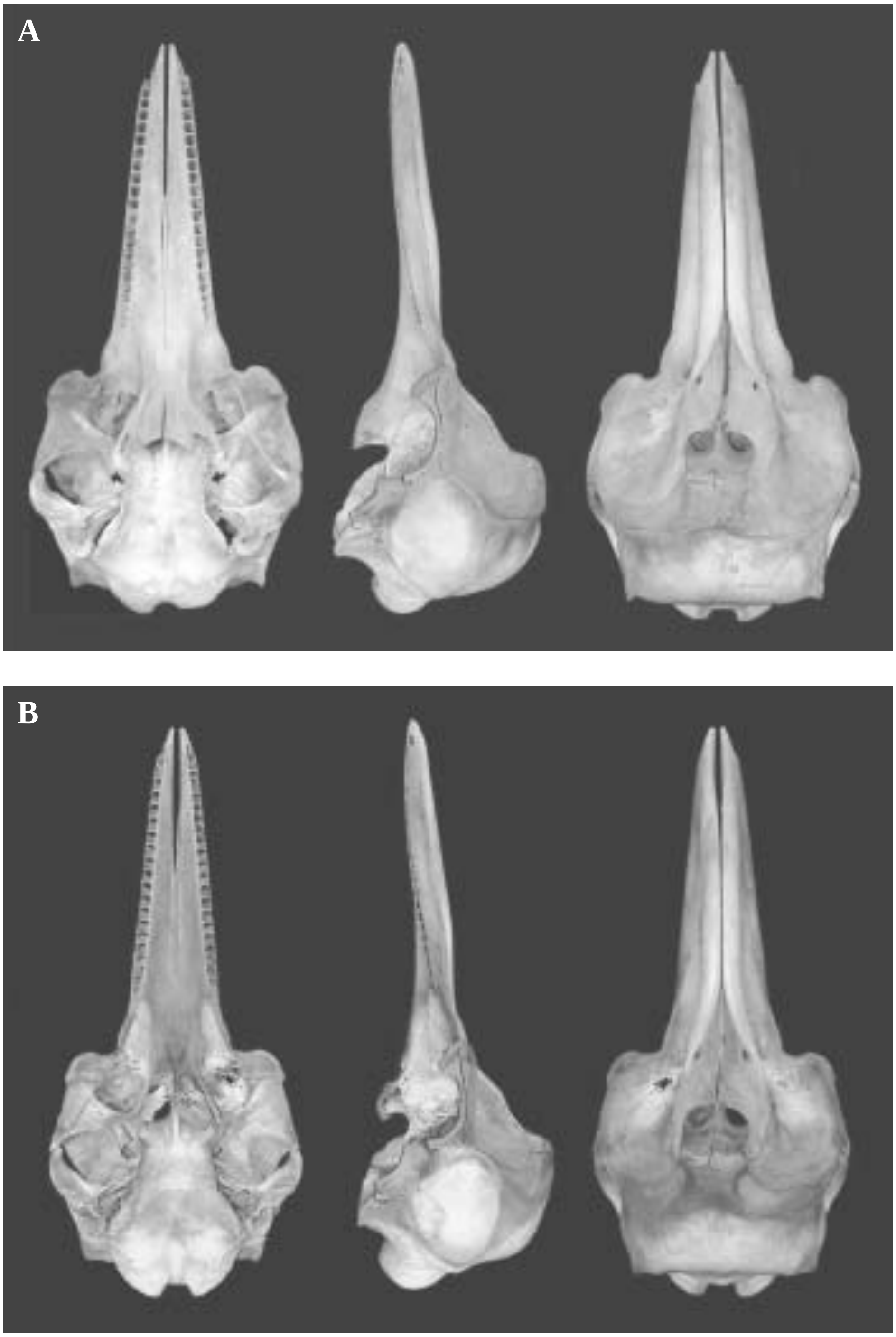

Fig. 6 Skull photographs of Mikura dolphins. A, M30166; B, M32733

Table 5A. List of cranial measurements and meristics used in Table 5B. Nos. 41-43 were taken from Wang *et al*., 2000a, No 44. was introduced in the present study.

1	Condylobasal length
2	Length of rostrum
3	Width of rostrum at base
4	Width of rostrum at 60 mm anterior to line across hindmost limits of antorbital notches
5	Width of rostrum at midlength
6	Width of premaxllaries at midlength rostrum
7	Width of rostrum at 3/4 length, measured from posterior end
8	Distance from tip of rostrum to external nares
9	Distance from tip of rostrum to internal nares
10	Greatest preorbital width
11	Greatest postorbital width
12	Least supraorbital width
13	Greatest width of external nares
14	Greatest width across zygomatic processes of squamosal
15	Greatest width of premaxillaries
16	Greatest parietal width, within posttemporal fossae
17	Vertical external height of braincase from midline of basisphenoid to summit of supraoccipital, but not including supraoccipital crest
19	Greatest length of left posttemporal fossa, measured to external margin of raised suture.
20	Greatest width of left posttemporal fossa, at right angles to greatest length
21	Major diameter of left temporal fossa proper
22	Minor diameter of left temporal fossa proper
23	Projection of premaxillaries beyond maxillaries measured from tip of rostrum to line across foremost tips of maxillaries visible in dorsal view
24	Distance from foremost end of junction between nasals to hind most point of margin of supraoccipital crest
25	Length of left orbit-from apex of preorbital process of frontal to apex of post-orbital process
26	Length of antorbital process of left lacrimal
27	Greatest width of internal nares
28	Greatest length of left pterygoid
30	Greatest length of bulla of left tympanoperiotic
31	Greatest length of periotic of left tympanoperiotic
32	Length of upper left tooth row-from hindmost margin of hindmost alveolus to tip of rostrum
33	Number of teeth- upper left
34	Number of teeth- upper right
35	Number of teeth- lower left
36	Number of teeth- lower right
37	Length of lower left tooth row- form hindmost margin of hindmost alveolus to top of mandible
38	Greatest length of left ramus
39	Greatest height of left ramus at right angles to greatest length
40	Length of left madibular fossa, measured to mesial rim of internal surface of condyle
41	GWBS/JW
42	WAS/JW
43	TPC/JW
44	Minimum width of pterygo-palatine complex

Table 5B. Measurement values and %Md of seven bottlenose dolphins.

	Offshore bottlenose dolphins									Mikura dolphins				%Md
	M30117	M32417	M32418	M32431	M32469	M32763	M32530	Av	Xo	M30133	M32733	Av	Xm	
1	556	512	516	506	562	536	530	546		501	481	491		
2	306	276	291	285	319	301.5	293	304.875	55.8326	294	286.5	290.25	59.123	0.06
3	143.7	143.4	135	138.5	153.4	151.5	142	147.65	27.0495	136.5	118.8	127.65	25.972	-0.04
4	116.8	106	100.7	98	104.5	108.6	110.7	110.15	20.1874	87.2	77.4	82.3	16.7483	-0.19
5	92.7	86	84.3	74.5	79.5	88.4	90	87.65	16.0731	69.3	61	65.15	13.2571	-0.19
6	44.7	49.3	52	43	43	44	54	46.425	8.52211	41	33.5	37.25	7.57414	-0.12
7	67.4	59	61.5	59	55	70.4	62.5	63.825	11.7089	53.5	45.5	49.5	10.0691	-0.15
8	367.5	333.5	344	338	376	356	341	360.125	65.9396	338.5	329	333.75	67.982	0.03
9	375	350.5	355	345.5	375	369.5	356	368.875	67.5696	347	331	339	69.0382	0.02
10	265	243	231.5	226.5	246	251	238.5	250.125	45.8156	215	195	205	41.7274	-0.09
11	287	273.5	254	252	261	271	266	271.25	49.7021	240	219.5	229.75	46.7691	-0.06
12	260	239	228	224	241	245.5	238.5	246.25	45.1118	214	190.5	202.25	41.1598	-0.09
13	58.6	56.5	59.8	55.9	56.4	59.1	61	58.775	10.7777	58	55.2	56.6	11.5265	0.07
14	289.5	273.5	256	257	270	270	271	275.125	50.4041	246	219	232.5	47.316	-0.06
15	99.3	98	97.4	87.5	101.6	94.3	99.5	98.675	18.0762	89.3	82.2	85.75	17.4569	-0.03
16	219	202.5	200	212.7	214	187	224.5	211.125	38.6783	184.5	170	177.25	36.0847	-0.07
17	223.5	203.5	205	199	208.5	211	203	211.5	38.7413	184	169.6	176.8	35.9932	-0.07
19	133	114.9	118	108	112	109	108.3	115.575	21.1549	107	107	107	21.8013	0.03
20	87.3	78.3	74.6	72	80.6	76	74	79.475	14.5461	80	76	78	15.8842	0.09
21	65	63	55.5	54	63	59	56	-	-	64	61	62.5	12.7282	-
22	60	60	54.5	49	48	53	50	-	-	43	41	42	8.55337	-
23	16.4	15	17.5	10	9.3	12.7	-	-	-	29.9+	29	-	-	-
24	37	25.3	31	35.4	28.6	20	29.5	28.775	5.26026	43	31	37	7.51387	0.35
25	72.5	78.9	70	71	73	73	71	72.375	13.2611	68.2	61.4	64.8	13.1889	-0.01
26	59.5	73.1	58.5	53	53.5	60	61	58.5	10.7311	44.3	38.5	41.4	8.42324	-0.24
27	91	83	73	74.5	84.3	78	81.6	83.725	15.3288	68.9	65.4	67.15	13.6746	-0.11
28	85.5	82	79.4	74.2	85.5	88	70	82.25	15.0542	63.9	58.2	61.05	12.4271	-0.19
30	35	34	33.3	35.5	36	39.1	33.2	35.825	6.5649	36.5	35.5	36	7.33294	0.11
31	42	40.8	37.5	33	38.3	38.5	37.4	39.05	7.15209	31.5	30.8	31.15	6.34538	-0.12
32	245	228	242	245	273	249	-	191.75	34.7741	250	231	240.5	48.9626	0.34
33	19	21	25	23	-	25	-	-	-	25+a	25	-	-	-
34	19	21	24	21	-	24	-	-	-	25+a	23	-	-	-
35	23	23	21	20	-	25	-	-	-	25	23	24	4.88586	-
36	22	23	22	22	-	24	-	-	-	21	24	22.5	4.59061	-
37	248	251.5	243.2	242.7	267.5	254	238	251.875	46.124	248	227	237.5	48.3472	0.05
38	472	453.5	443	445	479	469	455	468.75	85.8681	428	411	419.5	85.4381	-0.01
39	108	102	82	93	94	97.2	93	98.05	17.958	82	82.3	82.15	16.7387	-0.07
40	157.8	148.4	134	146.6	155.8	157	159.5	157.525	28.8723	135	143.4	139.2	28.3795	-0.02
41	35	28.9	38.8	29.3	41.3	42	53.5	42.95	7.89347	32.4	32.4	32.4	6.60152	-0.18
42	93	89.7	83	82	89.8	84.3	95	90.525	16.5894	70	66.6	68.3	13.9091	-0.18
43	180	158	173	158	188	173	174	178.75	32.7331	185.1	183.5	184.3	37.5479	0.14
44	61	52	53	54	56.5	55.4	53	56.475	10.3401	40.7	36.3	38.5	7.83527	-0.28

dolphin population and not foreign passers by.

Dentinal GLG counts of these dolphins were 7.5 (M30133) and 4.5 (M32733). Various findings indicate that they were young, *i.e.*, smaller body length, un-fused epiphyses in most of the thoracic and lumbar vertebrae, and fairly large thymus. These data account for the absence of ventral spots in these dolphins, that are usually found in adult Mikura bottlenose dolphins.

The facts that these two dolphins died young without significant pathological change and they have indication of some orexia (results of the two of the necropsies indicate that they were foraging until a few hours before they died) suggest that they died accidentally.

Female *Tursiops truncatus* matures at about 12 years of age with body length around 235 cm (Sergeant *et al*., 1972), and *Tursiops aduncus* 3.5 to 11 GLGs in dentine with body length between

Table 6 Vertebral counts and condition of epiphyses in Mikura dolphins.

	M30133		M32733	
C	7		7	
Th	12		12	
Th-L	0		0	
L	14		15	
Ca	25+1		26+1	
Total	33		34	
epiphyses	C1-C7	all fused	C1-C5	all fused
	Th1-Ca12	all open	C6	fused anteriory, open posteriory
	Ca13-Ca16	open anteriory, fused posteiory	C7-Ca16	all open
	ca17-	all fused	Ca17-	all fused

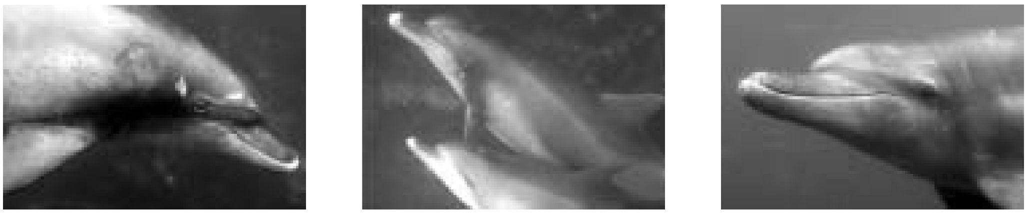

Fig. 7 Photographs of Mikura dolphins

2020–2310 mm (Ross, 1977), indicating that M30133 is within this range. If M30133 was already sexually matured, there is a possibility that the strongly contrasted layers in the 7th GLG (at 6 years old) may have been associated with reproductive cycles of females, labor and lactation (Myrick, Jr., 1991). The data sheet compiled by the Mikura Jima Bando Iruka Kenkyukai contains no data indicating that M30133 (Kushidango) was pregnant or sighted with neonate.

Morphological considerations. As was mentioned above, the animal examined in this article were not physically mature judged from several findings. It would therefore be not fruitful to make detailed comparison of measurements. We can, however, summarize several characters we found on their external and skeletal morphology. The beak is slender and long in Mikura dolphins. %Md values were calculated for the measurements of Mikura bottlenose dolphins and also for those of offshore bottlenose dolphins, to evaluate the mean difference in proportion to the body length or to condylobasal length. Among %Md values for the external body measurements Nos. 3, 4, 9, 27, 2, and 25 are largest and Nos. 10, 24, 26, 29, 19, and 5 are large. These measurements are for the beak length, head length, position of the flipper, flipper length and width of the both flukes. Mikura dolphins have relatively long beak (3, 4 and 9), and long head (2, 5 and 10). We do not make detailed comparison of the measurements because both two the specimens we examined were fairly young. Beak length, however, normally shows rather positive partial growth in various cetacean species, whereas proportional length of the head could be longer in younger animals. Thus, our result, showing that the Mikura dolphins have longer beak, has less possibility of overestimation. The beak can be still longer proportionately but the head could be shorter in adults. It would be safe to describe in Mikura bottlenose dolphins that the beak is longer than those of offshore bottlenose dolphins.

Among the five characters Ross (1977) listed as discriminating *truncatus*-type and *aduncus*-type bottlenose dolphins, 1) and 2) are not compatible and 4) and 5) are applicable. We propose following six characters significantly different between the two groups. a) In ventral view at the base of rostrum, palatine-pterygoid complex is pinched much more strongly at the anterior portion of the pterygoid in Mikura dolphins; b) At the vertex, nasals and postero-medial portion of both frontals form a complex which is much more clearly demarcated and elevated in offshore bottlenose dolphins; c) Supraoccipital has bilateral bulges on the dorso-caudal aspect in Mikura dolphins; d) Maxilla has an antero-mediad ridge (linea terminalis rostrae; terminal line of beak) from antorbital notch obliquely to almost middle of the beak. This ridge is longer and less prominent in Mikura dolphins whereas shorter and robust in offshore bottlenose dolphins; e) The lateral contour lines of maxillae are more straight in Mikura dolphins and outwardly curved in offshore bottlenose dolphins; f) Muscular ridge along the suture between frontals and supraoccipital is more prominent in offshore bottlenose dolphiins.

Skull measurements. Skull measurements were compared similarly as the external measurements. Measurement No. 24 indicate that the bony external nare is larger in the Mikura dolphin than that of the offshore bottlenose dolphin. Nos. 4, 5, 6, and 7 were smaller in Mikura dolphins depicting that their beak is narrower in the middle of its length. No. 43 is larger in Mikura dolphins, which indicates the premaxillary convexity (sensu Ross, 1977) is located more posterior and accounts for the longer beak in Mikura dolphins. This convexity or elevation suggests the region where connective tissue embracing the melon attaches firmly and has much to do with the ridge mentioned above (terminal line of the beak), which shows the line where the inner thickened layer of connective tissue delimiting antero-lateral border of melon anchors on each side of the beak. No. 26 indicates anterolateral length of lacrimal is much smaller in Mikura dolphins. Nos. 27, 41, and 44 are much smaller in Mikura dolphiins that might have some relation with smaller air way around the internal nare.

Stomach contents. Stomach contents of two Mikura dolphins are listed in Table 3. Prey species are mostly epi-pelagic fish and cephalopods. Both *Cypselurus agoo* and *Albunea symnista* were commonly seen around Mikura Jima. *Albunea symnista* is benthic crustacean found on the sea bed close to the island not deeper than 30 m. Cephalopods confirmed contain meso-pelagic species.

Molecualr phylogeny. In recent years, genetic distinctiveness of the *aduncus*-type bottlenose dolphin from the *truncatus*-type bottlenose dolphin has become recognized (LeDuc & Curry, 1998; Wang *et al*., 1999). Our results on analyses concerning the control region of mitochondrial DNA are summarized as follows, *truncatus*-type bottlenose dolphins from waters around Japan formed a cluster with *truncatus*-type of Chinese waters, whereas Mikura dolphins formed the cluster with *aduncus*-type bottlenose dolphins from Chinese waters. Two types of *Tursiops* are clearly distinguished on the molecular phylogenetic tree, and the individuals of the Mikura dolphins are not positioned at the base of *aduncus*-type cluster, but at the end of the cluster of *aduncus*-type (Fig. 5). This result indicates that Mikura dolphins has close affinity with the *aduncus*-type of Chinese waters rather than with the group of *truncatus*-type dolphins of geographically closer regions. The base substitution rate between *truncatus*-type of Japan waters vs. Mikura dolphins is 5 to 7%, on the other hand, the base substitution rate of *aduncus*-type bottlenose dolphins of Chinese waters vs. Mikura dolphins is 2%. These results suggest that the Mikura dolphins have great possibility of having the same genetic structure with *aduncus*-type bottlenose dolphins in Chinese waters.

In order to clarify systematics of these *Tursiops* cf. *aduncus* populations in the seas around China and Japan, further comparative works of populations including those from the type locality is necessary. Proper systematic positioning of these bottlenose dolphins, based on sufficient data of external morphology, skeletal morphology, molecular phylogeny, food habit, parasite specificity, ecology, behavior and so forth will give us better understanding on the species.

The regional distributions of *aduncus*-type bottlenose dolphins in the seas around Japan were reported from Amakusa Shoto, Amami Shoto, and Ogasawara Shoto, identified on the bases of external feature, cranial morphology and other characters (Miyazaki & Nakayama, 1989; Uchida, 1994; Kasuya *et al*., 1997; Shirakihara *et al*., 2002). The *aduncas*-type bottlenose dolphin population of Mikura Jima has been investigated by the Mikura Jima Bandoiruka Kenkyukai since 1994. Resulted from their efforts about 130 individuals are recognized annually, and cumulative number of identified individuals is about 220. On the other hand, there are sporadic findings of *aduncus*-type bottlenose dolphins in various regions adjacent to Mikura Jima, such as Toshima or Tateyama, some of which stay around the area for years (Fujita, personal communication). Some of the Tateyama dolphins were recognized as those registered in the Mikura Jima dolphin database. Individual recognition of the dolphin population facilitates analyses on structure of the society and geographical movement of the constituent dolphins.

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要　　約

御蔵島沿岸で死亡した2個体のイルカの外部形態，剖検所見，胃内容物，ミトコンドリアDNA，頭骨の形態学などについて調査した．これらは形態学的にはvan Bree (1966) やRoss (1977) あるいはWang *et al*., (2000a, 2000b) の*Tursiops aduncus*と類似点が多い．またミトコンドリアDNA調節領域の塩基配列からは，中国および台湾近海の*aduncus*-type *Tursiops* (Wang *et al*., 1999) と同じ系統に含まれることを確認した．ただし，2個体とも若い個体であり，厳密な形態学的議論を行うには成熟個体の標本の蓄積が必要である．また，*Tursiops* cf. *aduncus*の位置付けを含むgenus *Tursiops*の系統についてはさらに検討の必要がある．御蔵島では御蔵島バンドウイルカ研究会が，個体識別データベースを作成している．個体データの蓄積は，群の社会構造や離合集散を把握する上で不可欠である．

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Errata 正誤表

p.260 line 2	*truncatus* type	→	*truncatus*-type
p.261 line 17	Miyake	→	Mikura
p.263 caption of Fig.4 line1	*aduncus* and *truncatus*	→	*aduncus*-type and *truncatus*-type
p.263 Fig.5 (A)	*aduncas* type	→	*aduncus*-type
p.263 Fig.5 (A)	*truncatus* type	→	*truncatus*-type
p.263 Fig.5 (B)	*aduncas* type	→	*aduncus*-type
p.263 Fig.5 (B)	*truncatus* type	→	*truncatus*-type
p.263 caption of Fig.5 line1	*aduncus* type and *truncatus* type	→	*aduncus*-type and *truncatus*-type
p.264 line 17	dolphiins	→	dolphins
p.269 line 11	dolphiins	→	dolphins
p.269 line 21	dolphiins	→	dolphins
p.270 line 6	*aduncas*	→	*aduncus*
p.271 line 5	*tursiops* forms	→	*truncatus* forms
p.272 line 3	Wang, J.-P.	→	Wang, J. Y.
p.272 line 7	Wang, J.-P.	→	Wang, J. Y.